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Poster

493. Patterning of Brain

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 493.01/A1

Topic: A.01. Brain Patterning

Support: NIMH00023

Title: ERBB3-regulation of Bergmann glia proliferation is required for cerebellar lamination

Authors: *A. SATHYAMURTHY, D.-M. YIN, A. BARIK, C. SHEN, J. BEAN, W.-C. XIONG, L. MEI
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Abstract: The cerebellum controls motor movement and has recently been implicated in higher functions including cognition and emotion. Fundamental to cerebellar function is the unique laminar organization. During development, granule cell precursors (GCPs) proliferate in the external granule layer (EGL) beneath the glia limitans and migrate along the Bergman glial (BG) scaffold into the internal granule layer (IGL). Yet, little is known about BG proliferation and scaffold formation. We studied the role of ERBB3, a receptor of neuregulin 1 (NRG1), in cerebellar lamination. GFAP::Cre;Erbb3F/F mice that lack ERBB3 in both radial glia and neurons exhibited impairments in balance and motor-coordination. Cerebellar lamination was aberrant; Purkinje neurons (PNs) were misplaced; and granule neurons (GNs) failed to migrate into the IGL and formed ectopic clusters in the ML. Interestingly, these phenotypes were not observed in Math1::CreERT2; Erbb3F/F mice where the Erbb3 gene was deleted in GNs, suggesting that loss of Erbb3 in cells other than GNs and PNs contributes to lamination defects in GFAP::Cre;Erbb3F/F mice. Moreover, we found that ERBB3 was expressed in cerebellar astroglial cells and was required for their proliferation in culture. These observations support a working model where cerebellar BG require ERBB3 for proliferation. Diminished BG proliferation in the absence of ERBB3 causes reduction and disorganization of BG scaffold that is required for GN migration and cerebellar lamination. These observations identify a novel, cell autonomous role for ERBB3 in BG proliferation and cerebellar lamination.

Disclosures: **A. Sathyamurthy:** None. **D. Yin:** None. **A. Barik:** None. **C. Shen:** None. **J. Bean:** None. **W. Xiong:** None. **L. Mei:** None.

Poster

493. Patterning of Brain

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Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

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Topic: A.01. Brain Patterning

Support: Deutsche Forschungsgemeinschaft SFB629 project A2

Title: The motorized Rho GAP Myosin IXa is important for radial glia function

Authors: *S. THELEN, M. BÄHLER
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Abstract: Proper function of neural progenitor cells depends on the development of specific cell-cell adhesion structures and the establishment of an apico-basal polarity. Coordinated distribution of plasma membrane components and dynamic reorganization of the actin cytoskeleton are crucial to establish and maintain this polarity. It is regulated by signaling networks and protein complexes that include Rho family GTPases and polarity complexes. Among the regulators of the GTPase RhoA is the mammalian class IX myosin Myo9a. Constitutive deletion of Myo9a expression in mice leads to severe postnatal hydrocephalus formation (Abouhamed et al., 2009). The underlying molecular mechanism is not exactly known. Leakage of lysophosphatidic acid (LPA) into embryonic brain tissue through traumatic injury of blood vessels has been proposed to induce hydrocephalus through the induction of neuronal rosettes. LPA can induce Rho activity just as the loss of a RhoGAP. Therefore, we examined the role of the motorized RhoGAP Myo9a during neurogenesis in the developing mouse cerebral cortex. In radial glia cells of the developing telencephalon Myo9a localizes to the contractile actin ring along the apical junctional complex. As revealed by immunofluorescence stainings and electron microscopy images, its loss leads to thickening of F-actin bundles and a reorganization of adherens junction (AJ) components. In WT cortices, N-Cadherin labeling appeared as a prominent apical band along the ventricular surface. Upon loss of Myo9a, the N-Cadherin band appeared diffuse, indicating loss of the normal AJ organization. Consistent with this, aberrant localization of megalin, an apical membrane receptor, was observed in Myo9a-/- radial glia. These results demonstrate that loss of the motorized RhoGAP Myo9a impairs the proper morphological arrangement of cell-polarity and cell-fate cues in radial glia cells. The numbers of basal mitotic progenitors originating from asymmetric cell divisions of radial glia cells were increased by 3-fold in Myo9a-/- cortices. However, the total numbers of Pax6+ radial glia and Tbr2+ basal progenitors were unaltered. Except for a few aberrantly positioned neurons in the developing telencephalon, loss of normal radial glia morphology did not grossly interfere with

neurogenesis as indicated by normal layering of the cortex at birth. Mere inhibition of the Rho downstream effector ROCK did not rescue the phenotype. In conclusion, Myo9a is important for proper morphology and division of neural precursor cells, but is dispensable for neurogenesis. The exact molecular mechanism(s) by which Myo9a-RhoGAP affects AJ in radial glial cells remains to be investigated.

Disclosures: S. Thelen: None. **M. Bähler:** None.

Poster

493. Patterning of Brain

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Topic: A.01. Brain Patterning

Support: FAPESP 2013/12928-8

Title: Gap junction channels has different patterns of distributtion and plays specific roles during the chick brain development

Authors: *V. PASCHON, G. S. V. HIGA, E. R. KINJO, C. M. FURTADO, N. V. SANTOS, A. H. KIHARA
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Abstract: Gap junction channels formed by connexins (Cx) may play essential roles in some processes that occur during neuronal development, such as apoptosis and calcium wave spread. Although Cx expression was described in the chick retina, their expression in the visual areas of the brain is largely unknown. The present study was undertaken to determine the distribution pattern of Cx36, Cx43, and Cx45 by immunofluorescence, Western Blot, and real time PCR during the development of the chick Optic Tectum (OTe). We also measured the coupling level in each age by scrape loading (dye uptake). Our results showed that Cx43 was mostly expressed in the adult chick OTe (P15 – 100%) co-localizing with radial glial cells, while E5 was only 15-30% ($P<0,05$). The Cx43 phosphorylated isoform was only present in P15 suggesting formation of functional gap junction channels. Cx36 was up-regulated during the development. We observed low levels at E5 (0,19% $P<0,05$), which increased during the development. At E15 and P15 we observed specific neuronal layers expressing Cx36. Cx45 was highly expressed in the beginning of the development and was maintained during the development. Our results about scrape loading showed that the neurobiotin dye was uptake in all developmental stages, while the

Lucifer Yellow dye was spread only after E15 showing a transient pattern. In addition, E10 neuronal and glial cell culture of chick OTe was incubated with quinine, a specific blocker for Cx36 50 and 100μM, for 24hours to observe specific changes related with neuritogeneses and synaptogenesis. In conclusion, we observed an important modulation of Cxs during the development, which is probably related to particular roles in the brain function and maintenance of homeostasis during development of the chick OTe. Cx45 may have important roles during the neurogenesis and in the control of proliferative processes. Cx36 is upregulated during the development revealing important roles in the late development. On the other hand, Cx43 may be important after cell differentiation. It was find functional gap junction channels in the hole development and adult. Finally, the blockade of specific Cxs alters the synaptogenesis and neuritogenesis *in vitro*. Financial **Support:** FAPESP.

Disclosures: V. Paschon: None. **G.S.V. Higa:** None. **E.R. Kinjo:** None. **C.M. Furtado:** None. **N.V. Santos:** None. **A.H. Kihara:** None.

Poster

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Cell Innovation

CREST

Title: RNA Binding Protein Sfpq is required for the expression of schizophrenia-related long neuronal genes

Authors: *A. TAKEUCHI[1], K. IIDA[1], K. NINOMIYA[1], M. ITO[2], K. OHNO[2], M. HAGIWARA[1]
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Abstract: Mammalian neuronal cells express long pre-mRNAs which are vulnerable to misregualtion of transcriptional elongation or processing but the neuron-specific regulation of long pre-mRNAs is still largely unknown. Here we found that RNA-binding protein Sfpq (splicing factor, proline/glutaminerich) is essential for neuronal genes to fully express extremely long pre-mRNAs. Sfpq was specifically expressed in differentiated neurons during development and disruption of Sfpq caused massive apoptosis in embryonic mouse brains. Genome-wide Sfpq binding analysis using *in vivo* iCLIP (cross-linking immunoprecipitation) showed saw-tooth binding patterns on entire pre-mRNAs of more than 7400 genes with low sequence specificity, which suggests co-transcriptional binding of Sfpq to entire pre-mRNAs. In transcriptome analysis of Sfpq-/- brains, the expression of Sfpq-bound pre-mRNAs longer than 100 kilobases was significantly down-regulated. Gene Ontology analysis demonstrated these Sfpq target long genes have essential functions for late development stage of brains, including cell adhesion, neuronal migration, axon guidance, and synapse formation. Mutation of Sfpq target long genes are shown to have association with several neurodevelopmental disorders including schizophrenia. To further address the *in vivo* function of Sfpq, we examined the heterozygous mutant mice and performed comprehensive behavioral test battery. Sfpq-heterozygous mutant mice were viable and they appeared grossly normal, including the morphology of brains. However, Sfpq +/- mutant mice displayed schizophrenia-like abnormal behaviors in fear and anxiety-related behavioral test and Pre-pulse inhibition test. These data indicate that the RNA binding protein Sfpq is required for the expression of long pre-mRNAs which play essential roles for neuronal development and that the impairment of Sfpq-dependent gene expression could be a cause of neurodevelopmental and psychiatric disorders.

Disclosures: **A. Takeuchi:** None. **K. Iida:** None. **K. Ninomiya:** None. **M. Ito:** None. **K. Ohno:** None. **M. Hagiwara:** None.

Poster

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Topic: A.01. Brain Patterning

Support: NIH Grant 5T32NS007098-33

Title: Fgf signal transduction through frs in the developing telencephalon

Authors: *N. KAMATKAR[1,2], G. GUTIN[3], J. HÉBERT[2,3]
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Abstract: Fibroblast growth factors (FGFs) are a family of secreted proteins utilized for different processes including survival and specification of cells during embryonic brain development. How these factors induce these different processes in a spatial and temporal manner is an essential question in understanding FGF signaling. Our insight into FGF signal transduction has largely been deduced from *in vitro* experiments that have found several intracellular transducers that directly bind to the receptor including FGF Receptor Substrate proteins 2α/β (FRS2/3). To understand the underlying mechanism of FGF signaling in the developing telencephalon through FRS2/3, we inhibited signaling through FRS2/3 via two complementary approaches. First, we inhibited the binding of the adaptor proteins FRS2/3 to the receptor intracellularly, and we observed a severe phenotype in which the telencephalic precursor cells do not survive which results in a loss of the telencephalon. We suspect that there are other intracellular signaling transducers that are unable to bind to the mutated receptor since when we conditionally delete the adaptors FRS2/3 themselves, we find that there is a less severe phenotype in which the telencephalon is largely formed albeit reduced in size with failure to properly differentiate cells in the medial ganglionic eminence (MGE). Thus, FGF signaling through FRS2/3 during early development of the telencephalon is required for only a subset of functions including properly differentiating neurons in the MGE.

Disclosures: **N. Kamatkar:** None. **G. Gutin:** None. **J. Hébert:** None.

Poster

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Topic: A.01. Brain Patterning

Support: Supported by Grants from NIH and The Kavli Foundation

Title: Origin and secondary expansion of the transient subplate zone in the developing cerebrum of human and nonhuman primates

Authors: *A. DUQUE[1], Z. KRSNIK[3], P. RAKIC[2], I. KOSTOVIC[3]
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Abstract: Because of its exceptionally large size it may not be coincidence that the subplate (SP) was originally discovered, and its basic functions proposed, based on the analysis of the developing cortex in human and nonhuman primates (NHPs) (Kostovic and Moliver, 1974; Rakic, 1977). Furthermore, the generally accepted hypothesis that the SP evolves from the deeper stratum of the primordial preplate layer (PP) after it becomes separated from the marginal zone (MZ) neurons situated above, by the arrival of the cortical plate neurons from the ventricular zone (VZ), was also based on histological studies of human fetal brain tissue (Marin Padilla, 1978). However, most of the subsequent research on the genesis, function and fate of the SP was carried out in non-primate species such as rodents and carnivores (e.g. Allendoerfer and Shatz, 1994; Kanold and Luhmann, 2010) and its origin and evolutionary expansion in human remains elusive. Here we take advantage of the large and slowly developing SP in the macaque monkey to examine the time of origin, mode of migration, settling pattern and fate of the SP in primates. Monkey embryos were exposed to the DNA replication marker H3-Thymidine (TdR) at early embryonic ages and sacrificed at different intervals to follow changes in positions and fates of the postmitotic cells. Surprisingly, we found that most SP neurons generated in the VZ initially migrate radially to the cortical plate together with the prospective layer 6 neurons. Only subsequently, during mid-gestation, they become secondarily displaced within the widening SP zone which expands by growth of the neuropil, due mainly to invasion of basal forebrain, thalamocortical and cortico-cortical axons. Examination of the SP in the embryonic human cerebral wall at corresponding fetal ages, indicates even larger displacement of SP cells, particularly subjacent to the association cortical areas and underneath the summit of the folia which contains the voluminous contingent of early cortico-cortical connections. Overall our data sheds new insight into the origin and fate of the SP in primates, and illustrate the dynamic nature of this transient zone, which has been associated with disorders of the highest cognitive functions, such as autism and schizophrenia (Akbarian et al., 1996; Kostovic et al., 2011; Hoerder-Suabedissen et al., 2013).

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Poster

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Topic: A.01. Brain Patterning

Support: Sharon Stewart Trust

Title: Implications of pax6 in pituitary gland function and development

Authors: *K. JOHNSON, C. BLATCHER, *. LAUDERDALE
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Abstract: Aniridia is a congenital condition characterized by the absence of the iris that is caused by a semidominant mutation in the transcription factor Pax6. The eye phenotypes of this disorder have been well characterized, but recent studies have shown that Aniridic patients over the age of 18 have a higher propensity for obesity, polycystic ovarian disease, infertility, and severe eczema when compared to their non-Aniridic siblings. It is possible that these symptoms are a result of a Pax6 deficiency in the neuroendocrine regions of the brain, such as the pituitary gland. During development, Pax6 expression in the pituitary gland begins at e9.0 in the primordial anterior pituitary gland (Rathke's Pouch). This expression becomes restricted to the dorsal anterior pituitary by e11.5 and persists into adulthood. Therefore, it is plausible that a reduction in PAX6 could result in a change in pituitary hormone cell numbers and thus explain the endocrine symptoms that affect people with Aniridia. To test this possibility, we have used flow cytometry to measure pituitary cell numbers in the mouse model for Aniridia, Small eye, which have a deficiency in one copy of Pax6. Our data suggests that in adult mice a reduction in levels of PAX6 does not result in significant differences in cell numbers when compared to wild-type. Previous studies have reported changes in levels of Adrenocorticotropin Hormone and Thyroid Stimulating Hormone levels in people with Aniridia. However, based on the data presented here, it is unlikely that the hormone changes are a consequence of an increase or reduction in hormone-producing cells of the pituitary gland. Alternatively, it may be that Pax6 is indirectly affecting hormone production and release by regulating the enzymes that are responsible for generating the active form of the hormones, prohormone convertase (PC) 1 and 2. Future studies will therefore focus on the potential relationship between Pax6 and PC 1 and PC2, and on the possibility that Pax 6 affects the hypothalamic hormones.

Disclosures: K. Johnson: None. ***. Lauderdale:** None. **C. Blatcher:** None.

Poster

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Support: NIH grant 5R01MH081187

Title: The C-terminal region domain of reelin is required for arrest of cortical neuronal migration and formation of the hippocampal dentate gyrus

Authors: *S. HA[1], D. R. BEIER[1,2]
[1]Developmental Biol. and Regenerative Med., Seattle Children's Res. Inst., Seattle, WA;
[2]Brigham and Women's Hosp., Boston, MA

Abstract: We have previously described a forward genetic screen for cortical lamination defects and identification of a novel hypomorpic allele of *reelin* (Cereb. Cortex 2013 doi: 10.1093/cercor/bht209). This mutant, named *Reln* ^{CTRdel}, carries a splice-site mutation that results in a truncation of the C-terminal region (CTR) domain. The mutant expresses normal levels of secreted reelin protein, distinguishing *Reln* ^{CTRdel} from *Orleans reeler,* which has a larger C-terminal truncation that causes a secretion defect and phenocopies *reeler*. In contrast, *Reln* ^{CTRdel} displays remarkably distinct phenotypes from *reeler*. First, the size and foliation of the cerebellum is normal, and this mutant is not ataxic. Second, the mutant does not have the inversion of cortical layers; however, both superficial and deep layer neurons migrate beyond normal boundaries. This abnormality, in the absence of layer inversion, strongly supports the hypothesis that that *reelin* may act as a stop signal for migrating cortical neurons. Third, while lamination in *Cornu Ammonis* region of the hippocampus is normal, the dentate gyrus morphology is severely disturbed; most of the infrapyramidal blade is absent, while the suprapyramidal blade is present and better laminated compared to *reeler*. We are exploring the specific process that is disturbed in this mutant. We do observe increased *Dab1* expression, likely due to impaired negative feedback, which suggests defect in reelin signaling. In summary, CTR-truncated *reelin* still can carry out certain developmental processes (i.e. inside-out cortical lamination, normal cerebellar foliation and function, and development of the CA region and suprapyramidal blade). This unexpectedly enables us to elucidate a requirement of the *reelin* CTR domain in specific developmental processes: arrest of cortical neuronal migration and formation of dentate gyrus infrapyramidal blade.

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

Poster

493. Patterning of Brain

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Support: NIH Grant R01 EY022987

NIH Grant T32 EY015387

Title: Changes in the functional organization of the neocortex following lesions to visual cortex early in development

Authors: *L. A. KRUBITZER[1], J. C. DOOLEY[2]
[1]UC Davis, DAVIS, CA; [2]UC Davis, Davis, CA

Abstract: The neocortex is a highly dynamic structure that can be functionally optimized both in its organization and connectivity to generate appropriate behavior in an ever-changing social and physical environment. While many factors are known to contribute to this plasticity, the limit of this plasticity following early cortical injury is not well known. To better understand the time-course and extent of recovery of the neocortex, our laboratory has previously used the short-tailed opossum (Monodelphis domestica) as a model organism to investigate cortical reorganization. Opossums are born extremely early in development, which has allowed us to investigate the effects of lesioning the caudal pole of cortex (which would normally develop into primary visual cortex and other visual areas) before thalamocortical afferents have innervated cortex (postnatal day 4, P4). We found that early lesions result in a rostrally-shifted, compressed representation of somatosensory and auditory cortex and a very small, caudally-located visual area when animals were examined as adults. Thalamocortical projections had also shifted rostrally so that inputs from the major sensory nuclei correctly projected to their cortical targets (Huffman et al., 1999). The studies presented here demonstrate if and how plastic changes to the neocortex differ when cortex is bilaterally lesioned just after thalamocortical axons have innervated the neocortex (P12 in opossums). Following lesions, animals were allowed to develop to adulthood, and the functional organization of the neocortex was accessed using electrophysiological recording techniques. Unlike animals lesioned at P4, P12 lesioned animals do not show a compression or a rostral shift of somatosensory, auditory and visual areas. Additionally, only a small portion of cortex in which neurons responded to visual stimulation was found at the caudal-most portion of remaining cortex in some, but not all cases. Somatosensory cortex in these animals had a normal topography, with body and forepaw representations located medially and the face representation located laterally within somatosensory cortex. These results indicate that the extent of cross-modal plasticity following cortical insult is severely restricted once thalamocortical afferents have innervated the cortical subplate. Importantly, the persistence of a visual cortex is highly dependent on when cortical injury occurs relative to the development of thalamocortical axons.

Disclosures: L.A. Krubitzer: None. **J.C. Dooley:** None.

Poster

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Title: Suppressor of Fused (SuFu) controls the development of cerebellar granule neurons

Authors: *T. JIWANI[1,2], J. J. KIM[1,3], L. ROTIN[1], N. D. ROSENBLUM[1,2,3]
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Abstract: Cerebellar granule cells (GCs) are neurons that coordinate motor function, memory and learning. GC development is tightly controlled by the Sonic Hedgehog (SHH) signaling pathway. The pathway regulator, Suppressor of Fused (SUFU) modulates the levels of SHH effectors by stabilizing GLI activators and promoting the synthesis of GLI3 repressor (Gli3R), in a context dependent manner. Genetic deficiency of SUFU in all cerebellar cells resulted in abnormal patterning and cell differentiation, and reduced cerebellar size, suggesting a deleterious effect on GCs (Kim et al., 2011). However, the mechanisms by which SUFU controls GC development, specifically, are undefined. Here we investigate our hypothesis that SUFU controls GC development by regulating the activity of the SHH signaling pathway. We deleted SuFu specifically from the GC lineage using Cre recombinase driven by the Math1 promoter in mice. Mutant cerebella contained an increased number of Pax6-positive GC precursors, with a higher rate of GC proliferation demonstrated via *in situ* BrdU incorporation assays. A subset of mutant GCs also failed to migrate to their final destination in the mature cerebellum (internal granule layer), though they correctly expressed differentiation markers, such as NeuN and Tag1. Interestingly, the GFAP-positive Bergmann glia (BG) scaffold, a regularly arranged meshwork of neuron-supportive cells, was consistently disrupted in areas containing GCs that failed to migrate. Ectopic BLBP-expressing BG cell bodies were also observed in the GC precursor zone (external granular layer) in mutant cerebella, suggesting defects in BG patterning and

differentiation. As SuFu was not deleted from the BG lineage, these findings uncover a novel non-cell autonomous function of SuFu. Finally, constitutively expressing GLI3R (transgenically) in SUFU deficient mice fully rescued defects in GC proliferation, but not migration, suggesting complex molecular interactions downstream of SUFU in GC development. We conclude that (i) SUFU controls the number of GC precursors by cell-autonomously regulating GC proliferation and migration, in a GLI3R-dependent and -independent manner, respectively; and (ii) SUFU non-cell autonomously regulates the patterning of the BG lineage. We speculate that SUFU controls cerebellar development via SHH-dependent and -independent mechanisms.

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Poster

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Topic: A.01. Brain Patterning

Title: Behavioral examinations of forebrain-specific Ctgf knockout mice

Authors: *H.-C. CHANG[1], K.-C. CHEN[2], I.-S. YU[3], L.-J. LEE[2,4]
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Abstract: Connective tissue growth factor (CTGF), a member of CCN family, is important for the development of connective tissue. In the cerebral cortex, CTGF is expressed in subplate neurons. However, the roles of CTGF in the brain are still largely unknown. To evaluate the functions of CTGF in the brain, a forebrain-specific *Ctgf* knockout mouse model was generated by crossing *Ctgf*$^{flox/flox}$ mice with *Emx1-Cre* transgenic mice. In *Ctgf*$^{flox/flox}$; *Emx1-Cre* mice, the expression of Ctgf in the subplate was abolished while these mice are still fertile and develop normally. In the present study, the behavioral phenotypes of *Ctgf*$^{flox/flox}$; *Emx1-Cre* mice were examined. Wildtype and mutant mice exhibited comparable locomotor activity in an open field and similar sensorimotor gating ability in response to acoustic startle stimuli. The olfactory function was evaluated in buried food test and the latency to uncover food pellet was equivalent between genotypes. However, the mutant mice tended to spend more time in the closed arms of an elevated plus maze and display less immobility time in the forced swimming test. Since some emotion-related behaviors are affected in forebrain-specific *Ctgf* knockout mouse model, our data suggest a novel function of subplate-expressed Ctgf in adult brain.

Disclosures: **H. Chang:** None. **I. Yu:** None. **K. Chen:** None. **L. Lee:** None.

Poster

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Topic: A.01. Brain Patterning

Title: Role of hypoxic environment during neocortical development

Authors: *A. HONDA
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Abstract: During neocortical development, a multitude of cellular events must all occur with exquisite spatiotemporal control. Its location within the uterus when embryonic cardiovascular development and placental blood flow are not well-established, leads to a hypoxic environment. Despite this hypoxia, the embryonic cells are able to undergo remarkable growth and differentiation. Hypoxia-inducible factor (HIF) is known to play critical roles as molecular sensors of oxygen pressure, and HIF-1alpha has been reported to be crucial for normal brain development. Additionally, HIF may acts cooperatively with other signaling molecules such as Notch-Hes, thereby influencing a wide range of processes including tumor malignancy and neural progenitor maintenance and differentiation. In this study, at first we measured oxygen pressure level in the embryonic neocortex with needle type micro oxygen sensor. We found that the oxygen pressure level was around 1% to 2.5% at embryonic day of 12 (E12) to E15, and this oxygen level was generally known as physiological hypoxia. Next, a chemical reagent pimonidazole, which is activated in hypoxic cells, was utilized to confirm the localization of hypoxic cells in the developing neocortex, and we detected the activated pimonidazone around the ventricular zone. Furthermore, we performed transient transfection experiments in the mouse neuroblastoma Neuro2a cell line using plasmids containing HIF- responsive element (HIFRE) region fused to the luciferase reporter gene, and observed that HIFRE-luciferase activity was significantly increased in the hypoxic condition compared with that in the normoxic condition. Also, we found that hypoxic environment significantly increased Hes1 promoter activity, whereas hypoxia significantly suppressed both Neurogenin2 and NeuroD1 promoter activity in the Neuro2a cell line. In addition, the HIF target genes such as Igfbp5 preferentially expressed in the ventricular zone, and these HIF target genes were highly correlated with Notch-Hes

signaling. These results suggest that hypoxic environment plays a critical role in the regulation neural differentiation during neocortical development.

Disclosures: A. Honda: None.

Poster

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Title: Docosahexaenoic acid and its ethanolamide in the brain: Possible role in early development and neuroprotection of the dopaminergic system

Authors: *S. SONTI, S. J. GATLEY, R. I. DUCLOS, R. H. LORING, K. QIAN
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Abstract: Docosahexaenoic acid (DHA, 22:6), an omega-3 long chain fatty acid (LCFA), constitutes the primary structural LCFA of the brain. It is esterified as phosphatidylserine (PS) and phosphatidylethanolamine (PE) phospholipids localized primarily in the inner leaflet of cell membranes. Ca2+-independent phospholipase A2 (iPLA2) releases DHA from the membrane phospholipids, and DHA prevents apoptosis, promotes neuronal differentiation and activates neuronal gene expression by functioning as a ligand at the retinoid X receptor. DHA thereby plays an important role in neuronal survival and maturation. Due to its roles in both membrane architecture and cellular transcription, DHA is critical for proper development of brain. For example, recent evidence establishes the role of DHA in the development of the hippocampus and the dorsal root ganglia. Based on this background, we are investigating the possible role of DHA in the development and neuroprotection of the mid-brain. In parallel efforts, we are evaluating the uptake of exogenous DHA and related compounds by the brain *in vivo*, and the effects of DHA on a dopaminergic cell line. Our lab has previously shown that LCFA ethanolamides (N-acylethanolamines) enter the brain much faster than their corresponding free LCFA's (Abstract#: 639.25, 2012). In the brain, the ethanolamides are rapidly converted to free LCFA's by the enzyme fatty acid amide hydrolase (FAAH). DHA ethanolamide (DHEA) has been termed "synaptamide" (PMID: 21810478). We hypothesized that the ethanolamides might generally be useful to deliver LCFA's to the brain, in other words, to act as prodrugs. To test this

hypothesis, we synthesized the radiolabelled N-acylethanolamine of DHA, [C-14]docosahexaenoylethanolamine ([C-14]DHEA). Preliminary experiments confirmed that following intravenous administration the radiotracer was found in the mid-brain. Parallel *in vitro* studies on the IRB3AN27 (mesencephalic dopaminergic) cell line showed increased neuritogenesis following treatment with DHA, suggesting a possible role of DHA in the development and/or maturation of mid-brain dopaminergic system. Studies are in progress to confirm this role of DHA in the development of mid brain dopaminergic system and to evaluate the potential therapeutic effects of DHA and DHEA using a suitable animal model.

Disclosures: S. Sonti: None. **S.J. Gatley:** None. **R.I. Duclos:** None. **R.H. Loring:** None. **K. Qian:** None.

Poster

493. Patterning of Brain

Location: Halls A-C

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Program#/Poster#: 493.14/A14

Topic: A.01. Brain Patterning

Support: UDEM grant UIN14011

UDEM grant UIN13053

Title: The transcription factor ebf2 regulates gene expression driven by the murine prepro-orexin promoter

Authors: A. SANCHEZ-GARCIA[1], V. C. ZOMOSA-SIGNORET[1], C. DEVEZE[2], R. ORTIZ-LOPEZ[1], *R. VIDALTAMAYO[2]
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Abstract: Orexins (hypocretins) are peptide neurotransmitters that regulate wake-sleep cycle and arousal, promoting wakefulness and inhibiting REM sleep. We have previously demonstrated that KO mice lacking ebf2, a member of the bHLH family of transcription factors, show a narcoleptic phenotype, characterized by direct to REM transitions from the wake state and cataplexic episodes. This phenotype is due to the loss of 80% of the orexinergic neurons in the lateral hypothalamus and decreased levels of expression of orexin A in the remnant cells. We also identified two olf1-like binding sites in the putative minimal promoter region (comprising

roughly 450 bp upstream of the transcription initiation site) of the prepro-orexin genes from several mammalian species. Here, we analyze the regulation of expression of luciferase driven by the putative promoter region of the murine prepro-orexin gene. We generated luciferase-expressing constructs based on the pNifty3-Lucia plasmid vector (Invivogen, CA) containing synthetic sequences derived from the publicly available prepro-orexin gene sequence (MGI ID 1202306, Hcrt; NCBI: NM_010410.2). We transfected our reporter constructs into a stable, modified HEK 293 cell line, overexpressing mouse ebf2. Ebf2-expressing cells show a 7-fold increase in luciferase signals over levels observed in Wt HEK293 cells (2091±243 vs. 293±24 luminescence units, respectively; mean ± SEM; $P<0.001$, Bonferroni-corrected ANOVA followed by Holm-Sidak post-hoc test). Deleting both putative olf1 sites in the prepro-orexin promoter prevents the increase in luciferase expression and luminescence reverts to near Wt levels (188±17; $P<0.001$ vs. ebf2 cells). Our results suggest that ebf2 regulates prepro-orexin gene expression through binding to olf1 sites present in its promoter region.

Disclosures: **A. Sanchez-Garcia:** None. **V.C. Zomosa-Signoret:** None. **C. Deveze:** None. **R. Ortiz-Lopez:** None. **R. Vidaltamayo:** None.

Poster

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Topic: A.01. Brain Patterning

Support: KAKENHI 25430032

Title: Protocadherin 10 expression pattern in the developing mouse cerebellar cortex

Authors: S. VIBULYASECK[1], S. HIRANO[2], *I. SUGIHARA[1]
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Abstract: The cerebellum is compartmentalized into some tens of areas that have distinct axonal projection patterns, and thus that are presumably involved in different functions. In each compartment, a subset of Purkinje cells will have distinct expression levels of certain molecules, such as aldolase C (=zebrin II), which can be used as markers for the compartment. Because aldolase C is expressed only in the mature cerebellum, we investigated whether protocadherin 10 (Pcdh10), which is expressed during cerebellar development, could also act as a molecular

marker. By using heterozygote samples of the knock-in mouse strain (OL-KO, Lexicon Pharmaceuticals, Inc., USA; Uemura et al., 2007), in which Pchd10 gene expression is visualized by the reporter molecule beta-galactosidase, we were able to follow the evolution of cerebellar compartments through successive developmental stages. Whole mount preparations of the cerebellum from embryonic day 13.5 up to adult were used to investigate the alternating striped pattern of Pcdh10 expression throughout development. In the mature cerebellum (~adult), high expression areas were clearly compartmentalized into multiple stripes mainly in the vermis, paravermis, and paraflocculus. Although these compartments could be roughly traced through development, they were differently organized in shape and were merged in some cases in the immature cerebellum (down to E13.5). Consequently, the appearance of the mature striped pattern of Pcdh10 expression in the adult cerebellum was quite different from that in the embryonic immature cerebellum. We are now investigating the details of the developmental changes in Pcdh10 compartments. We also compared Pcdh10 compartments and Aldolase C compartments precisely in serial sections at postnatal day 16, when Aldolase C compartments start to stabilize. We are studying the spatial relationship between Aldolase C and Pcdh10 compartments. Initial results suggest that the compartments defined by these markers are exactly matched in some areas but not in others. The results indicate that the fine molecular compartments of the adult cerebellar cortex have a developmental lineage from immature early compartments in the embryonic cerebellum.

Disclosures: S. Vibulyaseck: None. **S. Hirano:** None. **I. Sugihara:** None.

Poster

493. Patterning of Brain

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Topic: A.01. Brain Patterning

Support: NIH Grant MH070596

NIH Grant 5K12GM10277902

Title: The role of FGF receptor adaptor proteins during embryonic brain development

Authors: *C. A. BLACKWOOD[1], G. GUTIN[1], N. KAMATKAR[1], K. LEE[2], F. WANG[3], G. FISHELL[4], M. GOLDFARB[2], J. HEBERT[1]
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Abstract: During early development, the Fibroblast Growth Factor (FGF) signaling pathway is one of the major inductive pathways that contribute to the ability of cells to organize into complex structures such as the telencephalon and its dorsal derivative, the cortex. For example, in early development FGFs are required for the survival of telencephalic precursors, while at later stages they maintain "stem-cellness" of radial glial cells in the neocortex. However, the role of the adaptor proteins that interact with FGF receptors to transduce signals and promote these various functions remain poorly understood *in vivo*. The FGF signaling adaptor proteins, FRS and Crk, are leading candidates for transmitting FGF signals during telencephalon development. We utilized conditional FRS and Crk knockout mice to study the roles of FRS and Crk genes during early and late development of the telencephalon. We found that the FRS genes are not critical for all aspects of FGF signaling during telencephalon development.

Disclosures: C.A. Blackwood: None. **G. Gutin:** None. **N. Kamatkar:** None. **K. Lee:** None. **F. Wang:** None. **G. Fishell:** None. **M. Goldfarb:** None. **J. Hebert:** None.

Poster

493. Patterning of Brain

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Topic: A.01. Brain Patterning

Title: Acupuncture on GB34 activates the precentral gyrus and prefrontal cortex in Parkinson's disease

Authors: *L. M. GYU[1], S. YEO, 30's[2], S. LIM, 50's[2], C. ILHWAN, 30's[2], M. VAN DEN NOORTC[3], P. BOSCH[4], G.-H. JAHNG[5]
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Abstract: Acupuncture on GB34 activates the precentral gyrus and prefrontal cortex in Parkinson's disease

Disclosures: **L.M. Gyu:** None. **S. Yeo:** None. **S. Lim:** None. **C. ilhwan:** None. **M. van den Noortc:** None. **P. Bosch:** None. **G. Jahng:** None.

Poster

493. Patterning of Brain

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Topic: A.01. Brain Patterning

Title: Morphometric study of the barrel cortex of forebrain-specific Ctgf knockout mice

Authors: *K.-C. CHEN[1], H.-Y. CHEN[2], H.-C. CHANG[2], I.-S. YU[3], L.-J. LEE[2,4]
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Abstract: Abstract Cconnective tissue growth factor (CTGF), a critical player in connective tissue development, is exclusively expressed in the cortical subplate. To explore the function of CTGF in the brain, a forebrain-specific *Ctgf* knockout mouse model was generated by crossing *Ctgf* $^{flox/flox}$ mice with *Emx1-Cre* transgenic mice in which the expression of *Cre* is prenatally initiated. The expression of Ctgf in the subplate was abolished in *Ctgf* $^{flox/flox}$; *Emx1-Cre* mice. The densities of cortical neurons were estimated in adult mice (10-12 weeks and 16-18 weeks). Besides, since Ctgf-expressing subplate neurons send axons to the center of each cortical barrel in which spiny stellate cells preferentially orient their dendrites toward it. The dendritic arborization of layer 4 spiny stellate cells in the barrel cortex was revealed using Golgi stain and the morphometric parameters of dendrites were measured. The densities of cortical neurons and dendritic arbors of layer 4 spiny stellate cells in the barrel cortex were comparable between genotypes in adult mice. Our data indicated that the cytoarchetecture of barrel cortex in adult brain is not affected by forebrain-specific deletion of *Ctgf* from the embryonic period.

Disclosures: **K. Chen:** None. **H. Chen:** None. **H. Chang:** None. **I. Yu:** None. **L. Lee:** Other; Neurobiology and Cognitive Science Center.

Poster

493. Patterning of Brain

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Topic: A.01. Brain Patterning

Support: JSPS KAKENHI Grant Number 24659085

Title: Functional analysis of the Reelin-Dab1 signal pathway in the developing zebrafish nervous system

Authors: *S. KIKKAWA, T. SUMIMOTO, T. SUMI, Y. SAKIHAMA, T. TERASHIMA
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Abstract: Reelin is an extracellular matrix protein highly conserved among vertebrates and is known to control neuronal migration during mammalian brain development. The intracellular protein disabled homolog 1 (Dab1) has been identified as a downstream adaptor protein in the Reelin signal. Spontaneous mutants for reelin and dab1, as well as targeted knockout mice, have been reported to share their characteristic laminar malformation in the brain cortices, which includes the largely inverted outside-in structure of the cerebral neocortex and distinctive hypoplasia of the cerebellar cortex. Whereas a large body of evidence has been accumulating in mammals, little is known about the roles of these molecules in the neural development of non-mammalian vertebrates. Zebrafish embryo is an established model organism bearing robustness to experimental manipulation and transparency feasible for visualization of developmental processes, including neuronal migration, nerve nucleus formation, and axonal outgrowth, in a living organism. To investigate functions of the Reelin-Dab1 signal pathway in neural development of non-mammalian vertebrates, we cloned their zebrafish homologues, including reelin and dab1 (with two isoforms, dab1a and dab1b). Immunohistochemical studies revealed that the Reelin protein is highly expressed throughout the CNS, including the telencephalon, optic tectum, cerebellum, hindbrain, and spinal cord, and that the expression pattern changes dynamically during embryogenesis. Next, we found that gene silencing using morpholino antisense oligonucleotides (MOs) against reelin or dab1a, but not dab1b, causes migration defects in the facial and vagus motor neurons in the developing hindbrain, defects in peripheral nerve pathfinding and fasciculation, and defects in posterior migration of the lateral line neuromasts . We also observed migration defects in cerebellar Purkinje, but not granule, cells in reelin and dab1a morphants. In addition, these Purkinje cells seem to undergo the retarded neuronal differentiation and/or development. Our results demonstrate that the Reelin-Dab1 signal pathway, known to mainly control neuronal migration in mammals, plays more diverse roles in zebrafish neural development.

Disclosures: **S. Kikkawa:** None. **T. Sumimoto:** None. **T. Sumi:** None. **Y. Sakihama:** None. **T. Terashima:** None.

Poster

493. Patterning of Brain

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Topic: A.01. Brain Patterning

Support: NIGMS F32 Postdoctoral Fellowship GM105202

Title: Distal enhancers and chromatin state in forebrain development

Authors: ***A. S. NORD**, C. ATTANASIO, J. A. AKIYAMA, A. HOLT, R. HOSSEINI, S. PHOUANENAV, I. PLAJZER-FRICK, M. SHOUKRY, V. AFZAL, E. M. RUBIN, L. A. PENNACCHIO, A. VISEL
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Abstract: Control of gene expression is critical during brain development. This is evidenced by genetic studies of neurodevelopmental disorders, where implicated genes converge in transcriptional regulation and chromatin remodeling pathways. Enhancers are distal regulatory DNA elements that activate expression of target genes. The genome-wide location and activity of enhancers can be predicted via ChIP-seq targeting histone modifications. We profiled the enhancer-associated histone mark H3K27ac in mouse forebrain across seven developmental stages. Complementary to time course analysis, we use data from additional histone modifications (e.g. H3K4me1, H3K4me3, and H3K27me3) to identify active and repressed chromatin states in the embryonic forebrain, and used transgenic assays to validate enhancer activity *in vivo* in the mouse brain. The results reveal regulatory circuitry associated with processes such as neuronal proliferation, lineage specification, and synaptic development and provide an entry point for examining the role of regulatory control systems in evolution and disorders of the brain. This integrated functional genomic study implicated many thousands of non-coding sequences, as well as active and repressive chromatin states, in orchestrating transcriptional regulation underlying forebrain development and function.

Disclosures: **A.S. Nord:** None. **J.A. Akiyama:** None. **A. Holt:** None. **R. Hosseini:** None. **S. Phouanenav:** None. **I. Plajzer-Frick:** None. **M. Shoukry:** None. **V. Afzal:** None. **E.M. Rubin:** None. **L.A. Pennacchio:** None. **A. Visel:** None. **C. Attanasio:** None.

Poster

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CIRM TG2-01153

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Sontag Foundation

Title: Mixed lineage leukemia 1 (MLL1) chromatin modifying factor maintains neural stem cell regional identity in the developing and postnatal mouse brain

Authors: *R. N. DELGADO, R. D. SALINAS, D. A. LIM
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Abstract: The adult subventricular (SVZ) zone contains neural stem cells (NSCs) that produce neurons throughout life. NSCs in the SVZ are regionally heterogeneous such that those located ventrally generate different subtypes of neurons than those located dorsally. Whether epigenetic mechanisms underlie the maintenance of the regional identity of NSCs is not known. Microarray analysis of dorsal and ventral V-SVZ NSCs revealed an enrichment of homeotic transcription factors consistent with the embryonic origins of each region. Specifically, ventral SVZ NSCs were enriched for Nkx2.1, a transcription factor required to specify the ventral telencephalon during embryogenesis. While sonic hedgehog (Shh) signaling is required to induce and maintain Nkx2.1 expression early in neural development, inhibition of Shh signaling by cyclopamine and vismodegib did not reduce Nkx2.1 expression in postnatal SVZ NSCs, suggesting that Nkx2.1 expression in the SVZ was being maintained epigenetically. Chromatin immunoprecipitation sequencing (ChIP-seq) analysis of dorsal and ventral SVZ NSCs revealed that many of the regionally expressed genes, including Nkx2.1, were enriched with H3K4me3, a chromatin mark associated with active transcription. Furthermore, Mixed-lineage leukemia 1 (MLL1) protein, a trithorax group member and H3K4 methytransferase, was enriched at the Nkx2.1 locus in ventral, but not dorsal SVZ NSCs. Conditional deletion of MLL1 in ventral SVZ NSC cultures resulted in loss of Nkx2.1 expression, but the Shh signaling pathway remained intact, indicating that MLL1 is required for the maintenance of Shh-independent Nkx2.1 expression. Using Nkx2.1-CreER fate tracing, we found that early embryonic Nkx2.1+ cells are direct precursors to

the Nkx2.1+ NSCs of the postnatal SVZ. We next investigated whether MLL1 is also required for Nkx2.1 expression during embryonic development. While conditional deletion of MLL1 with the Nestin-Cre transgene did not result in loss of Nkx2.1 expression at embryonic day 13.5 (E13.5), by E18.5, the expression of Nkx2.1 in the developing ventral telencephalon was greatly diminished. These data suggest that MLL1 is not required for the establishment, but rather the maintenance of Nkx2.1 expression in the embryonic and postnatal brain. These data thus illustrate how the positional information provided by extrinsic cellular signals such as morphogens becomes epigenetically encoded into regionally distinct cell types. More generally, these results help explain how complex developmental information is properly maintained despite rapid tissue growth and/or dynamic changes in morphogen gradients.

Disclosures: **R.N. Delgado:** None. **R.D. Salinas:** None. **D.A. Lim:** None.

Poster

493. Patterning of Brain

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

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Topic: A.07. Development of Motor, Sensory, and Limbic Systems

Support: Eunice Shriver Kennedy NICHD Grant R01HD078561, R21HD069001

Research Fellowship of the Ralph Schlaeger Foundation

Title: Emerging cerebellar pathways in humans from newborns to young adults using high angular resolution diffusion MR tractography

Authors: T. J. RE[1,2], K. IM[3], M. J. PALDINO[4], A. RIGHINI[5], P. E. GRANT[4], *E. TAKAHASHI[3]
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Abstract: We aimed to describe the evolution of the cerebellar pathways of the inferior (ICP), middle (MCP), and superior (SCP) cerebellar peduncles in developing human subjects ranging from newborn to young adult, using high angular resolution diffusion imaging (HARDI)

tractography. The ICP primarily contains fibers from/to the inferior olivary (IO) nucleus related to proprioceptive sensory and motor vestibular inputs as well as outputs for balance and posture maintenance. The MCP contains two dissociated pathways: 1) pathways from the rostral pons to the inferior cerebellum (MCP-1) attributed with a sensory-motor function and 2) pathways from the caudal pons to the superior cerebellum (MCP-2) attributed with a higher cognitive function. The SCP pathways are the primarily output pathways from the cerebellum. Number and volume of all studied pathways increased progressively with age with an initial plateau varying across pathways (approximately: ICP at 3Y, MCP-1 at 5Y, MCP-2 at 3Y, SCP at 3Y). MCP-1 and MCP-2 pathways were distinctly identifiable starting from the earliest ages, and the volume of MCP-1 was consistently greater than the volume of MCP-2 (2-way ANOVA, $p < 0.05$). Mean ADC for all studied pathways decreased in the first 5 years then plateaued. ADC values for ICP and SCP remained significantly higher than those of the MCP for all age groups ($p > 0.01$). Mean FA increased until a plateau of 5 years (chi-square test, $p < 0.05$). ICP showed significantly lower mean FA than all other tracks ($p < 0.01$), while MCP-1 showed significantly higher mean FA than all tracks ($p < 0.01$). ICP tracks were not detected until about 2 months (M) post-term and showed a surge in development from 2M to 6M post-term. At approximately 6 years of age, these tracks begin to branch more extensively. The absence of tractography pathways is likely to indicate lower myelination and lower density of axons, suggesting lower maturity of the pathways. Interestingly, the ICP, involving primary sensorimotor functions appear to begin maturing later, although complete more rapidly, than the SCP and MCP pathways. This may suggest that output pathways to the IO nucleus may play a role in the maturation of IO neurons and therefore axons which compose the ICP. Early extra-uterine proprioception and balance experiences may also contribute to the rapid development of the ICP 2M to 6M post-term. The dissociated maturation patterns of the two MCP pathways suggest that although there are pathways related to higher cognitive functions, primary motor functions are dominant in the cerebellum. We hope this work may represent an initial step towards the creation of an age-based reference atlas for developing cerebellar tracts.

Disclosures: T.J. Re: None. **E. Takahashi:** None. **K. Im:** None. **M.J. Paldino:** None. **A. Righini:** None. **P.E. Grant:** None.

Poster

493. Patterning of Brain

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Topic: A.07. Development of Motor, Sensory, and Limbic Systems

Support: Eunice Shriver Kennedy NICHD Grant R01HD078561, R21HD069001

NCRR Shared Instrumentation Grant Program (NIH S10RR023401, S10RR019307, and S10RR023043)

Title: Emerging callosal pathways in mice and humans using high angular resolution diffusion MRI (HARDI) tractography

Authors: *J. W. SONG[1], G. DAI[2], A. C. R. SCOTT[3], E. TAKAHASHI[3]
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Abstract: Little is understood about the early development of callosal connections in human brains. In mice, the corpus callosum pathways are thought to be pioneered by axons from the cingulate cortex, which help guide axons across the midline. Although extensive work has been carried out in mice, the spatiotemporal development of the callosal pathways in the developing human brain remains elusive. The aims of this study are to describe during human fetal, newborn, and toddler ages 1) the timing of the emergence of pioneer axons for callosal pathways and their course as they cross the midline, 2) specify the location within the mesial wall of the brain from where the pioneer axons for callosal pathways originate, 3) determine whether callosal axons are distinguishable from the population of pioneer axons by HARDI tractography, and 4) perform a comparative neuroanatomic analysis between mouse and human brains by tractography of the developing callosal connections. We first confirmed in mouse brains that the HARDI technique can detect pathways that likely correspond to pioneer axons and permanent callosal axons in the cingulate gyrus from embryonic day (E) 14.5. Overall axonal development patterns were consistent with previously reported orders and organization of emerging fiber pathways in mice. In humans at 15-17 gestational weeks (GW), most of the pathways running through the superior/inferior mesial wall were radially oriented to the brain surface and terminated in the ventricular zone. At 17GW, a few pathways from the superior mesial wall emerged and connected to the contralateral hemisphere through the corpus callosum. Interestingly, additional tractography pathways arising from more lateral regions of the brain also led to the corpus callosum. At 20GW, pathways connecting both hemispheres from/to the inferior mesial wall (cingulate gyrus) emerged and were observed in all of the anterior, middle, and posterior brain regions, while pathways from/to the superior mesial wall connecting both hemispheres were not observed at this stage. Only a few, local short pathways connecting the superior and inferior mesial walls within a hemisphere were observed. At 22GW, pathways corresponding to the callosal pathways from/to the cingulate gyrus emerged. At 30GW, short, local pathways were clearly identifiable by tractography. From 40GW to 2 years of age, tractography pathways from/to the superior/inferior mesial walls revealed a similar organization

as the 30GW. These results suggest that HARDI can image potential pioneer axons as early as 17GW in humans and identify developing pathways that correspond to permanent callosal pathways in the mesial wall of the brain.

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Poster

493. Patterning of Brain

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Topic: A.04. Stem Cells

Support: NUHS seed fund: R-181-000-157-112

Title: Maternal diabetes alters expression of microRNAs that regulate genes critical for neural tube development

Authors: *T. DHEEN, S. SHYAMASUNDAR, S. S. W. TAY, B. BAY
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Abstract: Maternal diabetes causes neural tube defects (NTDs) in embryos and neuropsychological deficits in infants. Over the years, several metabolic pathways and genes have been identified to be deregulated by maternal diabetes although the exact mechanism behind this remains unknown. Recently, micro RNAs have been shown to be critical for brain development and maturation. Therefore, we hypothesized that maternal diabetes alters the expression of miRNAs that regulate genes involved in biological pathways critical for neural tube development and closure. To address this, we performed miRNA expression profiling in neural stem cells (NSCs) isolated from the forebrain of embryos exposed to hyperglycemia *in vivo*. Since maternal diabetes results in fetal hypoglycemia/hyperglycemia or hypoxia (induced by hyperglycemia), we exposed NSCs *in vitro* to low glucose, high glucose or hypoxia. Distinct miRNA expression patterns were observed in NSCs from different groups. Pathway analysis identified biological pathways that were targeted by differentially expressed miRNAs in the different groups. From this, we identified 7 common pathways (namely MAPK signalling, focal adhesion, actin cytoskeleton, wnt signalling, neurotrophin signalling, ErbB signalling and axon guidance) that were predicted to be deregulated in NSCs from various groups. The biological pathways identified by pathway analysis have already been shown to be associated with or critical for neural tube development. Our results indicate that altered miRNA expression induced

by maternal diabetes may form the basis for deregulation of several metabolic pathways resulting in NTDs. Overall, our results provide novel insights into the mechanism of maternal diabetes induced NTDs and altered brain function in infants of diabetic mothers.

Disclosures: **T. Dheen:** None. **S. Shyamasundar:** None. **S.S.W. Tay:** None. **B. Bay:** None.

Poster

494. Brain Patterning and Cell Death

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Topic: A.01. Brain Patterning

Support: NIH Grant 3R01MH095147-0251

Title: Calcineurin regulated development of cortical interneurons

Authors: ***R. PRIYA**[1], I. GRAEF[2], G. FISHELL[1]
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Abstract: Our laboratory has identified a role for activity in the maturation of specific classes of cortical interneurons. Neuronal activity typically functions through activation of calcium-signaling cascades, however the contribution of any specific calcium signaling cascade or a calcium effector molecule in the development of interneurons has yet to be established. We observe that Calcineurin, a calcium dependent serine threonine phosphatase, is expressed in both medial and caudal ganglionic eminence-derived cortical interneurons. Loss of Calcineurin using a pan interneuronal driver decreases the survival of the animal to an average lifespan of 21 days. The overall body size of the animal is smaller, however the removal has no effect on the brain size. Interestingly, while at P6 the numbers of cortical interneurons is unaffected in conditional Calcineurin animals, by P21 it is 20% higher than found in age matched wild type littermates. This result suggests that the increase in the number of cortical interneurons observed at P21 is due to disruption in intrinsic cell death. Interestingly, in calcineurin knockout animals despite the overall increase in interneuron numbers, none of the subtype specific markers seem to be increased. In fact, paradoxically Parvalbumin and Reelin markers was found to be reduced. This suggests that calcineurin removal is also affecting the maturation of interneurons.

Disclosures: **R. Priya:** None. **I. Graef:** None. **G. Fishell:** None.

Poster

494. Brain Patterning and Cell Death

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 494.02/A26

Topic: A.01. Brain Patterning

Support: NIH MH068482

Title: Neuronal cell death during the pre- to postnatal transition in mice

Authors: *M. MOSLEY, C. SHAH, N. FORGER
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Abstract: Naturally occurring neuronal cell death is a widespread feature of brain development. Two waves of death have previously been described. The first occurs near proliferative zones, peaks around embryonic (E) day 14 in the mouse, and eliminates many neuronal precursor cells soon after they are generated. The second wave, known as postmitotic cell death, occurs after neurons have migrated and begun to make connections, and is thought to be largely postnatal. We previously generated an "atlas" of cell death in the postnatal mouse brain, using immunohistochemical detection of activated caspase-3 as a marker for dying cells (Ahern et al., 2013). Of the ~30 areas of the forebrain quantified from the atlas, many had elevated levels of cell death at postnatal day (P)1. This raised the question of whether peak cell death in these regions actually occurs prenatally; few studies have systematically examined cell death during the embryonic to postnatal transition. To address this, we generated timed-pregnant mice and collected male and female offspring on E17, E18, E19, P0 and P1. In most forebrain areas, there were very few activated caspase-3 cells prior to birth. This confirms that postmitotic cell death is predominantly postnatal in the forebrain. Of the regions in which postnatal cell death was previously quantified, the amygdala and hippocampus were the only areas to show appreciable levels of activated caspase-3 prenatally. In addition, however, we found striking accumulations of activated caspase-3 cells dorsal and ventral to the corpus callosum in the subcallosal sling and indusium griseum/cingulate cortex prenatally. Grayscale thresholding revealed significant effects of age in these regions ($p=0.0003$ and $p<0.0001$, respectively). The subcallosal sling showed a 2.5-fold increase between E17 and E18 and remained somewhat constant to P1. In the indusium grisium/cingulate cell group, a sharp, 3-fold, increase in caspase-3 immunoreactivity was observed between E17 and E18 followed by a return to baseline one day later. We also observed a significant age-by-sex interaction ($p<0.05$) in this region, due to higher cell death in males than in females on E18 and greater cell death in females on E19. Because microglia are suggested to play an active role in neuronal cell death, we stained adjacent sections for the microglial marker,

Iba1. We observed dense concentrations of microglia in the indusium griseum/cingulate cortex and subcallosal sling on both E18 and E19. We are currently determining the phenotype of the unusual clusters of dying cells, as well as effects of manipulating microglial activity on cell death in these regions.

Disclosures: M. Mosley: None. **C. Shah:** None. **N. Forger:** None.

Poster

494. Brain Patterning and Cell Death

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Topic: A.01. Brain Patterning

Support: CONACYT 179234

PAPIIT-DGAPA, UNAM IN206213

Title: Glutathione content modulates the effect of reactive oxygen species produced during cerebellar granule neurons development

Authors: *J. MORAN, M. OLGUIN
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Abstract: Reactive oxygen species (ROS) have been associated with neuropathologies and neurodegenerative diseases, as well as to different physiological processes in the nervous system acting as modulators of normal brain function. During development, ROS participate in some processes of neuronal differentiation and programmed cell death. In this study we evaluated the role of glutathione and the levels of ROS in neuronal development by using a model of cerebellar granule neurons (CGN). We cultured CGN from 0 to 8 days *in vitro* (DIV) under physiologic concentrations of KCl (5 mM, K5) or depolarizing concentrations of KCl (25 mM, K25) that is believed to mimic the presynaptic trophic action exerted by mossy fibers on the survival and maturation of CGN. We found a gradual increase in the levels of ROS during the first 2 DIV, which was sustained until 3 DIV under both K5 and K25 conditions. After this time (3 DIV), the levels of ROS returned to the basal levels in CGN cultured in K25, but not in K5, where ROS levels continued increasing from 3 to 5 DIV. In contrast to K25, and in coincidence with the increment of ROS levels observed in K5, the cell viability and the mitochondrial activity diminished significantly at 4 and 5 DIV. In addition, the levels of reduced glutathione (GSH)

increased two fold from 0 to 1 DIV under K5 and K25 conditions, preceding the peak of ROS observed at 2 and 3 DIV. These levels of GSH remained high in K25 until 5 DIV, and at 8 DIV they returned to the basal levels detected at 0 DIV. In contrast, the levels of GSH diminished from 3 to 5 DIV in K5. At 5 DIV, the levels of GSH were 30% higher in K25 than in K5. To determine the relevance of the transitory high GSH levels during CGN development, we inhibited the synthesis of GSH during different periods of time. When GSH synthesis was inhibited during 48 h, the totality of CGN died at 2 DIV, but no cell death occurs when treatment is delayed 1 or 2 DIV. This condition was completely reverted by the antioxidant Euk-134. On the other hand, when ROS levels were reduced by antioxidants or inhibitors of the ROS-producing complex NADPH-oxidase, the expression of the proteins MAP2 and TAU were reduced at 1 and 2 DIV, but not at 3 DIV. These results suggest that the regulation of ROS could influence different events of CGN development. At early times of development, the production of ROS, which is highly regulated by the GSH system, seems to be critical for neuronal maturation. During a second stage of CGN development, ROS levels reach a low basal state to allow cell survival, otherwise this would lead to the activation of the programmed cell death.

Disclosures: J. Moran: None. **M. Olguin:** None.

Poster

494. Brain Patterning and Cell Death

Location: Halls A-C

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Program#/Poster#: 494.04/A28

Topic: A.01. Brain Patterning

Title: Oxygen level-dependent effects of sod2 deficiency on the development of cultured cerebral cortical neurons

Authors: D. LU, Y. YANG, M. MATTSON, *A. CHENG
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Abstract: Superoxide dismutases (SODs), including cytosolic and extracellular SODs (SOD1 and SOD3) and mitochondrial SOD (SOD2), are major antioxidant defense enzymes. Inactivation of SOD2 results in neonatal lethality. However, the function of SOD2 in early neuronal development is unknown. In the present study we found that deletion of SOD2 reduced embryonic brain development. During the first 24 hours in culture, primary cortical neurons established from embryonic day 15 wild type (WT) and SOD2-/- mice appeared morphologically

similar. During the ensuing two days in culture SOD2-/- neurons exhibited a profound reduction of neurite outgrowth and subsequently died such that less than 10% of the SOD2-/- neurons remained alive at culture day 5. Mitochondrial mass and mitochondrial distributions to neurites were significantly reduced and the level of reactive oxygen species was dramatically increased in SOD2-/- neurons on culture day 3 compared to WT neurons. Moreover, the expression of the mRNAs encoding PGC1alpha, a transcription factor critical for mitochondrial biogenesis, and NRF1 and TFAM (two PGC-1alpha[[Unsupported Character - Symbol Font ]]target genes) were significantly reduced. Attempts to rescue SOD2-/- neurons with neurotrophic factors (FGF2 and BDNF), antioxidants (N-acetylcysteine, TMP and Tiron), an alternative energy substrate (pyruvate) and a mitochondrial membrane permeability pore inhibitor (cyclosporine) failed. However, maintenance of the neurons in a reduced oxygen atmosphere (3% O_2) abolished the adverse effects of SOD2 deficiency on neurite outgrowth and cell survival that occurred in the usual 20% O_2 atmosphere. Relative to the O_2 level neurons are exposed to *in vivo* (2-5%), the 20% O_2 level typically used for cell cultures is hyperoxic . Our findings therefore suggest that SOD2 plays a particularly important role in enabling neurite outgrowth and cell survival under conditions where superoxide production is high. Supported by the Intramural Research Program of the National Institute on Aging. Key Words: SOD2, neurite outgrowth, mitochondrial biogenesis, neuronal death, ROS.

Disclosures: D. Lu: None. **Y. Yang:** None. **M. Mattson:** None. **A. Cheng:** None.

Poster

494. Brain Patterning and Cell Death

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Support: European Research Council Grant 243153

The Wellcome Trust International Senior Research Fellowship 090946/Z/09/Z

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Title: Abhydrolase domain-containing protein 4 (ABHD4) controls adherens junction-dependent cell survival in radial glial cells

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Abstract: Anandamide (AEA) is a lipid mediator, which exerts its physiological effects in the brain by activating CB1 cannabinoid receptors and the non-selective cation channel TRPV1. This endocannabinoid/endovanilloid molecule is also present in the embryonic forebrain and emerging evidence indicates that anandamide signaling regulates several fundamental aspects of cortical development including proliferation and neuroblast migration. Interestingly, NAPE-PLD, the enzyme which was proposed to be the predominant anandamide-synthesizing enzyme in the brain is not expressed until postnatal ages raising the possibility that anandamide may be synthesized via an alternative synthesis route in the developing forebrain. Therefore, in the present study, we first tested the hypothesis whether ABHD4, a serine hydrolase involved in the metabolism of N-acyl phospholipids, including a potential lipid precursor for anandamide is expressed in the embryonic forebrain. We found that ABHD4 mRNA expression is highly expressed by radial glial cells (RGCs) in the ventricular zone throughout prenatal development. Despite this restricted expression pattern, which was validated in ABHD4 knockout animals, we could not detect any defects in processes regulated by RGCs including proliferation, neuroblast migration or adult cortical lamination in ABHD4 knockout cortices. On the other hand, the disappearance of ABHD4 expression from sister neuroblasts implied that the function of ABHD4 may interfere with neuroblast migration. Indeed, ectopic expression of wild-type ABHD4, but not of a hydrolase-dead ABHD4 mutant in postmitotic neuroblasts arrested migration and induced cell death. Importantly, ABHD4-induced cell death did not require CB1 receptors or TRPV1 channels indicating that the pro-apoptotic function of ABHD4 is independent of endocannabinoid or endovanilloid signaling. Because the absence of ABHD4 alone did not cause developmental defects, but ABHD4 needs to be downregulated in the sister neuroblasts, we then asked the question whether this serine hydrolase controls a specific cell death process in radial glial cells. Notably, *in utero* disassembly of adherens junctions in the ventricular zone by using a dominant-negative version of N-cadherin induced robust cell death in wild-type, but not in ABHD knockout embryios. In contrast, basal developmental or genotoxic stress-induced apoptosis did not require ABHD4 activity. Taken together, these findings demonstrate that ABHD4 plays a specific stand-by guardian function in radial glial cells by controlling adherens-junction-dependent cell survival during cortical development.

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Poster

494. Brain Patterning and Cell Death

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Topic: A.01. Brain Patterning

Support: Wellcome Trust grant 057700

Title: The small GTPase RhoQ is a target of the MLK-JNK-c-Jun pathway in sympathetic neurons

Authors: *J. HAM, M. KRISTIANSEN, R. HUGHES
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Abstract: Developing sympathetic neurons depend on nerve growth factor (NGF) for survival and die by apoptosis after NGF withdrawal. This process requires *de novo* gene expression and we used Affymetrix exon arrays to study the pattern of expression of all known genes in sympathetic neurons at 16 hours after NGF withdrawal compared to +NGF as a control (Kristiansen et al., 2011, *BMC Genomics* 12: 551). We selected 16 hours because this was previously defined as the transcriptional commitment point and induced genes known to be required for NGF withdrawal-induced death, e.g. *c-jun, bim, egln3*, are expressed at a high level at this time. By including a mixed lineage kinase (MLK) inhibitor, CEP-11004, in our experimental design we identified which of the genes induced after NGF withdrawal are potential targets of the MLK-JNK-c-Jun pathway, which promotes apoptosis following NGF deprivation. In these microarray experiments we found that the level of the *rhoq* mRNA increased by 2.5 fold after NGF withdrawal and this increase was completely inhibited by CEP-11004 suggesting that the *rhoq* gene could be a downstream target of the JNK pathway. The *rhoq* gene encodes a small GTPase (RhoQ, ras homologue family member Q) similar to Cdc42 and Rac1 but with some distinct properties. The RhoQ protein can activate various effectors including protein kinases that activate the JNK pathway and this can increase the phosphorylation and transcriptional activity of c-Jun. We confirmed in real time Q-PCR experiments that the *rhoq* mRNA increases in level after NGF withdrawal. Furthermore, in immunoblotting and immunocytochemistry experiments we found that NGF withdrawal causes a significant increase in the level of the RhoQ protein. To investigate the function of RhoQ in this context, we microinjected a pcDNA1-RhoQ expression vector into sympathetic neurons. We observed that overexpression of RhoQ in the presence of NGF induces neuronal death as efficiently as expression of a constitutively active form of MLK3. Finally, bioinformatic analysis

of the DNA sequence upstream of the first exon of the rat *rhoq* gene identified conserved AP-1 and ATF sites, which are within regions bound by c-Jun in ChIP-seq experiments published by the ENCODE project consortium. These results suggest that *rhoq* could be a direct target of c-Jun in sympathetic neurons. We are currently studying the role of RhoQ in the NGF withdrawal-induced death pathway and will present the results of these experiments.

Disclosures: J. Ham: None. **M. Kristiansen:** None. **R. Hughes:** None.

Poster

494. Brain Patterning and Cell Death

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Topic: A.01. Brain Patterning

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Title: Methylmercury exposure affects cell proliferation and death but not neural progenitor specification in xenopus laevis

Authors: *R. HUYCK[1], M. NAGARKAR[2], N. OLSEN[3], S. E. CLAMONS[1], M. S. SAHA[1]
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Abstract: Methylmercury (MeHg) is a ubiquitous environmental toxin with neurotoxic properties. To investigate the impact of MeHg exposure during early nervous system development, we exposed *Xenopus laevis* embryos to MeHg introduced in solution and assayed developmental response using a graded phenotype scale. We then evaluated the expression of the neural patterning genes Sox2, Delta, and En2, and the cell proliferation marker, PCNA, using *in situ* hybridization (ISH) and whole transcriptome microarray; and labeled patterns of apoptosis using TUNEL assay. *X. laevis* embryos showed a range of embryological disruption in both a dose and embryo density dependent manner, including failure of brain development, with a TD_{50} of ~0.05 microg/g MeHg and an LD_{50} of ~0.1 microg/g MeHg at densities of one embryo per ml of MeHg solution. Neural patterning genes did not show changes in gene expression even at the highest non-lethal doses by either ISH or microarray. In contrast, PCNA expression was

negatively correlated with MeHg dose at stages past gastrulation, while the amount of stained apoptotic cells in epithelial and neural regions was conversely increased. Partek pathway analysis on the differentially expressed genes identified by microarray similarly showed a significant impact of MeHg exposure on cell cycle and apoptotic pathways. These results suggest that disruption of neural development by MeHg may not be directly due to a loss of early neural patterning gene transcription, but rather related to impairment of cell proliferation during neurogenesis and an increase in cell death throughout the developing nervous system.

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Poster

494. Brain Patterning and Cell Death

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Topic: A.01. Brain Patterning

Support: NIMH Contract HHSN-271-2008-0047-C to the Allen Institute for Brain Science (Seattle, WA)

Title: Spatiotemporal expression atlas of brain development in non-human primate

Authors: *T. BAKKEN[1], J. MILLER[1], S.-L. DING[1], S. SUNKIN[1], K. SMITH[1], L. NG[1], A. SZAFER[1], J. GOLDY[1], C. LEE[1], A. EBBERT[1], R. DALLEY[1], N. DEE[1], J. ROYALL[1], P. D. PARKER[1], Z. RILEY[1], Z. MOLNAR[2], R. HEVNER[3], D. AMARAL[4], M. HAWRYLYCZ[1], A. JONES[1], J. PHILLIPS[1], P. WOHNOUTKA[1], C. DANG[1], A. BERNARD[1], J. HOHMANN[1], E. LEIN[1]
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Abstract: Existing gene expression atlases of the developing human brain have focused on attaining either fine spatial resolution at few timepoints or broader temporal resolution at low spatial resolution, largely due to tissue limitations. Non-human primates make ideal model systems for studying human brain development due to their close evolutionary proximity to humans, protracted development and maturation, and availability of multiple specimens from precisely specified ages. We present here a transcriptional atlas of the developing rhesus macaque brain that provides both high spatial and temporal resolution by using laser

microdissection and DNA microarray-based profiling across ten prenatal and postnatal timepoints, providing a window into understanding the role of genes associated with primate brain development, human evolution and neurodevelopmental diseases. A major focus was to probe the molecular underpinnings of events in cortical development, including progenitor and post-mitotic excitatory neuron development, thalamocortical projection specificity, and GABAergic neuron development by selectively assaying layers of several cortical areas (including V1) and associated subcortical structures including the dLGN and ganglionic eminences. Six prenatal time points correspond with ages of peak neurogenesis for different layers in V1, and four postnatal time points correspond to key phases of postnatal development: infancy, childhood, adolescence and early adulthood. We find extensive variation between cortical layers and across developmental stages, as well as evidence of a global transcriptional "clock," involving dramatic rates of gene expression change in all assayed regions throughout prenatal and early postnatal development. Interestingly, these rates were consistently 2 to 3-fold higher in macaque, mirroring the more rapid developmental trajectory compared to humans. We find a great deal of conservation of laminar and areal patterns between human and macaque, substantiating its value for studying the molecular basis of human cortical development. Finally, we used the anatomical specificity of connections between dLGN and layer 4Cb of V1 to search for genes that may mediate this connectivity, and find shared selective expression in parvocellular layers of dLGN and L4Cb of V1. Several of these genes (LRRTM1, EPHA3, CNTN5) have a known role in axon guidance and cell adhesion and may contribute to the target specificity of thalamocortical afferents in V1. This dataset is publicly accessible as part of the NIH Blueprint NHP Atlas at www.blueprintnhpatlas.org.

Disclosures: **T. Bakken:** None. **J. Miller:** None. **S. Ding:** None. **S. Sunkin:** None. **K. Smith:** None. **L. Ng:** None. **A. Szafer:** None. **J. Goldy:** None. **C. Lee:** None. **A. Ebbert:** None. **R. Dalley:** None. **N. Dee:** None. **J. Royall:** None. **P.D. Parker:** None. **Z. Riley:** None. **Z. Molnar:** None. **R. Hevner:** None. **D. Amaral:** None. **M. Hawrylycz:** None. **A. Jones:** None. **J. Phillips:** None. **P. Wohnoutka:** None. **C. Dang:** None. **A. Bernard:** None. **J. Hohmann:** None. **E. Lein:** None.

Poster

494. Brain Patterning and Cell Death

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Topic: A.01. Brain Patterning

Support: MRC UK

Taiwan Study Aborad Studentship

Title: The regulatory role of Pax6 on cortical progenitor cell proliferation

Authors: *Y.-T. HUANG[1,2], D. MI[2], K. RUISU[4], A. PANTAZI[3], J. O. MASON[2], D. J. PRICE[2]
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Abstract: Forebrain development is controlled by a set of transcription factors which are expressed in dynamic spatiotemporal patterns in the embryonic forebrain and are known to regulate complex gene networks. Pax6 is a transcription factor that regulates corticogenesis and mutations affecting Pax6 protein levels cause neurodevelopmental defects in eyes and forebrain in both humans and mice. In previous studies, it is shown that the graded expression pattern of Pax6 protein, which is high rostro-laterally to low caudo-medially in the cerebral cortex, is critical for its control of cell cycle progression and proliferation of cortical progenitors. However the underlying mechanisms are still unclear. Based on a microarray analysis, we identified a number of cell cycle-related candidate genes that may be affected by Pax6. One such gene, *Cell division cycle associated 7 (Cdca7)* is expressed in a counter-gradient against that of Pax6. In addition, *Cdca7* mRNA expressions is upregulated in *Pax6* null (*Small eye*) mutants and downregulated in mice that overexpress PAX6 (*PAX77*). There are several potential Pax6 binding motifs close to Cdca7 gene. One of them is proven to be physically bound by Pax6 *in vivo* by chromatin immunoprecipitation. Promoter luciferase assays using fragments combining four suspect binding motifs show that Pax6 is functionally critical. Cdca7 is a Myc and E2F1direct target and is upregulated in some tumours but its biological role is not fully understood. Current work using *in utero* electroporation to overexpress Cdca7 around the pallial-subpallial boundary (PSPB), where Cdca7 expression levels are normally lowest, to test the effects on the cell cycle of cortical progenitor cells in this region.

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Poster

494. Brain Patterning and Cell Death

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University Paris-Sud

Ecole des Neurosciences de Paris (ENP)

Fondation pour la Recherche Médicale (FRM)

ZF-Health (FP7/2010-2015, grant agreement number 242048))

Title: A revised view of the regionalization of the ventral secondary prosencephalon based on morphogenetic analysis in zebrafish

Authors: *K. YAMAMOTO, P. AFFATICATI, B. RIZZI, C. BUREAU, N. PEYRIÉRAS, C. PASQUALINI, M. DEMARQUE, P. VERNIER
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Abstract: Regionalization of the forebrain is complex and highly dynamic event during the development. Since some of the processes are conserved among different vertebrate groups, notably at the developmental time point referred to as the “phylotypic stage”, we took advantage of zebrafish embryos to examine in 3D the secondary prosencephalon at 24, 30, and 48 hours post-fertilization. We systematically analyzed the i) localization of cells undergoing proliferation and differentiation and ii) expression of developmental genes, in relation to the ventricular organization. Based on the general distribution of DAPI staining in link with ventricular morphology, three distinct cellular masses were identified: ones containing future telencephalon, preoptic area, and hypothalamus. These regions were bordered by radial glia (GFAP+) and nerve fibers (acetylated tubulin+). The preoptic area was organized around the optic recess of the third ventricle, and the expression of ccna2 (marker of proliferating cells) and elavl3 (marker of differentiating cells) transcripts, and HuC/D protein (marker of differentiated cells) were organized in a centrifugal manner around the optic recess. A proneural gene neurogenin 1, transcription factors such as dlx2a and sim1a/otpb were also symmetrically expressed around the optic recess. These results suggest that the region containing the zebrafish preoptic area behaves as a morphogenetic unit, independent from the telencephalon and hypothalamus. We named this new morphogenetic unit the "ventral optic recess region" (vORR), as it is organized around the ventral part of the optic recess. Our results lead to reconsider the boundary of the telencephalon and the hypothalamus within the framework of the prosomeric model, and we propose possible homologous areas of the vORR between teleosts and tetrapods.

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Poster

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Support: NIHR21 NSO73585

Title: The expression and function of Sonic hedgehog in the human fetal telencephalon

Authors: *F. MEMI, N. RADONJIC, J. A. ORTEGA, N. ZECEVIC
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Abstract: Sonic hedgehog (Shh) signalling plays a crucial role in the development of the mammalian nervous system regulating ventral patterning, proliferation, differentiation and survival of progenitor cells along the entire neuraxis. The expression pattern of Shh in the rodent ventral telencephalon and its role in the specification of GABAergic interneurons has been thoroughly characterized. Still, little is known about the expression and function of this morphogen in the human forebrain. Here, we provide, for the first time, a detailed description of the Shh expression pattern in the developing human brain, focusing in the dorsal (cortical) part of the telencephalon, using *in situ* hybridization. Furthermore, we identify the cells producing Shh to be projection and inhibitory neurons as well as neuronal progenitors such as Radial Glial Cells (RGCs), using a combination of *in situ* hybridization and cell-type specific immunostaining. Next, we determined Shh concentration in the human fetal brain and compared the levels of Shh between ventral and dorsal telencephalon. Finally, to reveal the role of Shh signalling in the developing human cerebral cortex, we treated enriched cortical RGCs *in vitro* with exogenous Shh and its inhibitor cyclopamine. Control dorsal RGCs generated cortical interneuron progenitors expressing Nkx2.1 and Lhx6, and calretinin-positive (CalR+) cells, the only GABAergic neurons present in RGC cultures at this developmental stage. Treatment of dorsal RGC cultures with exogenous Shh, increased the pool of Nkx2.1+ progenitors, decreased levels of Lhx6 expression, and suppressed the generation of CalR+ cells. On the other hand, the blockade of endogenous Shh signalling by cyclopamine led to an increase in number of CalR+ cells in dorsal RGCs cultures, but did not affect Nkx2.1 expression. These results support the idea that in human forebrain Shh plays an important role in specification of cortical interneuron progenitors generated from dorsal RGCs.

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Poster

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USC Faculty Startup Funds

Title: Maternal hyposerotonemia alters placental physiology and biogenic amine function in fetal brain tissue of the B6.129(Cg)-SIc6a4tm1Kpl/J serotonin transporter knockout (SERT-KO) mouse line

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Abstract: Serotonin (5-HT) is a monoamine neurotransmitter fundamentally important for brain development, and recent studies have indicated the placenta, not the maternal blood or the fetal brain itself, is the source of 5-HT to the fetus in early development. Lower circulating levels of serotonin are associated with a variety of clinical psychopathologies including anxiety and depression. However, it is unclear how decreased circulating levels of 5-HT (hyposerotonemia) in mothers with such pathologies might affect placental levels of 5-HT and other biogenic amine signaling, or fetal brain development. Using a genetic mouse model for hyposerotonemia, B6.129(Cg)-*Slc6a*^{4tm1Kpl}*/J* serotonin transporter knockout (SERT-KO) mice were bred heterozygous (HET) by HET to generate wildtype (WT), HET, and knockout (KO) experimental genotypes. Females of each genotype were bred with HET males, and tissue was harvested at four different timepoints during embryonic development (E12.5, E14.5, E16.5, E18.5) as part of ongoing studies. High performance liquid chromatography (HPLC) analysis of forebrain, hindbrain, and placenta samples has measured concentrations of 11 biogenic amines, their precursors and metabolites in tissues. Thirty-three significant differences have been detected in biogenic amine levels when comparing maternal and fetal genotype combinations, indicating that SERT deletion has widespread effects on multiple neurotransmitter functions. Fetal brain tissue

from KO mothers had higher levels of both noradrenaline (NA) and 5-HIAA (main serotonin metabolite) than fetal brain tissue from HET mothers in a region specific manner. Increases in NA levels and higher concentrations of 5-HIAA, which indicates increased turnover rate of serotonin, are both associated with psychopathologies such as anxiety and depression. Placental tissues also showed marked decreases in 5-HT, NA, and 5-HTP in KO mothers as compared to HET mothers, regardless of fetal genotype, indicating a critical role for maternal availability of serotonin. Finding multiple differences in concentrations of biogenic amines due to 5-HT availability warrants further research regarding the effects of maternal 5-HT on placental physiology and fetal brain development.

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Poster

494. Brain Patterning and Cell Death

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Fondation pour la Recherche Medicale

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INSERM

CNRS

Title: Morphological features of early born GABA hub neurons: From embryo to adulthood

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Abstract: Early Born GABA neurons (EBGNs) are major components of the hippocampal circuit since at early postnatal stages they form a subpopulation of “hub neurons” transiently supporting CA3 network synchronization (Picardo et al., Neuron, 2011). One way to understand

the cellular mechanisms by which EBGNs coordinate neuronal activities as well as their developmental role is to examine the evolution of their connectome throughout life from the early perinatal period to adulthood. To this aim we have performed a thorough anatomical investigation of EBGNs labelled with EGFP/TdTomato using inducible genetic fate-mapping techniques. Postnatal day 3 appears to be a critical time point for the maturation of these cells, which coincides with the emergence of the first synapse-driven correlated activities within hippocampus. Indeed, we observed a spectacular development of the dendritic and axonal arborisation of EBGNs at P3, together with a higher expression of KCC2 at the membrane, further supporting their advanced maturation stage. EBGNs persist into adulthood. Locally, hippocampal EBGNs target principally dendrites of principal cells. Most importantly, hippocampal EBGNs include a significant proportion of cells with an extrahippocampal GABAergic projection to the septum but no other extrahippocampal target regions. Notably, half of the EBGNs from the dorsal CA1 at the rostral level project to the septum. Conversely, a quarter of EBGNs were targeted by septal afferents, as well as by inputs from the entorhinal cortex. Last, we found that some EBGNs outside from the hippocampus, like in the septum or the entorhinal cortex also displayed a long-range projection, this time to the hippocampus. We propose that a subpopulation of EBGNs develop into a network of GABAergic projection neurons in many brain areas, thus making them lifelong candidates to support information flow across distant structures

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Poster

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Title: Alterations of the forebrain in the Lhx2 and Lhx2/Lhx9 knockout mice

Authors: *A. ABELLAN[1], Y. ZHAO[2], E. DESFILIS[1], H. WESTPHAL[2], L. MEDINA[1]
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Abstract: The paralog genes Lhx2 and Lhx9 are expressed in specific and comparable patterns in the forebrain of different vertebrates. Studies in mouse and zebrafish showed that Lhx2 regulates forebrain development at multiple levels, including patterning, cell differentiation and proliferation, and axonal guidance (Porter et al., 1997; Seth et al., 2006; Saha et al., 2007; Zhao et al., 2010; Peukert et al., 2011). Lhx2 has been shown to be essential for the development of the neurohypophysis, the retina, and the telencephalon (Porter et al., 1997; Zhao et al., 2010). In the telencephalon of Lhx2-/- mice, there is agenesis of the hippocampal formation and severe malformation of the neocortex (Porter et al., 1997). In zebrafish, additional roles have been described for Lhx2 in the formation of the preoptic/commissural region (Seh et al., 2006), and for both Lhx2 and Lhx9 in the formation of the thalamus (Peukert et al., 2011). The roles of Lhx2 and/or Lhx9 in the thalamus, or Lhx2 in the preoptic region remain unexplored in mouse. We analyzed the role of both Lhx2 and Lhx9 in forebrain development by studying the mRNA expression of several developmental regulatory genes in the embryonic forebrain of Lhx2-/-, Lhx2-/-/Lhx9+/- and Lhx2-/-/Lhx9-/- mice. Our results show an enlargement of the ventrolateral pallium in the Lhx2-/- mouse, visualized with Dbx1, Tbr1, Lhx9 and Lmo3, accompanied by displacements of the boundaries separating this region from the subpallium or the dorsal pallium. We observed a distinct calretinin-immunolabeled area at the surface of the ventrolateral pallium, extending dorsally. This labelling does not include olfactory fibers (Saha et al, 2007), but possibly represents cells incoming from the enlarged prethalamic eminence. At the preoptic region, the anterior commissure was not formed. In the diencephalon of Lhx2-/- mice, the thalamic size was smaller compared to adjacent regions, although this region showed expression of Lef1. However, the thalamus was more severely affected in the absence of both Lhx2 and Lhx9, and showed no expression of Lef1 or Id4. The defect was accompanied of a ventricular enlargement. The malformation was more severe in the Lhx2-/-/Lhx9-/- than in Lhx2-/-/Lhx9+/-, suggesting a dose-dependent effect of Lhx9 in the formation of the thalamus.

Disclosures: **A. Abellan:** None. **E. Desfilis:** None. **L. Medina:** None. **H. Westphal:** None. **Y. Zhao:** None.

Poster

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MRC

Title: The regulation of Notch ligands Dll1 and Jag1 by Pax6 during cortical development

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Abstract: The regulation of gene expression resulting in the formation of the mammalian cerebral cortex is tightly regulated by a group of transcription factors. The deletion of any one of these transcription factors results in numerous defects whose nature and severity depends on the role of the transcription factor in the regulation of complex gene regulatory networks involved in development. There is currently relatively little knowledge about the gene networks that these transcription factors control and how they exert their regulatory effects. The paired-box transcription factor *Pax6* has been identified as a master regulator of gene networks involved in cortical development and its deletion results in numerous cortical defects such as an abnormally thin cortical plate and a vastly expanded proliferative zone. Previous work in our lab identified a list of candidate genes that are likely to be regulated by *Pax6* in the developing cortex. Members of the *Notch* signalling pathway were potential *Pax6* targets of particular interest since *Notch* signalling plays a crucial role in the maintenance of neural progenitor cells during development and consequently plays a critical role during corticogenesis. Our work aims to identify the regulatory relationship between *Pax6* and *Notch* ligands *Dll1* and *Jag1* during cortical development. Double label analysis of both gene and protein expression has provided insight into the relationship between *Pax6* and *Dll1* in progenitor cell subpopulations during cortical development. *In situ* hybridisation and qPCR results confirmed that loss of *Pax6* causes loss of *Dll1* expressing cells and downregulation of *Jag1*, indicating that both ligands are regulated by *Pax6.* Bioinformatic screening suggests that *Jag1* is a likely candidate to be a direct target of *Pax6.* Ongoing work aims to confirm *Jag1* as a direct target by visualisation of reporter gene expression produced by predicted enhancer elements driven by *Pax6*.

Disclosures: E.F. Dora: None. **I. Simpson:** None. **J.O. Mason:** None. **D.J. Price:** None.

Poster

494. Brain Patterning and Cell Death

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Topic: A.01. Brain Patterning

Title: The lateral cortical stream in embryonic and early fetal development of the human insular cortex

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Abstract: The embryonic period sets the framework and pace for the subsequent patterning, proliferation and differentiation events of the cortex. The human insular lobe, thought to be important for sociocognitive processing, displays three main cytoarchitectonic divisions ranging from a ventral and rostral agranular neocortex (periallocortex), adjacent to the piriform cortex, to a dorsal and caudal granular neocortex, with transitional dysgranular areas in between. We study insula development in human embryos from Carnegie stage (CS) 16 (5.5 postconceptional weeks, PCW) to CS23 (8.5 PCW), and young fetuses (9-16PCW). CS17 marks the onset of the first, ventrally directed migration of Tbr1+ cells from the lateral edge of the pallial-subpallial boundary (PSB) toward piriform cortex and amygdala. In parallel, calretinin (CR) + interneurons migrate ventrally from the lateral ganglionic eminence (LGE). Both migrations form part of the lateral cortical stream (LCS). The LCS becomes more prominent in the following stages (CS 18-21) and contributes large numbers of Tbr1+ and CR+ cells to the prospective rostral insula, which is thus the earliest neocortical area to develop. At CS18, a subventricular zone (SVZ) appears in the cortex adjacent to the PSP, though not yet in dorsal and medial regions. Radial glia fibers guide the migration of the LCS toward the rostral insula. At CS 22/23, fibers of the internal capsule traverse LCS and PSB and enter the cortical intermediate zone. As the temporal horn of the lateral ventricle expands at 11/12 PCW, the PSB at the ventral ganglionic eminence gives rise to an ascending LCS which provides cells for the caudal insula, guided by radial glia from the PSB of the temporal lobe. Dorsal and ventral ganglionic eminences are connected by radial glia bridges which may serve as a substrate for neurons migrating in the LCR toward the prospective intermediate, dysgranular insula. At the posterior pole of the ganglionic eminences, the PSB is characterized by a highly developed SVZ, from where radial glia fibers direct migratory Tbr1/Tbr2+ neurons toward the caudal granular insula. We conclude that the agranular insula is the first neocortical region to develop; dysgranular and granular insular areas develop according to the progression of the ventricular system and the LCS, in concert with parietal and temporal areas. The agranular/dysgranular/granular character of the insula may be related to its proximity to the SVZ, with the dorsocaudal granular insula being closest to the highly developed SVZ of the parietal PSB.

Disclosures: G. Meyer: None. **M. Gonzalez-Gomez:** None. **C. Medina-Bolivar:** None.

Poster

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Topic: A.01. Brain Patterning

Support: Hopkins Brain Science Institute

Title: Regulation of the patterning and development of the hypothalamus and prethalamus by canonical Wnt signaling

Authors: *E. NEWMAN[1], H. WANG[1], D. WU[2], J. ZHANG[3], M. TAKETO[4], R. AWATRAMANI[5], S. BLACKSHAW[1]
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Abstract: We tested the role of canonical Wnt signaling in hypothalamic and prethalamic patterning through loss and gain of function experiments using the canonical Wnt effector beta-catenin. We selectively eliminated beta-catenin from the hypothalamus using an Nkx2.1-Cre line, which we have previously shown to be active in posterioventral hypothalamus beginning at E9.5. We examined Nkx2.1-Cre; beta-cateninlox/lox embryos at E12.5 using *in situ* hybridization. With loss of beta-catenin, posterior structures were lost or reduced, and anterior structures were expanded. Gain of function studies using Shh-Cre; βcat(ex3)lox/+ showed a complementary phenotype, in which posterior hypothalamic structures were expanded at the expense of anterior structures. We next used the Foxd1-Cre line, which is expressed earlier in development throughout both the prethalamus and hypothalamus, to investigate the effects of gain and loss of function of beta-catenin. We found that Foxd1-Cre; βcat(ex3)lox/l+ mice lost expression of most, but not all, hypothalamic and prethalamic markers. However, some posterior hypothalamic markers such as Pitx2 and Lhx5 showed expanded domains of expression. These data suggest that canonical Wnt signaling is coordinately patterning both the hypothalamus and prethalamus.

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Poster

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Title: Role of differential heparan sulfate sulfation in mouse forebrain Fgf8 signalling

Authors: *W. CHAN, J. M. CLEGG, D. J. PRICE, T. PRATT
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Abstract: Heparan sulfate proteoglycans (HSPGs) are cell surface/secreted molecules expressed by all cells consisting of linear carbohydrate side-chains attached to a core protein. HSPGs are involved in regulating key signalling pathways in the developing mammalian brain via sugar-protein interactions. It has been hypothesised that the specificity for the interaction between the HSPGs and its particular signalling pathway is encoded by its heparan sulfate (HS) side-chain. HS has a variety of structures due to postsynthetic modification during its biosynthesis. Hs2st and Hs6st1 are two enzymes involved in generating various HS structures where the former catalyses the specific sulfation at the 2-carbon molecule of the sugar backbone while the latter catalyses the sulfation of the 6-carbon. Hs2st and Hs6st1 have been shown to be important for mouse forebrain development but interestingly, Hs2st and Hs6st1 each play a different role. Hs6st1 play a unique role in Fgf8 signalling while Hs2st does not providing support for the existence of a HS code. Fgf8 is a secreted morphogen crucial for forebrain development where it functions to pattern the forebrain via regulated gradient formation. Fgf8 protein levels and the interpretation of the Fgf8 protein gradient are important for Fgf8 signalling. HS has been previously shown to be involved in these processes however, the role differential sulfation plays in these processes and particularly, the molecular mechanism(s) behind this has not been clearly resolved. Fgf8 signalling has been widely studied and its signalling mechanism well characterised. Yet, the conventional model of its signalling mechanism could not fully explain the observations preliminary to this work where Hs6st1 mutants have disrupted Fgf8 distribution, presenting a gap in our current understand of Fgf8 signalling. To begin filling this gap, we test our hypothesis that differential HS sulfation is involved in Fgf8 signalling regulation. Here, we used a combined *in vivo* and *in vitro* approach to investigate the formation of Fgf8 gradient over time. Comparing these data between wild-type and Hs2st or Hs6st1 mutants reveals differences that indicate differential HS sulfation is important in the formation of Fgf8 gradient in the forebrain. Furthermore, the data revealed several key aspects of how differential sulfation is

affecting Fgf8 gradient interpretation. This provides us with further insight into the role of HS in the complex but precise regulation of mouse forebrain development.

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Poster

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Title: Fate-labeled Islet1 progenitor cells uniquely contribute to mature interneuron subtypes in mouse neocortex

Authors: *F. SIDDIQI[1], A. L. TRAKIMAS[3], D. J. JOSEPH[2], T. T. CLARKE[2], E. D. MARSH[2,4], J. H. WOLFE[2,5]
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Abstract: The LIM homeodomain transcription factor, Islet1, is expressed in the ventral division of the lateral ganglionic eminence (vLGE) and is known to generate medium spiny neurons of striatum. It remains controversial whether or not Islet1-derived cells of the vLGE contribute to neocortical interneuron subtypes, which would indicate a larger unidentified role of Islet1 in forebrain development. Since Islet1 expression is mostly down-regulated in the postnatal brain, we used a Cre/loxP knock-in mouse line whereby the Islet1 gene locus drives expression of Cre recombinase crossed with a floxed R26-stop-CAG-tdTomato-WPRE mutant mouse line to trace the lineage of the Islet1-derived population. Results showed that at E11.5, tdTomato+ cells were localized to the LGE, yet three days later (E14.5) these DCX+ cells had tangentially migrated to the neocortex. Birth-dating with a S-phase marker showed many tdTomato+/EdU+ cells pulse labeled at E14.5. Co-staining with the MGE and CGE markers, Nkx-2.1 and CoupTF-II, respectively, showed that tdTomato+ cells did not overlap with these cortical interneuron progenitor pools. E14.5 isochronic transplantation of tdTomato+ microdissected tissue onto

littermate control organotypic slices showed that only LGE to LGE transplant resulted in cortical migration. In contrast, tdTomato+ cells isolated from the preoptic area only migrated locally in the basal ganglia. Lastly, phenotyping at P0 revealed labeled cells were present in olfactory bulb and at distinct locations from the known dLGE-derived ER81 interneuron pool. At P21 in the neocortex, tdTomato+ cells spanned layers II through VI and represented 18±1% of the larger GAD67+ population. A combination of neurochemical markers and electrophysiology determined that tdTomato+ cells were heterogeneous with fast spiking, regular spiking and rebound spiking cells within the population. Together, our data supports a model of the presence of a distinct Islet1+ progenitor population contributing to cortical interneuron diversity.

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Poster

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Topic: A.01. Brain Patterning

Title: Relationship between malformations of neuronal migration and variation of folia development in the cerebellar vermis of C57BL/6J mice

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Abstract: The complex neuronal circuitry of the cerebellum is embedded within its lamina and folia, which together play an important role in sensory and motor function. Studies in mouse models demonstrate that cerebellar lamination and folia development are under genetic control. The cerebellar vermis of C57BL/6 mice exhibit normal variation in the organization of anterior folia (lobules I-V) as well as malformations of neuronal migration of posterior folia (lobules VIII and IX; molecular layer heterotopia). However, the relationship between naturally-occurring variations in foliation and the presence of heterotopia has not been directly examined. In the present report, we analyze the frequency with which folia variation and heterotopia occur, and demonstrate that folia variation is more commonly found to coincide with heterotopia than alone. These data are relevant toward understanding normal cerebellar development and disorders affecting cerebellar foliation and lamination.

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Poster

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Title: Autism-associated Met tyrosine kinase receptor influences frontal cortical size and neuronal generation

Authors: ***J. M. SMITH**, E. M. POWELL
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Abstract: *MET*, the gene encoding the tyrosine kinase receptor for hepatocyte growth factor/scatter factor (HGF/SF), has been identified as a susceptibility locus for autism spectrum disorders (ASD). Both Met and HGF are expressed in the cerebral cortex during development, and HGF-Met signaling has been implicated in a number of cellular processes, including proliferation, migration, survival, and process formation. Alterations in HGF-Met signaling may therefore affect cortical development, potentially leading to neuroanatomical changes such as those thought to play a role in neurodevelopmental disorders such as ASD. We have previously found an expansion of the cortex at rostral levels in transgenic mice expressing a kinase-dead Met in the *Emx1* lineage. We have also found altered cortical lamination at the level of the somatosensory barrel fields. The embryonic loss of Met-signaling led to a permanent expansion of frontal cortical areas and increased white matter. We examined layer-specific marker expression in frontal cortex to determine if lamination was altered. To determine if the changes in cortical laminar structure could be due to changes the timing of neurogenesis, we also examined BrdU incorporation after a series of injections during early, middle, and late cortical neurogenesis. Our results suggest that prenatally impaired Met signaling alters neuronal generation and specification in a regionally-specific manner.

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Poster

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Title: Specification of select hypothalamic circuits and innate behaviors by the embryonic patterning gene, Dbx1

Authors: *K. SOKOLOWSKI[1], S. ESUMI[3], T. HIRATA[4], Y. KAMAL[2], T. TRAN[2], A. LAM[2], M. ZAGHLULA[2], S.-C. BRIGHTHAUPT[2], J. MARTINEZ CRUZ[2], S. GRIMBOVSKI[2], S. KNOBLACH[2], A. PIERANI[5], K. JONES[6], N. TAMAMAKI[3], N. SHAH[7], J. G. CORBIN[2]
[2]Ctr. for Neurosci. Res., [1]Children's Natl. Med. Ctr., Washington, DC; [3]Grad. Sch. of Med. Sci., Kumamoto-University, Kumamoto City, Japan; [4]Ehime Proteo-Medicine Res. Ctr., Ehime Univ., Ehime, Japan; [5]Inst. Jacques Monod, Univ. Paris Diderot, Paris, France; [6]Dept. of Biol., Howard Univ., Washington, DC; [7]Dept. of Anat., Univ. of California, San Francisco, CA

Abstract: The hypothalamus integrates information to coordinate the output of innate behaviors. However, the developmental programs for the generation of neural circuitry regulating these behaviors remains unknown. Here, we reveal that the homeodomain gene, Dbx1, is required for development of select subsets of hypothalamic neurons and innate behaviors. Despite widespread expression throughout the embryonic hypothalamic primordium, Dbx1 selectively functions in the specification of subgroups of neuronal populations within the feeding-associated arcuate and lateral hypothalamic nuclei. Consistent with this selective developmental role, Dbx1 hypothalamic-specific conditional knockout mice display sexually dimorphic alterations in energy homeostasis, without displaying defects in other hypothalamically regulated innate behaviors such as mating or conspecific aggression. Surprisingly, Dbx1-conditional knockout mice also have defects in innate fear responses. Thus, our findings link embryonic patterning to the emergence of subsets of motivational and aversive behaviors and suggest a common developmental genetic mechanism for specification of subsets of functionally related hypothalamic neurons.

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Poster

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Title: Concomitant expression of subcerebral- and callosal-specific genes defines a deep layer cell population in the postnatal mouse neocortex

Authors: *C. ALFANO[1,2], K. HARB[2], E. MAGRINELLI[2], N. LUKIANETS[2], A. ZUBIOLO[3], C. NICOLAS[2], E. DEBREUVE[4], G. SANDOZ[2], X. DESCOMBES[3], F. GRAMMONT[5], M. STUDER[2]
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Abstract: The mammalian cerebral cortex is subdivided into several tangential domains called functional areas, which are deputed to the elaboration of motor and sensory inputs, the selection and implementation of motor plans, and many other higher cognitive functions. Each functional area is constituted by six layers of projection glutamatergic neurons (PNs) with different morphologies, connectivity and molecular codes. Several transcription factors specifying different subclasses of neurons, such as callosal neurons (CPN, which target the contralateral neocortical hemisphere) and subcerebral projection neurons (SCPN, e.g. corticopontine and

corticospinal neurons) have been identified so far [1]. However, little is known about the mechanisms specifying their features in a time- and areal-specific manner. In this study, we show that a population of cells co-expressing molecular markers of CPN and SCPN neurons, such as Ctip2 and Satb2 (C/S+), becomes first specified in layers V and VI of rostro-medial mouse neocortex at perinatal stages and progressively increases between P0 and P21 in somatosensory areas. Since it was shown that Satb2 strongly inhibits the expression of Ctip2 [2, 3], we investigated the molecular mechanisms allowing their co-expression at different developmental stages and in different neocortical areas. Moreover, by the use of a transgenic line expressing GFP in layer V neurons and of vital dyes, we analysed morphology, connectivity and electrophysiological activity of this hybrid class of neurons at postnatal stages of development. Our study demonstrates that the co-expression of CPN and SCPN neuronal markers does not only characterize early phases of neuronal specification [1], but also defines a neuronal subpopulation with different areal-specific features and developmental timing in the mammalian neocortex. 1. Greig, L.C., et al., *Molecular logic of neocortical projection neuron specification, development and diversity.* Nat Rev Neurosci, 2013. **14**(11): p. 755-69. 2. Alcamo, E.A., et al., *Satb2 regulates callosal projection neuron identity in the developing cerebral cortex.* Neuron, 2008. **57**(3): p. 364-77. 3. Britanova, O., et al., *Satb2 is a postmitotic determinant for upper-layer neuron specification in the neocortex.* Neuron, 2008. **57**(3): p. 378-92.

Disclosures: C. Alfano: None. **K. Harb:** None. **E. Magrinelli:** None. **N. Lukianets:** None. **A. Zubiolo:** None. **C. Nicolas:** None. **E. Debreuve:** None. **G. Sandoz:** None. **X. Descombes:** None. **F. Grammont:** None. **M. Studer:** None.

Poster

494. Brain Patterning and Cell Death

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 494.24/A48

Topic: A.01. Brain Patterning

Support: Wellcome Trust

EASTBIO BBSRC PhD studentship

Title: Analysis of heparan sulfate function in the developing mammalian forebrain using systematic gene expression analysis

Authors: *H. PARKIN, J. CLEGG, J. MASON, T. PRATT
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Abstract: Heparan sulfate proteoglycans (HSPGs) are a family of molecules involved in regulating key signalling events required for normal mammalian brain development. It is thought that specificity of HSPGs for particular signalling processes is encoded by the heparan sulfate (HS) sugar side chains, which can be modified post-synthetically to yield huge variation in HS structure. Different sulfation patterns are generated by the action of enzymes such as the heparan sulfate sulfotransferases (HSTs) and sulfatases. Depending on the expression of these enzymes and the resulting heparan sulfate 'code', it is proposed that cells are then able to regulate signals they receive and send in the ligand rich extracellular environment of the developing forebrain. Following loss of the two HSTs Hs6st1 or Hs2st that add sulphate groups to specific positions on residues of the HS side chains, commissural tracts including the corpus callosum fail to develop normally during late mouse embryogenesis. The telencephalic midline environment is perturbed, with a striking mis-positioning of glial cell populations that normally act to guide axons towards the contralateral hemisphere. One hypothesis to explain this acallosal phenotype is a change in critical cell populations and processes at a time when the correct midline environment is being established, that may be identified by changes in gene expression. Given the function of HS these changes might correlate with alterations in signalling such as an increase in Fgf/ERK that has already been found. We performed RNA sequencing analysis on dissected midline regions of WT, Hs2st-/- and Hs6st1-/- mouse embryos at E16.5 and have identified lists of differentially expressed genes. We find changes in gene expression in cell populations that are known to be important for corpus callosum development, including callosal pioneer axons and the disrupted glial cells observed in HST-null embryos. By comparing the expression of these genes between WT and mutant embryos, we are gaining an insight into the underlying mechanisms of HS function.

Disclosures: H. Parkin: None. **J. Clegg:** None. **J. Mason:** None. **T. Pratt:** None.

Poster

494. Brain Patterning and Cell Death

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 494.25/A49

Topic: A.01. Brain Patterning

Support: KAKENHI

JSPS

GCOE

Title: The molecular mechanism of thalamic pattern formation during development

Authors: *H. EBISU[1,2], L. IWAI[2], T. MOMOI[3], H. KAWASAKI[1,2]
[1]Grad. Sch. of Med. Sci., Kanazawa Univ., Ishikawa, Japan; [2]Grad. school of Med., The Univ. of Tokyo, Tokyo, Japan; [3]Ctr. for Med. Sci., Intl. Univ. of Hlth. and Welfare, Tochigi, Japan

Abstract: The mammalian thalamus is an important brain region that transfers sensory information to the cerebral cortex. The thalamus is located in the diencephalon and contains dozens of morphologically and functionally distinct thalamic nuclei. Although the thalamic primodium differentiates into these distinct nuclei during development, the mechanisms underlying the formation of thalamic nuclei are still unclear. Here, we searched for candidate molecules that could mediate thalamic pattern formation using the Allen brain atlas. We focused on transcription factors expressed in the thalamic primodium with a graded expression pattern. We found that the expression of Foxp2 was low in the anterior thalamus and high in the posterior thalamus at embryonic day 11 when the identities of the thalamic nuclei are about to be distinguished. Loss-of-function studies revealed that antero-medial thalamic nuclei expanded, whereas postero-lateral thalamic nuclei shrank in Foxp2-mutant mice. Furthermore, using *in utero* electroporation, we found that Fgf8 signaling, which is thought to be important for thalamic pattern formation, suppressed Foxp2 expression in the thalamic primoidum. Our results suggest that Foxp2 is important for establishing the identities of thalamic nuclei.

Disclosures: H. Ebisu: None. **L. Iwai:** None. **T. Momoi:** None. **H. Kawasaki:** None.

Poster

494. Brain Patterning and Cell Death

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 494.26/A50

Topic: A.01. Brain Patterning

Title: EphA7 regulates striatal organization and function

Authors: C. J. GRIFFEY, *L. F. KROMER
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Abstract: In the developing nervous system, Eph receptor tyrosine kinases and their ephrin ligands regulate cellular interactions necessary to establish proper connections between brain regions, including neuronal migration, cell sorting, and axon guidance. However, the structural implications and correlated behaviors for which these molecules are responsible are less well known. For example, EphA7 receptors are reported to interact with ephrin-A5 ligands to regulate the topographic organization of thalamocortical and corticothalamic connections as well as the size of the somatosensory "barrel" cortex. In addition, neurons expressing EphA7 form a unique matrisome compartment within the striatum that exhibits a banded pattern. This distribution of EphA7+ matrisome neurons inversely correlates with the topographic organization of somatosensory inputs from ephrin-A5 expressing neurons in medial somatosensory (S1) cortex. However, it is currently unknown whether expression of EphA7 is essential for this organization of cortical afferents and whether EphA7 deletions result in behavioral abnormalities that are associated with corticostriatal functions. To address these questions, we evaluated anatomical changes within the striatum and identified behavioral abnormalities that occurred in EphA7-/- mice. Ephrin-A5 ligand binding histochemistry was used to evaluate changes in neuronal organization and axonal connections in the forebrain in the absence of EphA7 in 1-2 week postnatal mice (a time when EphA receptors are expressed at high levels in the forebrain). In EphA7-/- mice, there is a change from the normal striatal matrisome binding pattern observed in wild-type mice to a dispersed binding that extends across the entire striatal matrix compartment. Since all matrix neurons express EphA4, this suggests that EphA4 may function as a secondary binding partner for ephrin-A5 in the absence of EphA7. In addition, we observed that the pattern of ephrin-A5 binding is altered in the cortex, hippocampus and amygdala in EphA7-/- mice. Behavioral analysis of adult EphA7-/- mice indicates they exhibit motor impairments including hyperactivity and decreased coordination; diminished anxiety phenotypes in both elevated-plus maze and open field tests; and impaired contextual and cued fear conditioning. Taken together, these results support a developmental role for EphA7 in organizing striatal, limbic, and cortical structures and connections involved in regulating important cognitive and motor behaviors.

Disclosures: C.J. Griffey: None. **L.F. Kromer:** None.

Poster

495. Proliferation: Self-Renewal and Cell Cycle

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

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Topic: A.02. Neurogenesis and Gliogenesis

Support: NSF grant IOS-1257895 to MS

2014 WM Honors Fellowship to CT

HHMI Undergraduate Science Education Grant to the College of William and Mary (Student Research Award to CT)

Title: Compensation during neural development following Notch signaling perturbation in X. laevis

Authors: *C. TOCHENY[1], B. RABE[2], M. MCDONOUGH[1], T. ARALERE[1], J. HINES[1], M. SAHA[1]
[1]Col. of William and Mary, Williamsburg, VA; [2]Harvard Univ., Cambridge, MA

Abstract: During primary neurogenesis, the establishment of a balance between the proliferation of neural progenitor cells and the differentiation of neurons is crucial for the formation of a properly functioning nervous system. One of the pathways implicated in regulating this balance is the Notch pathway, a juxtacrine cell-signaling pathway that initiates the shift from a neural progenitor fate to a differentiated neuron through lateral inhibition. In order to determine the role of Notch signaling in neurotransmitter fate specification in Xenopus laevis, we unilaterally injected RNA constructs that overexpress or inactivate Notch signaling at the two cell stage. While these perturbations showed profound effects on the expression of neuronal gene markers at neural plate and neural tube stages of development on the injected side, expression of various neural marker genes equalized by the swimming tadpole stage, suggesting a compensatory response. To determine the molecular mechanism through which this compensation occurs during development, we performed microarray analysis as well as examining the expression patterns of early and late genes in the neural determination pathway. Our results suggest that the compensatory response involves both apoptosis to remove an excess of progenitor cells and a re-calibration of cell cycle dynamics, as well as modified regulation of genes involved in neuronal stabilization and determination.

Disclosures: C. Tocheny: None. **B. Rabe:** None. **T. Aralere:** None. **J. Hines:** None. **M. Saha:** None. **M. McDonough:** None.

Poster

495. Proliferation: Self-Renewal and Cell Cycle

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 495.02/A52

Topic: A.02. Neurogenesis and Gliogenesis

Support: Consolider- Ingenio fellowship 2010

Spanish Ministry of Economy and Competitivity (BFU2012-33473, CSD2007-00023)

European Research Council (ERC StG309633)

Title: Independent lineages of cortical progenitor cells established by founder cell seeding

Authors: M. MARTÍNEZ-MARTÍNEZ[1], A. CÁRDENAS[1], C. DE JUAN ROMERO[1], M. GÖTZ[2], *V. BORRELL[1]
[1]Inst. Neuroci Alicante, CSIC & Univ. Miguel Hernandez, San Juan de Alicante, Spain;
[2]Helmholtz Zentrum München, Neuherberg, Germany

Abstract: In the developing cerebral cortex the fibers of Radial Glia cells (RGCs) serve as guide and substrate for radially migrating neurons. In gyrencephalic species, like human and ferret, radial migration proceeds following the highly divergent trajectories of RG fibers, an arrangement that allows the expansion of the cerebral cortex in surface area and the formation of folds. This depends on the massive generation of basal Radial Glia Cells (bRGCs), particularly in the outer subventricular zone (OSVZ). Here we have investigated which are the cellular mechanisms involved in the generation of bRGCs in the developing ferret cortex. We find that at early postnatal stages bRGCs in the OSVZ are not produced by apical RGCs in the VZ (aRGCs), but only by self-amplification within the OSVZ. Unexpectedly, we find that this is completely different at embryonic stages, when aRGCs in the VZ undergo a brief period of massive generation of bRGC for both ISVZ and OSVZ. Hence, upon seeding the OSVZ with a small population of founder bRGCs, these establish a self-amplifying population independent from the VZ lineage. We are currently searching for molecules expressed differently in the VZ along cortical development that may underlie the transient generation of OSVZ-destined bRGCs. This is the first demonstration of the developmental origin of the OSVZ, and we identify for the first time the lineage independence between germinal layers within the developing mammalian cerebral cortex.

Disclosures: M. Martínez-Martínez: None. **A. Cárdenas:** None. **C. De Juan Romero:** None. **M. Götz:** None. **V. Borrell:** None.

Poster

495. Proliferation: Self-Renewal and Cell Cycle

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Program#/Poster#: 495.03/A53

Topic: A.02. Neurogenesis and Gliogenesis

Support: NSF IOS 0818259

NSF IOS 1121345

Title: Characterization of cells derived from adoptive transfer of hemocytes in crayfish brain

Authors: J. PLATTO, J. L. BENTON, *V. QUINAN, B. S. BELTZ
Neurosci. Program, Wellesley Col., Wellesley, MA

Abstract: As in mammals and other vertebrates, decapod crustaceans undergo life-long neurogenesis. In crayfish (Procambarus clarkii), the neuronal lineage underlying adult neurogenesis has been defined; this lineage is characterized by at least three generations of precursors that are spatially separated. The first-generation neuronal precursors are not self-renewing, but are never depleted; *in vitro* work has shown that hemocytes, and not other cell types, are attracted to and incorporated into the niche (Benton et al 2011). Thus, it has been hypothesized that neuronal precursors originate in a source extrinsic to the niche and that this source is part of the innate immune system. Adult-born neurons in the olfactory pathway in the crayfish brain differentiate by 6 weeks after BrdU labeling; these innervate the accessory and olfactory lobes (Sullivan and Beltz, 2005) and express appropriate transmitters (Sullivan et al., 2007). Labeled cells harvested from the hemolymph of donor crayfish and transferred to recipient crayfish become neuronal precursors. These go through the normal lineage events, and by 6 weeks after transfer express the transmitters appropriate to their locations in the brain. The current project seeks to characterize the morphology of the brain cells in P. clarkii that derive from adoptive transfers, by injection of dextran dyes into the accessory and olfactory lobes. These dyes retrogradely fill cells projecting to these regions, allowing visualization and characterization of the descendants of cells transferred from donor crayfish.

Disclosures: J. Platto: None. **V. Quinan:** None. **J.L. Benton:** None. **B.S. Beltz:** None.

Poster

495. Proliferation: Self-Renewal and Cell Cycle

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Topic: A.02. Neurogenesis and Gliogenesis

Support: NSF IOS 0818259

NSF IOS 1121345

Swedish Research Council 621-2011-4797

Title: The crustacean cytokine astakine 1: A link between the innate immune system and nervous system in crayfish

Authors: K. A. RAMOS[1], J. L. BENTON[1], J. LI[1], I. SÖDERHÄLL[3], *D. BAUER[2], B. S. BELTZ[1]
[1]Neurosci. Program, [2]Wellesley Col., Wellesley, MA; [3]Dept. of Comparative Physiol., Uppsala Univ., Uppsala, Sweden

Abstract: Freshwater crayfish (Pacifasticus leniusculus, Procambarus clarkii) live for up to twenty years and contain distinct hematopoietic tissues that synthesize hemocytes throughout the animal's lifetime. Astakines, crustacean cytokines belonging to the prokineticin family, are necessary for the synthesis and release of hemocytes from hematopoietic tissues (Lin et al., 2011, J Immunol 186:2073). In vertebrates, prokineticins are involved in diverse functions, including circadian regulation, angiogenesis, neuronal development, and adult neurogenesis. The goal of the present work is to define the role(s) of astakine 1 (AST1) in adult neurogenesis in the decapod crustacean brain. Immunoreactivity for AST1 was localized in the brains of crayfish using an antibody raised against the P. leniusculus form of the peptide. Fibers and varicosities within the olfactory and accessory lobes and associated interneuronal cell clusters express AST1 immunoreactivity. In both species, AST1 antibody also labels brain regions involved with circadian control. Immunoreactivities for AST1 and serotonin are generally expressed in the same synaptic areas in the brain, a finding that may be particularly relevant in the olfactory pathway, where adult-born neurons are integrated. The efficacy of recombinant P. leniusculus astakine (r-AST1) was tested in P. clarkii by injecting r-AST1 and sampling hemolymph at intervals after injection. At 12 hours post-r-AST1 injection, a ~75% increase in circulating hemocyte counts was documented. Western blot of P. clarkii AST1 shows that protein levels in the brain and hematopoietic tissue vary depending on time of day, with higher levels in the morning as previously reported for P. leniusculus (Watthanasurorot et al., 2011, Cell Mol Life Sci 68:315). R-AST1 injection also affects adult neurogenesis in the crayfish brain. The neuronal precursor lineage in adult crayfish is characterized by at least three generations of precursors that are spatially separated. R-AST1 regulates this lineage by increasing the total number of cells in the niche, as well as by increasing the numbers of 5-bromo-2'-deoxyuridine (BrdU)-labeled cells in all generations of the lineage. These studies demonstrate a close relationship between the innate immune system and adult neurogenesis in the crustacean brain. Further studies are examining the combined roles of serotonin and AST1 in regulating this link.

Disclosures: **K.A. Ramos:** None. **J.L. Benton:** None. **J. Li:** None. **I. Söderhäll:** None. **D. Bauer:** None. **B.S. Beltz:** None.

Poster

495. Proliferation: Self-Renewal and Cell Cycle

Location: Halls A-C

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Topic: A.02. Neurogenesis and Gliogenesis

Support: NIH Grant MH101188

Title: Microglial proliferation in the developing rat neocortex

Authors: A. KREUTZ[1], J. KEITER[2], S. SAYLOR[2], V. MARTINEZ-CERDEÑO[3], *S. C. NOCTOR[2]
[1]Neurosci. Grad. Program, [2]Psych & Behavioral Sci., UC Davis, SACRAMENTO, CA; [3]Pathology, UC Davis, Sacramento, CA

Abstract: Microglia are the immune cells of the CNS. We have found that in the normally developing brain microglia play a critical role in regulating the number of neural precursor cells in the fetal neocortex. Microglia first enter the cortex during early stages of forebrain development. However, they do not accumulate in significant numbers until the end of the neurogenic period, when they colonize the proliferative zones and their number increases substantially. How the number of microglia rises substantially is not understood. Since microglia specifically colonize proliferative zones we considered whether microglial precursor cells preferentially divide in the cortical proliferative zones to produce cortical microglia. Alternatively, it is possible that microglial precursor cells divide in peripheral sites to produce microglia that enter the cortex. To address these possibilities we examined the proliferative status of microglia in the developing rat neocortex from E11 through postnatal ages. To determine when and where microglia proliferate, we mapped out the distribution of actively dividing microglia in the embryonic rat cerebral cortex. Mitotic microglia were identified based on co-expression of the M-phase marker phosphohistone-3 (PH-3) and the microglial marker Iba1. The laminar distribution of microglia was determined using cytoarchitectonics and Tbr2 expression to define proliferative zones. Our results show that microglia arrive in the rat neocortex by E11, and their number increases until E19 when the Iba1+ population expands dramatically. We found that a steady low proportion of microglia were proliferative in the rat neocortex from embryonic through early postnatal ages. Interestingly, the mitotic microglia were not restricted to the proliferative zones, but instead were located in laminae across the cortical wall. These data suggest that the rapid rise in microglial number at E19 results primarily from increased microglial entry into the cortex, not proliferation. Unlike other CNS cell types, we find no evidence for specific proliferative zones for microglia.

Disclosures: **A. Kreutz:** None. **S.C. Noctor:** None. **J. Keiter:** None. **S. Saylor:** None. **V. Martinez-Cerdeño:** None.

Poster

495. Proliferation: Self-Renewal and Cell Cycle

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 495.06/A56

Topic: A.02. Neurogenesis and Gliogenesis

Title: Sleep deprivation effects over hippocampal neurogenesis and apoptosis after different recovery times in adult BalB/C male mice

Authors: *S. SOTO-RODRIGUEZ[1], G. LOPEZ-ARMAS[2], O. GONZALEZ-PEREZ[3], M. LUQUIN-DE ANDA[2], R. RAMOS-ZUÑIGA[2], R. GONZALEZ-CASTANEDA[2]
[1]Univ. De Guadalajara, Zapopan, Mexico; [2]Univ. de Guadalajara, Guadalajara, Mexico; [3]Univ. de Colima, Colima, Mexico

Abstract: Sleep deprivation (SD) is a stressful agent that actual society undergoes, with psychological and physio-logical repercussions. In mouse models it has been proven that SD affects the development in learning as well as memory tasks and it has been correlated with the decrease in the neurogenesis in the dentate gyrus (DG) but it remains unknown if this reduction is due to apoptosis and which cell line is the most affected. Most tests are applied immediately after SD, but it is unknown if these effects remain over long terms. The aim of this study was to analyze the effects of SD over proliferation in the DG at different stages of neurogenesis and gliogenesis in adult male mice, and evaluate the apoptosis in the hippocampus. SD was carried out in the inverted flowerpot for 72h, a single BrdU injection was applied at hour 70. After this time, animals were divided randomly in three groups. The first group was sacrificed immediately, and the remaining animals after a recovery period of 14 or 21 days. Neurogenesis was evaluated with inmmunohistochemistry (IHC) and apoptosis with a TUNEL test; brains were labeled to recognize between hemispheres. Progenitor cells and immature neurons were reduced in the SD animals, showing hemisphere differences. Apoptosis results showed no significant differences in DG, but there were differences in other hippocampal regions. Our results suggest that SD has a deleting effect in the dentate gyrus after 72h SD, affecting more one of the hemispheres, but this is not due to apoptosis, and this was persistent after a recovery period.

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Poster

495. Proliferation: Self-Renewal and Cell Cycle

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Topic: A.02. Neurogenesis and Gliogenesis

Support: R01-NS044363

5T32-AG020418

F31-DC012483

Title: Zinc-finger protein INSM1 regulates neurogenesis in spiral and vestibular ganglia and is transiently expressed in nascent outer hair cells

Authors: S. M. LORENZEN[1], *A. DUGGAN[1], A. OSIPOVICH[2], M. A. MAGNUSON[2], J. GARCÍA-AÑOVEROS[1]
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Abstract: Discussion of the development and differentiation of inner ear neurons and hair cells has centered on the bhlh transcription factors. These factors, while necessary, cannot alone account for proper differentiation of these cells. Furthermore, no factor has been described that is expressed by outer hair cells (OHCs) and not inner hair cells (IHCs) during early embryonic stages of inner ear development. INSM1 is a zinc-finger protein that is expressed throughout the developing nervous system in late neuronal progenitors and nascent neurons. In the embryonic cortex and olfactory epithelium, Insm1 promotes the transition of progenitors from apical, proliferative, and uncommitted to basal, terminally-dividing and neuron producing. In the embryonic mouse ear, not only is Insm1 expressed in neuronally committed progenitors and nascent neurons, but also in nascent OHCs. Insm1 expression pattern was assessed by *in situ* hybridization and Insm1GFP.Cre expression. Insm1 lineage analysis was conducted with Insm1GFP.Cre mouse crossed with reporter mice. Insm1 function was analyzed by comparing Insm1+/+ and Insm1-/- embryos. Immunohistochemistry was used to identify delaminating progenitors, nascent neurons, cells in mitosis, and cells undergoing apoptosis. In the otocyst,

delaminating and delaminated progenitors express Insm1, whereas, apically dividing progenitors do not. This pattern of expression is analogous to that in embryonic olfactory epithelium and cortex (basal/subventricular progenitors). Lineage analysis confirms that nearly all of the auditory and vestibular neurons come from cells which have expressed Insm1. In the absence of Insm1, spiral and vestibular ganglia have a 40% reduction in neurons, and accordingly the ganglia are smaller. To account for the decrease in neurons, we observe delaminated progenitors undergoing fewer mitoses, but no change in apoptosis in the progenitors or nascent neurons. We conclude that in the embryonic inner ear Insm1 regulates proliferation of delaminated neuronal progenitors and hence the production of neurons in the ear, a similar function to that in other embryonic neural epithelia. Unexpectedly, also found that nascent, but not mature, OHCs express Insm1, whereas IHCs never express Insm1. Thus far, we have not determined any abnormalities in the development of OHCs in the absence of Insm1. Insm1 is the earliest known gene expressed in OHCs versus IHCs, demonstrating that nascent OHCs initiate a unique differentiation program in the embryo, much earlier than previously believed. Furthermore, as a transcription factor, INSM1 may be an early regulator of the unique differentiation of OHCs.

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Poster

495. Proliferation: Self-Renewal and Cell Cycle

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Topic: A.02. Neurogenesis and Gliogenesis

Support: NCTR/FDA P00706 to SAF

Hornick Awards to LC

Title: Developmental estrogen treatment selectively increases proliferative activity in the 3rd ventricle stem cell niche of female rats at weaning

Authors: *Z. HE[1], L. CUI[2], M. G. PAULE[1], S. A. FERGUSON[1]
[1]Div. of Neurotoxicology, Natl. Ctr. For Toxicology Res., Jefferson, AR; [2]Neurol., Univ. of Arkansas for Med. Sci., Little Rock, AR

Abstract: Developmental estrogen treatment enlarges the sexually-dimorphic nucleus of the preoptic area (SDN-POA) in male and female weanling rats (He et al., 2012). Because stem cell activity at least partially accounts for postweaning SDN-POA development (He et al., 2013), the present study determined if neural stem cell activity in the 3rd ventricle stem cell niche (3VSCN, rostral end of the 3rd ventricle labeled with nestin-immunoreactivity, He et al., 2013) responds to estrogen treatment. Methods: Pregnant Sprague-Dawley rats were gavaged with ethinyl estradiol (EE2, 10 µg/kg/day) or vehicle (0.3% carboxymethylcellulose sodium salt) on gestational days 6-21 followed by gavage of offspring with the same dose from postnatal day (PND) 1 until weaning on PND 21. On PND 21, neural stem cell activity in the 3VSCN was assessed in 1 pup/sex/litter (n=5-7/sex/group). Stem cell markers, including nestin, Ki67, phosphohistone H3 (PHH3) and doublecortin (DCX), were combined to investigate neural stem cell activity in the 3VSCN with reference to the caudal 3rd ventricle (within-subject control) and to other well-accepted neural stem cell niches [i.e., the subventricular zone (SVZ) and subgranular zone (SGZ)] as the within-subject positive controls for immunoreactivity. Results: The 3VSCN was characterized by reproducible nestin-labeling showing long straight projections perpendicular to the ventricular wall along the rostral end of the 3rd ventricle. The 3VSCN scarcely expressed DCX, while both the SVZ and SGZ displayed robust DCX expression: the former showing immunoreactivity along the ventricular wall and the latter showing the deposit along the base of granular layer as well as in the hippocampal hilus. The SVZ exhibited vigorous expression of Ki67- and PHH3-positive immunoreactivity, but the 3VSCN displayed only a few Ki67-positive and PHH3-positive cells. Nevertheless, Ki67-positive cell counts in the 3VSCN were 2.2 to 5.8 times that of the caudal 3rd ventricle in PND 21 female and male rats: 2798±565/mm3 vs. 1254±305/mm3 for females and 4365±1033/mm3 vs. 753±345/mm3 for males, $p<0.05$, respectively. Estrogen treatment increased Ki67-positive cell counts in both sexes in both regions: 8992±1880/mm3 vs. 1390±284/mm3 and 8219±2118/mm3 vs. 1322±324/mm3 (3VSCN vs. caudal 3rd ventricle, female and male respectively) and was significant for females ($p<0.05$). Conclusion: Developmental estrogen treatment selectively increased proliferative cell counts in the 3VSCN of female rats at weaning. Neural stem cell activity in the 3VSCN may be one of the driving forces in the postweaning development of the SDN-POA in rats.

Disclosures: Z. He: None. **L. Cui:** None. **M.G. Paule:** None. **S.A. Ferguson:** None.

Poster

495. Proliferation: Self-Renewal and Cell Cycle

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Topic: A.02. Neurogenesis and Gliogenesis

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P30 EY07003

T32 EY013934

Research to Prevent Blindness, Inc.

Title: The function of Progranulin-a, a microglia-specific growth factor, during vertebrate retinal development

Authors: *C. WALSH[1,2], P. HITCHCOCK[2,1]
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Abstract: Progranulin is an evolutionarily conserved secreted growth factor well established for its role in embryogenesis, tumor development and wound repair. Although much is known about progranulin activity in non-neuronal tissues, the functions of progranulin within the central nervous system (CNS) are not well understood. In humans, mutations in the progranulin gene (*GRN*) cause neurodegeneration. In zebrafish, progranulin-a (*Pgrn-a*), syntenic to *GRN*, is expressed exclusively by peripheral macrophages and CNS microglia, and is strongly upregulated in retinal microglia after the selective death and regeneration of photoreceptors. There is a gap in our knowledge, however, regarding the functions of progranulin during developmental neurogenesis. Therefore, the purpose of this study was to determine how Pgrn-a regulates vertebrate retinal development, using zebrafish as a model. The work presented here combined reverse genetics and quantitative morphometric approaches, including *in vivo* and *in vitro* assays of cell proliferation and differentiation and expression studies, to elucidate the details by which Pgrn-a regulates retinal progenitor cell cycle. Knock down of Pgrn-a synthesis in zebrafish embryos results in the absence of neuronal differentiation in the retina, maintenance of retinal progenitors in a proliferating state, and altered cell cycle kinetics. Collectively, our data show Pgrn-a loss-of-function (LOF) lengthens retinal progenitor cell cycle. Specifically, the proportion of cells undergoing mitosis is significantly decreased in Pgrn-a LOF embryos. Relative to controls, the G2-phase, total cell cycle length and S-phase duration is significantly longer following Pgrn-a LOF. The expression of cell cycle promoters and inhibitors is correspondingly altered in experimental embryos. These data suggest that for retinal progenitors Pgrn-a functions as a mitogen to govern the transition from proliferation to differentiation, and that in the vertebrate retina, microglia-derived growth factors regulate aspects of developmental neurogenesis. Ongoing studies seek to elucidate the downstream signaling mechanism of Pgrn-a action on cell cycle kinetics.

Disclosures: C. Walsh: None. **P. Hitchcock:** None.

Poster

495. Proliferation: Self-Renewal and Cell Cycle

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 495.10/A60

Topic: A.02. Neurogenesis and Gliogenesis

Support: Spanish Ministry of Economy and Competitivity FPI fellowship to A.C

BFU2012-33473 grant to V.B.

Title: The role of direct neurogenesis in the development of the olfactory bulb

Authors: *A. CÁRDENAS[1], M. COGSWELL[2], C. DE JUAN ROMERO[1], A. TZIKA[3], M. MILINKOVITCH[3], L. MARTÍNEZ[1], M. TESSIER-LAVIGNE[4], S. RUSSEK[2], V. BORRELL[1]
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Abstract: The olfactory bulb (OB) develops as a unique specialization of the rostral pallium (OB primordium) from early stages of development. The onset of OB development coincides with changes in progenitor cell cycle parameters and early neurogenesis locally within the OB primordium, which have been suggested to be key for its initial evagination and growth. Here we show that the distinction between OB and neocortical development starts at the onset of neurogenesis, with changes in progenitor cell cycle parameters such as cycle lengthening and increased cycle exit, producing within a short developmental period a prominent accumulation of neurons that bulge rostrally. We find that these changes in progenitor cell dynamics involve mainly apical progenitors, and are linked to an equally important accumulation of newborn pallial neurons within the VZ of the OB primordium, virtually absent in the neocortex. Time-course and time-lapse analyses of progenitor cell lineages demonstrate that these changes are preceded by significant OB progenitor cell dynamics, including the abundant occurrence of direct neurogenesis from Radial Glia cells, which we show is nearly anecdotic in the neocortex at those stages. Hence, whereas in the neocortex the majority of neurons are born from intermediate progenitor cells through indirect neurogenesis, in the OB we find that a significant fraction of neurons are produced directly from apical progenitors, while maintaining similar rates of indirect neurogenesis as in the neocortex. Direct neurogenesis in the OB primordium is paralleled by a reduced self-amplification of RGCs, coupling an increased neurogenesis with a reduction in apical surface, which leads to OB evagination. In summary, the development of the OB is initiated by a transient peak of neurogenesis in a short developmental period driven by a high incidence of direct neurogenesis from RGCs. We are currently searching for candidate genes

differentially expressed between OB and neocortex that may regulate cell cycle dynamics as well as the fate of Radial Glial Cells towards a direct neurogenetic lineage.

Disclosures: **A. Cárdenas:** None. **M. Cogswell:** None. **C. De Juan Romero:** None. **A. Tzika:** None. **M. Milinkovitch:** None. **L. Martínez:** None. **M. Tessier-Lavigne:** None. **S. Russek:** None. **V. Borrell:** None.

Poster

495. Proliferation: Self-Renewal and Cell Cycle

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Topic: A.02. Neurogenesis and Gliogenesis

Support: University of Florida Age-related Memory Panel Grant through the Evelyn F. McKnight Brain Research Foundation to B.K.O

NIH Grant R37AG036800 to T.C.F.

Title: Neurogenesis declines in the olfactory bulbs and hippocampi of middle-aged and aged rats

Authors: ***J. MCGUINESS**[1,2], R. B. SPEISMAN[2], A. ASOKAN[2], A. RANI[1], A. KUMAR[1], T. C. FOSTER[1], B. K. ORMEROD[1,2]
[1]McKnight Brain Inst. and Dept. of Neurosci., [2]Biomed. Engin. Dept., Univ. of Florida, Gainesville, FL

Abstract: Identifying the mechanisms behind non-pathological cognitive aging is a critical step for developing preventative and ameliorative treatments that maintain or improve quality of life in the elderly. The production of new hippocampal and olfactory bulb neurons can diminish with age, but whether age-related changes in neurogenesis in these regions occur concomitantly or in a region-specific manner is unclear. In addition, age-related declines in hippocampal neurogenesis have been linked to changes in central cytokine and chemokine levels but whether these factors also impact olfactory bulb neurogenesis is also unclear. Here we quantified central and circulating chemokines/cytokines and new neuron numbers in the olfactory bulbs and hippocampi of young (4-6mo), middle-aged (10-12mo) and aged (18-20mo) male Fischer 344 rats injected with the cell synthesis marker bromodeoxyuridine (BrdU; 50 mg/kg every 24h for 3 days) 2 weeks after the rats were trained and tested in a rapid water maze. Although similar proportions of immature (DCX+), transitioning (DCX/NeuN+) and mature (NeuN+) neurons

were detected across age groups, significantly fewer new cells were found in the olfactory bulbs and hippocampi of middle-aged and aged rats relative to their young counterparts. We are currently testing the relationships between age-related changes in olfactory bulb neurogenesis, hippocampal neurogenesis and levels of circulating and central cytokines in memory-impaired and memory-unimpaired rats. Our data will add to the picture of how neuroinflammation impacts neurogenesis and cognition across age.

Disclosures: J. McGuiness: None. **R.B. Speisman:** None. **A. Asokan:** None. **A. Rani:** None. **A. Kumar:** None. **T.C. Foster:** None. **B.K. Ormerod:** None.

Poster

495. Proliferation: Self-Renewal and Cell Cycle

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Program#/Poster#: 495.12/A62

Topic: A.02. Neurogenesis and Gliogenesis

Support: NHRI-EX102-10260NI

Title: The role of Lhx2 in cortical neurogenesis

Authors: *H. CHIA-LING, L. CHIA-CHENG, N. SEAN, C. SHEN-JU
Inst. of Cell. and Organismic Biology, Acad, Taipei, Taiwan

Abstract: Abstract During corticogenesis, the balance between proliferation and differentiation of cortical progenitors is precisely regulated to give rise to an optimal number of cortical neurons. Lhx2, a LIM homeodomain transcription factor, was shown to be crucial for the formation of the cortex. Interestingly, *Lhx2* is expressed in a high-caudal-to-low-rostral gradient, which is opposite to the neurogenesis gradient in the developing dorsal telencephalon (dTel). Thus, it has been hypothesized that Lhx2 is involved in regulating cortical neurogenesis. The deletion of *Lhx2* in neural progenitors leads to a significantly smaller cortex with fewer progenitors and more neurons in the dorsal telencephalon at early developmental stages. We are currently comparing the proliferation and differentiation capacity of cortical progenitors from wild type and *Lhx2* conditional knockout (cKO) embryos. Our preliminary findings suggest that Lhx2 is required for the maintenance of cortical progenitors at their proliferative state. (This work is supported by NHRI-EX102-10260NI.)

Disclosures: H. Chia-Ling: None. **L. Chia-Cheng:** None. **N. Sean:** None. **C. Shen-Ju:** None.

Poster

495. Proliferation: Self-Renewal and Cell Cycle

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Program#/Poster#: 495.13/A63

Topic: A.02. Neurogenesis and Gliogenesis

Support: NIH Grant NS076640

March of Dimes

American Cancer Society

Title: The roles of cytokinesis and kinesin Kif20b in regulating cerebral cortex growth

Authors: K. M. JANISCH[1], V. VOCK[2], A. SHRESTHA[2], J. DARDICK[2], M. FLEMING[2], T. CUPP[2], C. GRIMSLEY-MYERS[2], *N. DWYER[3]
[1]Univ. of Virginia, Charlottesville, VA; [2]Univ. Virginia, Charlottesville, VA; [3]Cell Biol, Univ. Virginia, CHARLOTTESVLE, VA

Abstract: The proper development of the cerebral cortex depends upon precise temporal and spatial control of the cell divisions of neural stem cells. Early in development, sufficient numbers of symmetric divisions must occur to expand the neuroepithelium, and then asymmetric divisions must produce neurons with sequential layer fates and finally glia. During these divisions the neural stem cells maintain apical-basal polarity and epithelial integrity. It is thought that the fates of the daughter cells are determined by the partitioning of specific factors in the membrane and cytoplasm during cleavage. To control the segregation of fate determinants, the cells must precisely regulate mitotic spindle positioning, cytokinetic cleavage furrowing, and abscission. However, while many proteins affecting spindle orientation have been studied in mammalian cortex, much less is known about the cytokinesis events that actually carry out partitioning. This is crucial because neural stem cells undergo a special polarized form of cytokinesis. Their cleavage furrow ingresses from basal to apical, and forms a midbody at the apical membrane, which mediates abscission (severing of the daughter cells). We have undertaken quantitative analysis of cytokinesis in neural stem cells (apical progenitors) of the developing mouse cortex, and found differences between early and later stages of corticogenesis. Furthermore, we have identified a mouse mutant with microcephaly and defects in cytokinetic structures in the embryonic cortex. In mutant cortex, cytokinetic midbodies of dividing apical progenitors are often misaligned with the apical membrane. Those that are aligned tend to be wider than in controls. Apoptosis is increased four-fold in mutants. The subventricular zone and neuronal layers form, but are thin, and the mutants die at birth. The mutant has a hypomorphic mutation in

the vertebrate-specific Kinesin-6 family member Kif20b. This microtubule motor protein localizes within cleavage furrows and cytokinetic midbodies of control progenitor cells, but is reduced to undetectable levels in mutant cells. Kif20b was previously shown to be involved in cytokinetic abscission of human cells *in vitro*, but its precise function is not known. We hypothesize that in the developing cortex, Kif20b plays roles in midbody maturation and linkage to the apical membrane prior to abscission. In the Kif20b mutant, stochastic failures in abscission appear to lead to apoptosis, which depletes the progenitor pool and reduces neurogenesis. We are further analyzing the structure of neural progenitor midbodies, and testing whether candidate binding partners of Kif20b are mislocalized in mutant cells.

Disclosures: K.M. Janisch: None. **A. Shrestha:** None. **N. Dwyer:** None. **V. Vock:** None. **M. Fleming:** None. **T. Cupp:** None. **J. Dardick:** None. **C. Grimsley-Myers:** None.

Poster

495. Proliferation: Self-Renewal and Cell Cycle

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Topic: A.02. Neurogenesis and Gliogenesis

Support: Grant-in-Aid for Scientific Research

Title: Krüppel-like factor 5 plays important roles in self-renewal and differentiation of neural stem cells

Authors: A. KURODA[1], S. ISHIDA[1], *T. FUCHIGAMI[3], Y. HAYASHI[1], M. EMA[2], S. HITOSHI[1]
[1]Interactive Physiol., [2]Res. center for animal life science, Shiga university of medical science, Ohtsu, Japan; [3]Physiol., Japan, Ohtsu, Japan

Abstract: Krüppel-like factor (Klf) 5 is a member of Klf family of Zinc finger transcription factors, whose sequence and function are highly conserved in vertebrates. Among this family, Klf4 have been vigorously studied and there are so many reports about its roles in the induction of iPS cells by somatic cell reprogramming, in ES cell self-renewal, and in early embryogenesis, especially in inner-cell mass (ICM) development. In neural development, Klf4 also plays a significant role in the maintenance of neural stem cells (NSCs) by repressing proliferation of NSCs. Despite Klf5 can substitute, at least in part, for the roles of Klf4 in the early development, its roles in the neural development remain poorly understood. In this study, we performed the

neurosphere assay using Klf5 conditional knockout (cKO) mice and found that Klf5-deleted NSCs showed the impairment of self-renewal. We also observed that knockdown of Klf5 by *in utero* electroporation induced the premature differentiation and facilitated the radial migration of neural precursor cells of mouse embryonic brains. To understand the molecular mechanisms underlying the effects of Klf5 knockdown on the neurogenesis, microarray analysis was performed. Our results suggest that Klf5 plays important roles in the neural development and proliferation, migration and differentiation of neural precursor cells.

Disclosures: **A. Kuroda:** None. **S. Ishida:** None. **T. Fuchigami:** None. **Y. Hayashi:** None. **M. Ema:** None. **S. Hitoshi:** None.

Poster

495. Proliferation: Self-Renewal and Cell Cycle

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Topic: A.02. Neurogenesis and Gliogenesis

Support: NSF IOS 0818259

Swedish Research Council 621-2011-4797

NSF IOS 1121345

Title: Cells from the innate immune system generate adult-born neurons in crayfish

Authors: ***J. L. BENTON**[1], R. KERY[1], J. LI[1], C. NOONIN[2], I. SÖDERHÄLL[2], B. S. BELTZ[1]
[1]Neurosci. Program, Wellesley Col., Wellesley, MA; [2]Dept. of Comparative Physiol., Uppsala Univ., Uppsala, Sweden

Abstract: Neurogenesis continues in the brains of adult decapod crustaceans. However, the 1st-generation precursors that produce adult-born neurons, which reside in a neurogenic niche, are not self-renewing in the crayfish brain and must be replenished; the source of these neuronal precursors is unknown. Hemocytes, which are central players in innate defense mechanisms in crustaceans, are prime candidates. Here, we report that the number of cells composing the neurogenic niche in crayfish is tightly correlated with total hemocyte counts (THC) and can be manipulated by raising or lowering THC. Following adoptive transfer of ethynyl-2'-deoxyuridine (EdU)-labeled hemocytes, these cells populate the neurogenic niche containing the 1st-generation neuronal precursors. After a 7-week survival time, EdU-labeled cells are located

in brain clusters 9 and 10 where adult-born neurons differentiate, and express appropriate neurotransmitters. These findings demonstrate that the immune system is intimately involved in adult neurogenesis, and that adult-born neurons in crayfish can be derived from hemocytes *in vivo*.

Disclosures: J.L. Benton: None. **R. Kery:** None. **J. Li:** None. **C. Noonin:** None. **I. Söderhäll:** None. **B.S. Beltz:** None.

Poster

495. Proliferation: Self-Renewal and Cell Cycle

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Topic: A.02. Neurogenesis and Gliogenesis

Support: NSF IOS 0818259

NSF IOS 1121345

Title: Neuronal precursors in the crayfish Procambarus clarkii are replenished from a non-neuronal source

Authors: J. LI, E. COCKEY, J. PLATTO, J. L. BENTON, *B. S. BELTZ
Neurosci. Program, Wellesley Col., WELLESLEY, MA

Abstract: Adult neurogenesis - the birth and integration of new neurons in the adult brain - is common in both vertebrate and invertebrate species. Investigations in mammalian models suggest that stem cells producing adult-born neurons are self-renewing. Previous investigations in the crayfish, Procambarus clarkii, have found that although the pool of first-generation neuronal precursors in the neurogenic niche is never depleted, these cells are NOT self-renewing. Therefore, there must be a source of neuronal precursors extrinsic to the niche. We propose that the innate immune (hematopoietic) system is this source, based on *in vitro* studies showing that hemocytes (and not other cell types) are attracted to the niche. We further tested this hypothesis by examining the turnover rate of two key hematopoietic regions, the posterior hematopoietic tissue (HPT) and the newly discovered anterior proliferation center (APC). Both of these regions are highly proliferative and are potential sources of neuronal precursors. The turnover rate of cells in HPT and APC was tracked after a single 5-bromo-2'-deoxyuridine (BrdU) injection to label S-phase cells, followed by immunohistochemical detection. An overall decrease in the

number of BrdU-labeled cells in hematopoietic regions was observed over time, as well as a temporally correlated increase in BrdU-labeled cells in the hemolymph. In the niche, BrdU-labeled cells were observed for 4 days following the labeling period. This initial labeling period is followed by a 3-day gap when the niche is devoid of BrdU labeling. Unexpectedly, BrdU-labeled cells were again observed in the niche between 8 and 14 days after the BrdU exposure. As BrdU is no longer available for renewed labeling of neuronal precursors in the niche at this time, our interpretation is that the "second wave" of labeled cells must have incorporated the nucleoside while still in their source tissue. Many mechanisms underlying adult neurogenesis are conserved across a broad range of vertebrate and invertebrate species. Therefore, this work may have important implications for other organisms, as well as for diseases associated with adult neurogenesis.

Disclosures: J. Li: None. **B.S. Beltz:** None. **E. Cockey:** None. **J. Platto:** None. **J.L. Benton:** None.

Poster

495. Proliferation: Self-Renewal and Cell Cycle

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Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 495.17/A67

Topic: A.02. Neurogenesis and Gliogenesis

Title: Neurogenesis and *in vivo* cell cycle dynamics during the development of the DRG compared to the ENS

Authors: *D. G. GONSALVEZ, M. L. Y. FONG, K. N. CANE, L. A. STAMP, H. M. YOUNG, C. R. ANDERSON
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Abstract: To understand the *in vivo* proliferative behaviour of neural stem and progenitor cells outside the CNS, we have examined the *in vivo* cell cycle dynamics of the developing mouse dorsal root ganglia (DRG) and enteric nervous system (ENS). We have modified an existing double S-phase labelling approach by combining it with multiple label immunofluorescence to identify *in vivo* cell cycle parameters in identified populations of cells. With this approach we can simultaneously identify the growth fraction (GF), S-phase length (Ts) and cell cycle length (Tc) for individual embryos. To identify neural crest progenitor cells, committed neurons and proliferating cells we used antisera to Sox10, Hu, and Ki67 respectively. The changes to GF, Tc and Ts, in the developing DRG and ENS are summarised in Table 1. In the DRG, only Sox10

cells were cycling. From E9.5 to E12.5, Tc and Ts both increase in proportion. On E13.5 Tc increases further but Ts decreases, corresponding with significant numbers of Sox10 cells exiting the cell cycle. For Sox10 cells in the ENS, there was a significant increase in the length of Tc from E10.5 to E12.5, which then remained constant through E16.5. Over this period, the vast majority of Sox10 cells remained in the cell cycle. Together with a previous study (Gonsalvez et al 2013, J. Neuroscience, 33(14); 5969), this study shows that neural crest derivatives forming different parts of the peripheral nervous system each have different patterns of proliferation, despite their common origin. The approach used can be applied to identify key proliferation parameters *in vivo* from different CNS and neural crest-derived tissues in a single embryo. This approach will be useful in studying how *in vivo* cell cycle dynamics are altered in a range of neurocristopathies. Table 1: Proliferation parameters of Sox10 and Hu cells the developing DRG and ENS

Cells	**Age**	**GF**	**Tc (h)**	**Ts (h)**
DRG				
Sox10	*E9.5*	1.00 (4)	8.0 (4)	5.0 (4)
Sox10	*E10.5*	0.97 (4)	10.0 (4)	6.5 (4)
Sox10	*E11.5*	0.96 (4)	11.1 (4)	6.3 (4)
Sox10	*E12.5*	0.91 (4)	18.5 (4)	10.1 (4)
Sox10	*E13.5*	0.59 (5)	31.9 (5)	6.1 (4)
Sox10	*E14.5*	0.28 (4)	33.8 (3)	8.4 (3)
Sox10	*E16.5*	0.19 (3)	N/A	N/A
ENS				
Sox10	*E10.5*	1.00 (11)	14.6 (11)	6.8 (11)
Sox10	*E12.5*	0.99 (5)	19.2 (6)	9.5 (6)
Sox10	*E16.5*	0.89 (5)	20.8 (3)	6.1 (3)
Hu	*E10.5*	0.02 (7)	N/A	N/A
Hu	*E12.5*	0.05 (5)	N/A	N/A

Hu *E16.5* 0.05 (5) N/A N/A

No. in ( ) are No. of embryos used. N/A = ages where there were too few cycling cells to calculate Tc & Ts

Disclosures: D.G. Gonsalvez: None. **M.L.Y. Fong:** None. **K.N. Cane:** None. **L.A. Stamp:** None. **H.M. Young:** None. **C.R. Anderson:** None.

Poster

495. Proliferation: Self-Renewal and Cell Cycle

Location: Halls A-C

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Topic: A.02. Neurogenesis and Gliogenesis

Support: NIH Grant HD40182

AHA PD fellowship

Title: Initiation mechanism for apical nuclear migration in radial glial progenitors

Authors: *A. BAFFET, D. HU, R. VALLEE
Columbia Univ., New York, NY

Abstract: In the developing neocortex, neurons are formed from the division of neuronal progenitor cells, also called Radial Glial Progenitors (RGPs). RGPs undergo a long-mysterious cell cycle-dependent process known as Interkinetic Nuclear Migration (INM), during which their nuclei oscillate between the apical and the basal regions of the ventricular zone. This movement is dependent on the microtubule cytoskeleton and tightly linked to the cell cycle: The kinesin KIFf1A transports the nucleus basally during G1 and dynein transports the nucleus apically during G2. We recently reported an essential role for two dynein recruitment pathways to the nuclear pores, acting sequentially to drive G2 apical nuclear migration (Hu et al, Cell, 2013). The RanBP2-BicD2 pathway is activated early in G2 and is involved in long-range nuclear migration to the ventricular surface, whereas the Nup133-CENP-F pathway contributes specifically to the late stages of migration. Strikingly, inhibition of these factors also blocked mitotic entry and neuronal production, revealing a tight relationship between cell cycle, INM, and neurogenesis. The current study was initiated to identify cell cycle-dependent regulatory mechanisms responsible for triggering apical nuclear migration during G2. Using multiple protein kinase

pharmacological inhibitors in live cultured embryonic rat brain slices we found acute Cdk1 inhibition to block apical nuclear migration at its earliest stages, phenocopying dynein and BicD2 knockdown. Cdk1 inhibition also rapidly and reversibly interfered with recruitment of BicD2 and dynein to the G2 nuclear envelope. Dynein recruitment could be rescued by constitutively targeting the dynein-binding region of BicD2 to the nuclear envelope, indicating that Cdk1 primarily modulates the RanBP2-BicD2 interaction. Using *in vitro* protein kinase assays, we identify novel Cdk1 phosphorylation sites in RanBP2, which strongly enhance BicD2 binding. These results identify a mechanism to control dynein recruitment to the G2 nuclear envelope and initiate apical nuclear migration in brain progenitor cells.

Disclosures: **A. Baffet:** None. **D. Hu:** None. **R. Vallee:** None.

Poster

495. Proliferation: Self-Renewal and Cell Cycle

Location: Halls A-C

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Topic: A.02. Neurogenesis and Gliogenesis

Title: Basal radial glia in outer subventricular zone contributes to late-bone neurons in the marmoset cerebral cortex

Authors: *A. MURAYAMA[1,2], J. OKAHARA[3], E. SASAKI[3], H. OKANO[1,2]
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Abstract: The basal radial glia (bRG) is a type of progenitor in the subventricular zone (SVZ), which retains a basal process to the pial surface, sustains expression of radial glial markers and is capable of self-renewal. The bRGs occur at high relative abundance in the outer SVZ (OSVZ) of gyrencephalic animals, but lower of lissencephalic rodents, suggesting that the division of bRG in OSVZ is important to generate gyrencephalic brain. The common marmoset, Callithrix jacchus, is a primate that has a few gyrus and sulcus formations though the abundance of bRG cells is similar to that in human neocortex. In order to study the role of bRG in the developing marmoset brain, at first, we characterized the cell cycle of bRG using the technique of thymidine analog labeling, in combination with immunofluorescent staining. Secondary, we determined when the bRG cells build up in the OSVZ. Accordingly, the poor gyrification of marmoset's cerebral cortex is caused by the later birth of bRG than those of gyrencephalic animals such as macaque monkey. Furthermore, we labeled the dividing bRG with thymidine analogs when there

is an abundance of bRG in OSVZ according to previous data (Kelava et al., 2012). After birth, we performed histological analysis, showing that the bRG cells predominantly became the upper-layer neurons in the marmoset cerebral cortex.

Disclosures: **A. Murayama:** None. **J. Okahara:** None. **E. Sasaki:** None. **H. Okano:** None.

Poster

495. Proliferation: Self-Renewal and Cell Cycle

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Topic: A.02. Neurogenesis and Gliogenesis

Support: CNPq

CAPES

Proppi-UFF

Title: Coordinated cell cycle regulation by nucleotides and IGF-I in retinal progenitors

Authors: ***I. M. ORNELAS**, M. R. PEREIRA, T. M. SILVA, A. L. M. VENTURA
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Abstract: Aim: ADP induces proliferation of retinal progenitors through activation of P2Y1 receptors, MAPK and PI3K. Recent studies have also suggested the participation of growth factors in purinergic signaling. Here we evaluated the role of ADP and IGF-I in controlling cell cycle of retinal progenitors. Methods and results:To evaluate signaling pathways activated by ADP and IGF-I in retinal cells, primary cultures obtained from 7-day-old chick embryos were stimulated for 5 minutes and protein levels detected by western blot. Cultures treated with 250 μM ADP and 100 ng/ml IGF-I showed increased levels of phospho-Erk. However, only IGF-I could stimulate phosphorylation of IGF-I receptor, Akt and 4E-BP1. Besides, after IGF-I treatment we could detect, by immunofluorescence, phospho-Akt and PCNA double-labeled cells, indicating that IGF-I stimulate PI3K/Akt pathway in progenitor cells. Since PI3K/Akt pathway is involved in CDK1 regulation and G2/M progression of retinal progenitors, we evaluated if IGF-I or ADP could regulate CDK1 levels by RT-PCR and western blot. IGF-I treatment, but not ADP, significantly increased CDK1 expression at mRNA and protein levels. Consistent with this data, IGF-I promoted a significant decrease in histone-H3 phosphorylation, which was partially prevented by the addition of PI3K inhibitor. S-phase entry was evaluated by

[3H]-thymidine incorporation in cultures treated with IGF-I and/or ADP for 24 h. When cultures were maintained in absence of FBS (fetal bovine serum) nor ADP or IGF-I could promote entry in S-phase. However, co-treatment with both agonists promoted a significant increase in [3H]-thymidine incorporation. Besides, in absence of FBS, concomitant treatment with both agonists could also increase Cyclin D1 and Cyclin E levels. Furthermore, in presence of FBS, ADP could stimulate entry in S-phase and this effect was totally prevented by IGF-I receptor inhibitor AG-1024. Conclusions: These data suggest that ADP and IGF-I are extracellular factors that regulate cell cycle in retinal progenitors. While IGF-I controls CDK1 expression and G2/M progression, both ADP and IGF-I are required to S-phase entry and expression of G1-phase cyclins.

Disclosures: I.M. Ornelas: None. **M.R. Pereira:** None. **T.M. Silva:** None. **A.L.M. Ventura:** None.

Poster

495. Proliferation: Self-Renewal and Cell Cycle

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Topic: A.02. Neurogenesis and Gliogenesis

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MRC Grant-in Aid (U117570528)

Title: An intracellular mechanism integrating inputs from extracellular signals to activate hippocampal stem cells

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Abstract: Neural stem cells (or radial glia-like cells, RGLs) in the dentate gyrus (DG) of the adult hippocampus exist primarily in a quiescent state, in contrast to the highly proliferative NSCs in the embryonic nervous system. One of the main features of quiescent DG stem cells is their ability to re-enter the cell cycle upon reception of activation signals from the surrounding niche, and therefore, of their ability to respond to changing physiological demands as well as to aberrant pathological states. A number of environmental stimuli and signals have been shown to

influence stem cell activity. However, how stem cells integrate this information to generate an appropriate response is still unknown. Here we show that *Ascl1*, a member of the basic helix-loop-helix family of transcription factors known to promote progenitor fate commitment and differentiation in the developing mouse brain, is expressed by stem cells of the adult hippocampus, specifically when they become activated. Moreover, its expression is rapidly induced by neurogenic stimuli, with a faster kinetic than that of activation markers such as MCM2. Deleting *Ascl1* specifically in the neural stem cell population of the DG with a GlastCreERT2, tamoxifen-inducible line, results in the complete inability of RGLs to exit quiescence and divide, and consequently leads to an absolute block of neurogenesis in the adult DG. *Ascl1*-deficient cells remain in a state of quiescence over many months, and, most importantly, are unable to respond to neurogenic stimuli. By examining the genome-wide binding of *Ascl1* in adult hippocampal-derived neural stem cells, and the genes deregulated in *Ascl1*-deleted DG stem cells, we are learning how this factor controls stem cell activation and cell cycle progression. This work establishes the role of the transcription factor *Ascl1* in promoting the activation of neural stem cells and highlights the central function of *Ascl1* in integrating niche signals to regulate neural stem cell activity.

Disclosures: J. Andersen: None. **N. Urbán:** None. **A. Ito:** None. **A. Achimastou:** None. **B. Martynoga:** None. **M. Lebel:** None. **C. Göritz:** None. **J. Frisén:** None. **F. Guillemot:** None.

Poster

495. Proliferation: Self-Renewal and Cell Cycle

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 495.22/B4

Topic: A.02. Neurogenesis and Gliogenesis

Title: Down-regulated necdin expression triggers the proliferation of postmitotic neurons of the rat cortex

Authors: *S. LIU, R. LIU, H. QUE, J. YANG, Q. LIN, Y. LIU, S. YANG, S. JING
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Abstract: A widely accepted concept in neural biology field is that mature neurons are terminally differentiated cells and thus unable to re-enter cell cycles or to proliferate. The neurogenesis of the brains of adult animals reported by early articles is now believed to be the result of the proliferation of neural stem cells or neural progenitor cells. However, we found that down-regulated necdin expression by tri-iodo-L-thyronine (T3) and necdin RNAi can trigger the

proliferation of the purified primary neurons and the neurons *in situ* of adult rat cortex. The cultured primary neurons from fetal rat cortex were treated with (10^{-5} M) of arc-c for 96 hours and purified up to the concentration 99.99% and no nestin-positive cells is observed. The enriched primary neurons were then treated with T3 at different concentrations. Substantial amount of dividing neurons at different mitosis phases were observed. Low dose of T3 (2μg/ml) resulted in the division of parvocellular neurons and high dose of T3 (more than 50μg/ml) made magnocellular neurons proliferation. About 4.6±0.7% ShcC labeled neurons were checked out at G2M with flow cytometry when treated with 50μg/ml of T3. We further demonstrated that the dividing neurons possess various characteristics of postmitotic neurons, evidenced by specific markers, electrophysiology, ultrastructural and typical synapses between the dividing neurons and others. When different concentration of T3 was reversely administrated into the cortex of adult rat by microdialysis, a substantial amount of MAP2 and ShcC labeled neurons *in situ*, located around the microdialysis areas of layers III-VI, were triggered to divide. We further found that T3 triggers neuron division, is closely related to its ability to reduce the expression of necdin, alter the subcellular localization of E2F1, and to activate cyclin expression. Following, we demonstrated that down-regulation of necdin expression triggers the division of the enriched primary neurons and the cortex neurons *in situ* by necdin RNAi. The postmitotic neurons still possess the ability to proliferate under some conditions suggests the neurogenesis possibility and renews the hope in developing strategies for the cure of neural degenerative diseases. Key words: tri-iodo-L-thyronine, postmitotic neuron, proliferation

Disclosures: S. Liu: None. **R. Liu:** None. **H. Que:** None. **J. Yang:** None. **Q. Lin:** None. **Y. Liu:** None. **S. Yang:** None. **S. Jing:** None.

Poster

495. Proliferation: Self-Renewal and Cell Cycle

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 495.23/B5

Topic: A.04. Stem Cells

Support: Pearson Foundation

Lincy Foundation

Title: Retinal progenitors are stem cells and Hmga2 facilitates their self-renewal

Authors: *I. AHMAD, S. PARAMESWARAN, X. XIA
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Abstract: Retinal progenitors unlike neural stem cells do not self-renew under normal culture conditions *in vitro*. This observation is counterintuitive to a prolonged retinal histogenesis, which necessitates retinal progenitors to self-renew to sustain the temporal generation of diverse cell types. Here, we have examined the premise that the self-renewal property of retinal progenitors requires contribution from others cells in the histogenic environment. In the absence of the identity of the contributing cells in the developing retina this notion was tested in the presence of endothelial cells, known to support stem cells in neural and extra-neural niches. We used conventional neurosphere assays, lentivirus-mediated perturbation of gene expression, and molecular and biochemical analyses to test the hypothesis. We observed that a small subset of retinal progenitors were capable of clonal propagation and maintain multipotentiality of their parents in the presence of endothelial cells. The self-renewing features, also observed *in vivo*, involved multiple inter-cellular signaling pathways. Microarray analysis revealed that these pathways recruited high-mobility group protein AT-hook2 (Hmga2), an epigenetic regulator, whose expression progressively decreased in the developing retina, as progenitors were exhausted. Perturbation of expression of Hmga2 both *in vitro* and *in vivo* revealed that it was functionally involved in mediating the cell-extrinsic influences on the self-renewal of retinal progenitors. Our observations suggest that retinal progenitors possess non-cell autonomous self-renewal property, which is regulated by a molecular axis, underpinned by Hmga2.

Disclosures: I. Ahmad: None. **S. Parameswaran:** None. **X. Xia:** None.

Poster

495. Proliferation: Self-Renewal and Cell Cycle

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 495.24/B6

Topic: A.04. Stem Cells

Support: NIH P50 AA07611

M. Keck Foundation

Title: Genomic DNA methylation program of a neural stem cells differentiation

Authors: *F. C. ZHOU[1], C.-L. LO[2], A. LOSSIE[3]
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Abstract: The neural stem cell (NSC) restriction and differentiation require precise and orchestrated network of genetic transcription. What and how are the key genes transcribed from selected genome, and foremost what instructions are given to direct the complex transcription during neurulation is key to the understanding of the neuronal development. We have previously demonstrated that epigenomics of neural stem cells go through a critical DNA methylation diversification during initiation of differentiation (Zhou et al., 2011). In this study, comparing between undifferentiated and 3-day differentiated dorsal ganglia NSCs as model, we combined the analysis of genome-wide DNA methylation (by MeDIP-Chip) with the transcription (by microarray) to interrogate the involvement of the gene activities at epigenetic and transcriptional levels during the initial differentiation. The altered genes were further analyzed using Ingenuity pathway analysis (IPA) for canonical pathway and functional networking. We found that 691 genes were hypermethylated and 403 genes were hypomethylated in the differentiated cells as compared to the undifferentiated. Cross analysis of DNA methylation and transcription followed with IPA revealed a genes form a key network which involved critical cell signaling (including growth factors ECM protein, cytokines and hormone signaling pathways), cell cycle (e.g decrease of Cyclin D, Cyclin E), apoptosis (e.g. decrease of Bak, Bax), and Hedgehog, WNT, BMP, and TGF-β pathways. Our further analysis of neuronal specification pathways revealed many including Bmp4, Notch2, Hox and proneuron genes (ie. Ascl1) were also altered in expression or methylation. Finally, the influence of DNA methylation on transcription of a proneuron gene, Ascl1 was tested using miSeq methylation analysis followed by qRT-PCR to demonstrate the epigenetic influence of DNA methylation on transcription. Supported by P50 AA07611 to FCZ and M. Keck Foundation to FCZ and ACL.

Disclosures: F.C. Zhou: None. **C. Lo:** None. **A. Lossie:** None.

Poster

495. Proliferation: Self-Renewal and Cell Cycle

Location: Halls A-C

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Program#/Poster#: 495.25/B7

Topic: A.04. Stem Cells

Support: CNU Hospital Grant CRI10068-1

NRF Korea Grant 2010-0025501

Title: Neural differentiation of human adipose tissue-derived stem cells involves activation of the Wnt signalling

Authors: *S. JANG, J.-S. PARK, H.-S. JEONG
Physiol., Chonnam Natl. Univ. Med. Sch., Gwangju, Korea, Republic of

Abstract: Background: Stem cells are a powerful resource for cell-based transplantation therapies in neurodegenerative diseases, but understanding stem cell differentiation at the molecular level is not clear yet. Wnt signalling is involved in numerous events in animal development, including the proliferation of stem cells and the differentiation of stem cells to specific lineages. Based on this, we hypothesized that the Wnt pathway controls stem cell maintenance and neural differentiation. Results: We characterized the transcriptional expression of Wnt signalling genes during the neuronal differentiation of hADSCs followed our previous study. After neuronal induction, RT-PCR analysis was showed that the expressions of Wnt2, Wnt4, Wnt11 were decreased, but the expression of Wnt5a was increased compared with primary hADSCs. In addition, the expression level of most Fzds and LRP5/6 ligand were decreased, not Fzd3 and Fzd5. There were not changes the expression of b-catenin and GSK-3b. Interestingly, Wnt5a expression was highly increased in NI-hADSCs by real time RT-PCR analysis and western blot. Finally, we found that the JNK expression was increased after neuronal induction. Conclusion: Our studies indicate that the Wnt5a/JNK pathway modulated the neuronal differentiation binding Fzd3 and Fzd5 and directing Axin/GSK-3b in hADSCs.

Disclosures: **S. Jang:** None. **J. Park:** None. **H. Jeong:** None.

Poster

495. Proliferation: Self-Renewal and Cell Cycle

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Program#/Poster#: 495.26/B8

Topic: A.04. Stem Cells

Support: 1ZIAMH002468-26

Title: Global gene expression profiling of mesenchymal stem cells preconditioned with the neuroprotective agents lithium and valproic acid reveals involvement of novel genes and signaling pathways

Authors: *G. R. LINARES[1], D.-M. CHUANG[2]
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Abstract: Although stem cell-based therapy has emerged as a potential treatment for neurodegenerative diseases, donor cell loss after transplantation is a major drawback for this approach. One strategy to boost cell survival and efficacy is to precondition cells prior to transplantation with compounds that elicit a comprehensive increase in the expression of genes associated with trophic factors, energy metabolism, cell survival, and migration. Since the neuroprotective actions of the mood stabilizers lithium and valproic acid (VPA) induce multiple cell survival and anti-apoptotic pathways in neurons and other cell types, we hypothesized that preconditioning bone marrow-derived mesenchymal stem cells (MSCs) with lithium and VPA would enhance their biological and functional properties. To identify the genes and signaling pathways governing the beneficial effects of preconditioning, whole genome microarray analysis was performed on MSCs using an Agilent chip that contained 60,000 oligonucleotides. MSCs were incubated in the presence or absence of combined lithium and VPA (2.5 mM each) for 24 hrs. Of the 55,821 transcripts that hybridized to the array, 4,340 genes were differentially expressed with a fold change of 2.0 or higher ($P < 0.05$) in the lithium-VPA co-treatment group relative to control. Approximately 96% of the 4,340 genes were markedly upregulated, suggesting that lithium-VPA co-treatment exerts stimulatory biological effects on MSCs. The validity of the microarray data was confirmed with the increased expression of fibroblast growth factor-21 (2.3-fold) and matrix metallopeptidase related genes such as MMP-17 (2.6-fold) and Adam-2 (9.0-fold). Functional enrichment by gene ontology (GO) analysis revealed that genes clustered into 39 GO categories (false discovery rate p-value cut off = 0.10). We found robust expression (2-9 fold) of genes involved in growth factor, antioxidant, anti-apoptotic, chemokine/cytokine, migration, mitochondrial energy, and stress response signaling pathways compared to untreated MSCs. Furthermore, several expressed sequence tags (ESTs) and long non-coding RNAs (lncRNAs) with no known function were also identified as potential targets for lithium-VPA induced signaling pathways. Experiments are ongoing to validate the expression of selected genes by real time PCR. In conclusion, global gene expression profiling improved our understanding of the molecular pathways and mechanisms involved in mediating the beneficial actions of lithium-VPA on MSCs. Therefore, employing a preconditioning strategy utilizing these agents may prove useful for enhancing the therapeutic efficacy of stem cell-based therapy.

Disclosures: G.R. Linares: None. **D. Chuang:** None.

Poster

495. Proliferation: Self-Renewal and Cell Cycle

Location: Halls A-C

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Program#/Poster#: 495.27/B9

Topic: A.04. Stem Cells

Support: NIH Grant R01AG032383

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Welch Foundation Grant I-1660

Title: HDAC3 controls G2/M progression in adult neural stem/progenitor cells by regulating CDK1 levels

Authors: Y. JIANG, *J. HSIEH
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Abstract: The maintenance of the resident adult neural stem/progenitor cell (NSPC) pool depends on the precise balance of proliferation, differentiation, and maintenance of the undifferentiated state. Identifying the mechanisms that regulate this balance in adult hippocampal NSPCs can provide insight into basic stem cell self-renewal principles important for tissue homeostasis and preventing tumor formation. Pharmacological inhibition of histone deacetylases (HDACs), a class of histone-modifying enzymes, have promising effects in cancer cells, yet the specific roles of individual HDACs in stem cell proliferation is unclear. Here using conditional knockout (cKO) mice and *in vitro* cell culture, we show that histone deacetylase 3 (HDAC3) is required for the proliferation of adult NSPCs. Detailed cell cycle analysis of NSPCs from Hdac3 cKO mice reveals a defect in cell cycle progression through G2/M phase, but not S phase. Moreover, HDAC3 controls G2/M phase progression mainly through post-translational stabilization of the G2/M cyclin-dependent kinase-1 (CDK1). These results demonstrate that HDAC3 plays a critical role in maintaining the self-renewal of adult NSPC pool and suggest that strategies aimed at pharmacological modulation of HDAC3 may be beneficial for tissue regeneration and controlling tumor cell growth.

Disclosures: **Y. Jiang:** None. **J. Hsieh:** None.

Poster

496. Proliferation: Molecular Mechanisms

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 496.01/B10

Topic: A.02. Neurogenesis and Gliogenesis

Title: A multi-resource data integration approach: Identification of candidate genes regulating cell proliferation during CNS development

Authors: ***R. S. NOWAKOWSKI**[1], F. FREUDENBERG[2], C. M. VIED[1], A. A. S. F. RAPOSO[3], Y. WANG[4], D. FENG[5]
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Abstract: Neurons of the mammalian neocortex are produced by proliferating cells located in the ventricular zone (VZ) lining the lateral ventricles. This is a complex and sequential process, requiring precise control of cell cycle progression, fate commitment and differentiation. We have analyzed publicly available databases from mouse and human to identify candidate genes that are potentially involved in regulating early neocortical development and neurogenesis. We used a mouse *in situ* hybridization dataset (The Allen Institute for Brain Science, ~2,100 genes) to identify 13 genes (Cdon, Celsr1, Dbi, E2f5, Eomes, Hmgn2, Neurog2, Notch1, Pcnt, Sox3, Ssrp1, Tead2, Tgif2) with high correlation of expression in both time and space in the proliferating cells of the VZ of the neocortex at early stages of cortical development (E13.5 and E15.5) and then significantly reduced at later stages of development (E17.5 and onwards). Consistent with this approach, we found that the human homologs of 10 of these genes also show high levels of expression in the ventricular and subventricular zones in the human developing brain (microarray data from the BrainSpan Atlas, ~58,700 probes). Using human transcriptomic data (RNAseq data from Yale University on deposit at the Allen Institute, ~52,400 genes), we further confirmed 7 of these 10 candidates as being highly expressed in human neocortex at 8-9 weeks post-conception (a comparable time point to mouse early cortical development used above) and greatly reduced by 12 weeks post-conception. We then extended this gene set by including other genes that are highly expressed either in the human neocortex or mouse VZ. A

gene ontology search for both of these extended sets of genes indicates that the majority of the genes considered are involved in transcriptional regulation. Validation of the candidate genes is provided by the reported involvement of Celsr1, Neurog2, Notch1, Sox3, and Tgif2 in either early neuronal development or diseases resulting from the disruption of neurogenesis, further suggesting that Hmgn2 and Tead2 are involved in the regulation of early neocortical development. Taken together, our knowledge-based discovery method has uncovered a conserved set of 7 genes that are highly expressed at the right time and place during neocortex development, allowing the identification of two novel potential regulators of early stages of cell proliferation in both human and mouse neurogenesis.

Disclosures: R.S. Nowakowski: None. **F. Freudenberg:** None. **C.M. Vied:** None. **A.A.S.F. Raposo:** None. **Y. Wang:** None. **D. Feng:** None.

Poster

496. Proliferation: Molecular Mechanisms

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 496.02/B11

Topic: A.02. Neurogenesis and Gliogenesis

Support: Taipei City Hospital Grant, 99TPECH13

Title: Lipopolysaccharide-induced maternal cytokine changes and the effects on dopaminergic and serotonergic neuronal development

Authors: *S. WANG[1,2], K.-F. FAN[1], C.-Y. LIN[1]
[1]Inst. of Anat. and Cell Biol., Natl. Yang-Ming Univ., Taipei, Taiwan; [2]Dept. of Educ. and Res., Taipei City Hosp., Taipei, Taiwan

Abstract: Increasing evidences suggest that maternal infection during pregnancy may influence fetal brain development and leads to neurological disorders. In our previous studies we had used lipopolysaccharide (LPS) exposure to mimic maternal infection in rats. The offspring exposed to LPS during critical developmental window for dopaminergic and serotoninergic neurons showed a reduction in the number of dopaminergic and serotoninergic neurons and the dopamine (DA) and serotonin (5-HT) concentrations were decreased in various brain regions in adulthood. As a consequence, the offspring displayed more anxiety-like and depression-like behaviors and had increased corticosterone responses to stress; suggesting maternal immune activation during critical developmental window might pose a risk for psychiatric disorders in offspring. In the

current study, we set out to examine the maternal cytokine changes induced by LPS exposure and measured cytokine concentrations in the amniotic fluid. We also examined whether the developing dopaminergic and serotonergic neurons in embryo display cytokine receptors. The pregnant dams received a single *ip* injection of LPS on day 11 of gestation. Dams of the control group received a single *ip* injection of phosphate buffered saline (PBS) at the same time point. We collected maternal blood, amniotic fluid, and embryo samples at 2, 6, 12, 24 hours following LPS injection. The tissue narcosis factor α (TNFα), interleukin 1β (IL1β) and interleukin 6 (IL6) concentrations in the blood and amniotic fluid were determined by enzyme-linked immunosorbent assay (ELISA). In another group of dams we injected 5-bromo-2'-deoxyuridine (BrdU, 200 mg/kg, ip.) from E9-E11 (one injection per day) to label proliferating cells and had LPS or PBS injection on E12.We measured the number of serotoninergic and dopaminergic neurons and the number of BrdU-positive DA and 5-HT neurons on postnatal day 7, 14, 21 and 30. We found that there was a significant increase in TNFα, IL1β, and IL6 in maternal blood and amniotic fluid, however the peak time varies in each cytokine. The developing dopaminergic and serotonergic neurons express IL6 and TNFα receptors. There is a significant reduction in the proportion of BrdU-positive serotonergic and dopaminergic neurons in LPS rats; suggesting a reduction in cell proliferation. In summery, these data indicated that maternal cytokines induced by LPS exposure could reduce cell proliferation in DA and 5-HT neurons and the effect is long-lasting.

Disclosures: **S. Wang:** None. **K. Fan:** None. **C. Lin:** None.

Poster

496. Proliferation: Molecular Mechanisms

Location: Halls A-C

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Topic: A.02. Neurogenesis and Gliogenesis

Support: Grant-in-Aid for Exploratory Research, the Japan Society for the Promotion of Science

Grant-in-Aid for Scientific Research (B), the Japan Society for the Promotion of Science

Cancer Consortium, Kansai Medical University

Title: Identification of small cell lung cancer (SCLC)-specific miRNAs in blood as a tumor marker for the detection of SCLC

Authors: *M. SHIMOJO[1], Y. SHUDO[2], S. ITO[1]
[1]Dept. of Med. Chem., [2]Psycosomatic Med., Kansai Med. Univ., Hirakata/Osaka, Japan

Abstract: Small cell lung cancer (SCLC) is a highly malignant form of cancer, which originates from primitive neuroendocrine cells in the lung. SCLC cells express several autocrine neurotransmitters and neuropeptides and their respective receptors. It was reported that the expression of these neuronal markers is regulated by RE1-silencing transcription factor (REST). In SCLC cells, an SCLC-specific isoform of REST (sREST) is highly expressed while full-length REST expression is too low to detect, suggesting that the expression of sREST correlates with the pathogenesis of SCLC. We previously reported that the neural-specific SR-related protein of 100 kDa (nSR100) abnormally activates the alternative splicing of REST (Mol. Cancer Res. 11, 1258 (2013)) in SCLC cells. There is recently increasing evidence that miRNA expression plays a fundamental role in gene regulation and may contribute substantially to cancer progression through translational repression. We analyzed the miRNA expression in SCLC cells using microarray analysis, suggesting that some of down-regulated miRNAs are predicted to interact with nSR100 mRNA. Analysis of these down-regulated miRNAs in patients' serum showed that the expression of a specific miRNA was significantly higher in SCLC patients than in other tumor patients. Furthermore we have found the miRNAs in SCLC patients' serum were selectively incorporated in the exosome. SCLC cells cultured on Matrigel in the condition which causes the up-regulation of nSR100 expression showed that the secretion of the miRNA in exosome was increased, while the intracellular miRNA significantly decreased. Taken together these results suggest that the miRNA targeting nSR100 might be a novel SCLC-specific biomarker.

Disclosures: **M. Shimojo:** None. **Y. Shudo:** None. **S. Ito:** None.

Poster

496. Proliferation: Molecular Mechanisms

Location: Halls A-C

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Program#/Poster#: 496.04/B13

Topic: A.02. Neurogenesis and Gliogenesis

Title: An axon enriched microRNA with a neurodevelopmental role within the rodent cortex

Authors: A. KOS, N. OLDE LOOHUIS, H. VAN BOKHOVEN, G. MARTENS, S. KOLK, *A. ASCHRAFI
Donders Inst. For Brain, Cognition, and Behaviour, Nijmegen, Netherlands

Abstract: MicroRNAs (miRs) are small non-coding RNAs which can regulate gene networks through post-transcriptional modulation of gene expression. An axon-enriched miR was previously identified as a regulator of axonal outgrowth and oligodendrocyte differentiation. However, the role of this miR within the central nervous system and more specifically the cortex remains largely unknown. In the current study we characterized the function of this miR in cortical development and dissected its downstream signaling pathways. To monitor the spatio-temporal expression of this miR we used qPCR analysis to examine the quantity of this gene within several brain regions. This revealed that this miR is dynamically expressed throughout development in a wide range of brain regions including the cortex. Following this, we established primary cortical cultures wherein we inhibited this miR using a viral based sponge. In-vitro inhibition of this miR throughout development resulted in a significant decrease in neuronal complexity. To complement these findings we employed in-utero electroporation (IUE) in order to manipulate this miR in the developing mouse brain. Brains in which this miR was either up or down regulated displayed several abnormalities potentially due to changes in the cell-cycle status of newborn neurons. A combination of whole transcriptome RNA-sequencing and gene network classification of the altered genes allowed us to pinpoint miR targets likely responsible for the observed phenotype. Our results indicate that this miR has an important role in early cortical development, and possibly is capable of affecting multiple stages of this process by regulation several key downstream mRNA targets.

Disclosures: **A. Kos:** None. **H. van Bokhoven:** None. **G. Martens:** None. **S. Kolk:** None. **A. Aschrafi:** None. **N. Olde Loohuis:** None.

Poster

496. Proliferation: Molecular Mechanisms

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 496.05/B14

Topic: A.02. Neurogenesis and Gliogenesis

Title: Using RNA interference to examine junctional communication within the adult neurogenic niche

Authors: *M. SORRELL[1], D. LINZ[2], Y. TOMOYASU[2], K. KILLIAN[2]
[1]Biol., Miami Univ., Latonia, KY; [2]Biol., Miami Univ., Oxford, OH

Abstract: In adult crickets, new neurons arise from two clusters of mitotic cells within the mushroom bodies. Within mammalian neurogenic niches, cell-cell interactions play a role in

regulating neurogenesis during both early development and adulthood. Within insect neurogenic niches, we propose that septate junctions mediate such cell-cell communication. We hypothesize that septate junctions maintain an active adult neurogenic niche and that disruption of this cell-cell communication will decrease the mitotic activity of progenitor cells, and lead to their terminal differentiation. To test this, we used RNA interference (RNAi) to experimentally knockdown expression of the septate junction protein Lachesin. We focused on this protein since it had been previously reported that mitotic cells of the cricket neurogenic niche label with anti-*lachesin* antibodies (Malaterre et al., 2002, *J. Comp. Neurol*. 452:215). Newly emerged (day one) adult male crickets were given a 2µl intra-abdominal injection of dsRNA. The optimal concentration for an effective knockdown, and the duration of knockdown, was determined by sacrificing control and experimental animals at different times post-injection. Effectiveness of the RNAi was validated using quantitative real-time PCR. Two days after injection of 2.5µg dsRNA against *lachesin* into adult male crickets (*n*=6), brain *lachesin* transcript levels were decreased by approximately 60% compared to control crickets injected with 2.5µg dsRNA against *dsRed2* (*n*=6), a gene not found in insects. This 60% decrease in *lachesin* expression was still evident 30 days later. Decreasing the dose of *lachesin* dsRNA to 1µg (*n*=6) or 0.5µg (*n*=6) resulted in a decrease in *lachesin* expression of 71% and 40%, respectively, 30 days post-injection. As RNAi was determined to produce a long-term decrease in *lachesin* expression, we examined the effects of this knockdown on adult neurogenesis. Again, on day one of adulthood, male crickets received an intra-abdominal injection of 2.5µg of *lachesin* dsRNA (*n*=6) or *dsRed2* dsRNA (*n*=5). Twenty-nine days later, all crickets were injected with 10µl of 40 mg/ml 5-bromo-2'-deoxyuridine (BrdU) in saline. After 2 hr, brains were dissected and processed for BrdU immunocytochemistry. Preliminary results support our hypothesis, since *lachesin* knockdown led to a significant, 15% reduction in the number of mitotic cells in the neurogenic niches (Student's T-test, *p*=0.03). We plan to examine the effect of *lachesin* RNAi on the structure of the niche and to determine its impact on behavioral plasticity. This study provides insight into the mechanisms by which stem cells in adult neurogenic niches remain mitotically active.

Disclosures: **M. Sorrell:** None. **D. Linz:** None. **Y. Tomoyasu:** None. **K. Killian:** None.

Poster

496. Proliferation: Molecular Mechanisms

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Program#/Poster#: 496.06/B15

Topic: A.02. Neurogenesis and Gliogenesis

Support: NIH Grant DA024681

Title: Function analysis of centrosomes in the developing neocortex

Authors: *W. SHAO[1], R. INSOLERA[2], H. BAZZI[3], K. ANDERSON[3], S. SHI[3,2,1]
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Abstract: Centrosomes are essential for the proper development of the neocrotex, the dysfunction of which underlies several neurodevelopmental disorders, including microcephaly and ciliopathy. As demonstrated in our previous study, asymmetric inheritance of mature versus immature centrosomes dictates the cell fate of each daughter cell during asymmetric cell division of neuronal progenitors. Once neurons are born, the centrosome also directs neuronal migration by its repositioning into the leading process before nuclear translocation. However, the mechanisms underlying centrosomal behaviors in neurogenesis and neuronal migration remain elusive. I hypothesize centrosomes may regulate different aspects of neurogenesis and neuronal migration through different centirole-related structures in the developing neocortex. To begin with, we compared the phenotypes upon loss of primary cilia and centrosomes with a *Nestin::Cre* line to drive conditional knockout in the developing neocortex. In the cilia mutant *Ift88*$^{-/-}$, the cortex is surprisingly normal based on immunohistochemical analysis of neurogenesis and neuronal lamination. In contrast, the centrosomal mutant *Sas4*$^{-/-}$ is microcephalic with extensive apoptosis by E15.5. Upon full rescue by simultaneous removal of *p53*, Majority of Pax6+ cells cells delaminated and were ectopically present in the intermediate zones of the double mutant *Sas4*$^{-/-}$;*p53*$^{-/-}$. The ectopic progenitors do not possess any long processes and divide with randomized cleavage planes, indicating that they are not outer subventricular zone progenitors (oRGs). Further analysis demonstrated these progenitors are as capable as their normal counterparts in ventricular zone (VZ) to give rise to a more or less normal cortex. Taken together, centrosomes are required to anchor the radial glial progenitors and ensure a proper cleavage orientation. However, we also revealed that neuronal progenitors are remarkably plastic, in which they do not depend on the VZ as their essential stem cell niche and retain normal proliferation capability in spite of changed morphology and randomized cleavage orientations. We will examine *Sas4*$^{-/-}$;*p53*$^{-/-}$ mutant in more details to fully understand the functions of centrosomes in the developing neocortex.

Disclosures: **W. Shao:** None. **R. Insolera:** None. **H. Bazzi:** None. **K. Anderson:** None. **S. Shi:** None.

Poster

496. Proliferation: Molecular Mechanisms

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 496.07/B16

Topic: A.02. Neurogenesis and Gliogenesis

Support: NIH Grant HD40182

Title: Differential roles of Nde1 and Ndel1 in rat cortical development

Authors: *D. DOOBIN, S. KEMAL, R. VALLEE
Pathology and Cell Biol., Columbia Univ., New York, NY

Abstract: Radial glial progenitor cells in the developing neocortex undergo cell cycle dependent interkinetic nuclear migration (INM). We have found apical nuclear migration to require cytoplasmic dynein, recruited to the nuclear envelope by sequential G2-specific mechanisms (Hu et al. Cell. 2013, 154:1300-13), and basal nuclear migration to involve kinesin-3 (Tsai et al. Nat. Neurosci. 2010, 13:1463-71). A number of dynein regulatory factors are involved in brain development, including LIS1, NudE, and NudEL. The latter two are closely related (55% identity; 70% similarity), but knockout mice exhibit distinct brain developmental phenotypes. Using *in utero* electroporation of Nde1 and Ndel1 shRNAs, followed by fixed and live cell imaging, we tested the effects of NudE and NudEL RNAi on neurogenesis and migration. NudE RNAi resulted in strong inhibition of INM, with severe defects specifically in apical nuclear migration, and a complete failure to enter mitosis. In contrast, NudEL RNAi had no effect on INM, and cell division at the ventricular surface persisted. Either NudE or NudEL RNAi, however, led to an accumulation of multipolar cells in the intermediate zone. Further migration of postmitotic neurons into the cortical plate was also diminished, an effect rescued by RNAi-insensitive NudE expression. These results identify a specific role for NudE in INM, and suggest common roles for both NudE and NudEL during the multipolar to bipolar transition and subsequent migration. Supp. by NIH HD40182.

Disclosures: D. Doobin: None. **S. Kemal:** None. **R. Vallee:** None.

Poster

496. Proliferation: Molecular Mechanisms

Location: Halls A-C

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Program#/Poster#: 496.08/B17

Topic: A.02. Neurogenesis and Gliogenesis

Support: NSC 102-2311-B-010-007-MY3

Title: The role of rab18 in cerebellar development

Authors: *L.-S. KAO[1,3], P.-C. WU[3], H.-F. WU[1], C.-J. HONG[2]
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Abstract: Rab GTPase proteins belong to the superfamily of Ras small G protein family. They coordinate multiple processes of membrane trafficking, including regulation of vesicle formation, transport, budding, tethering and fusion. However, little is known about the physiological roles of Rab18 *in vivo*. Mutations of Rab18 are identified in patients with Warburg Micro Syndrome characterized by progressive limb spasticity, optic atrophy, hypoplasia of corpus callosum and cerebellum, suggesting that Rab18 is involved in neurodevelopment. Rab18 is also reported to be a candidate susceptibility gene of schizophrenia. Indeed, there is evidence for cerebellar pathology in schizophrenia, including decreased numbers of Purkinje cells and reduction of cerebellar volume. In cultured cells, Rab18 is shown to cycle between cytosol and secretory granules and interact with secretory granules to repress exocytosis upon activation of the regulated secretory pathway, suggesting a role of Rab18 as a brake for secretion. Therefore, it is possible that Rab18 may control some secretory factors crucial for neurodevelopment. In this study, the Rab18-mutant mice exhibited severe hind limbs ataxia and reduced cerebellar size by approximate 20%. We found that the foliation pattern of cerebellum was alterebd and the number of Purkinje cells was decreased in Rab18-mutant mice at P28. However, no significant differences were found in the thickness of granule cell layer and molecular layer as well as the Purkinje cell density. Furthermore, the proliferation of granule cells in the external granule cell layer of Rab18-mutant cerebella revealed no alterations. We will next focus on the deficits of cerebellum in Rab18-mutant mice and further elucidate functions and molecular mechanisms of Rab18. The study of the pathophysiological role of Rab18 during neurodevelopment will help to solve the mysteries of cerebellar malformations in human diseases.

Disclosures: L. Kao: None. **P. Wu:** None. **H. Wu:** None. **C. Hong:** None.

Poster

496. Proliferation: Molecular Mechanisms

Location: Halls A-C

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Program#/Poster#: 496.09/B18

Topic: A.02. Neurogenesis and Gliogenesis

Support: VA Merit Award to AKS (Dept of Veterans Affairs

Emerging Technology Funds (State of Texas)

Title: Promise of resveratrol for relieving cognitive and mood dysfunction, hippocampus oxidative stress and inflammation in gulf war illness

Authors: *B. HATTIANGADY[1,2,3], B. SHUAI[1,2,3], G. A. SHETTY[1,2,3], V. MISHRA[1,2,3], M. KODALI[1,2,3], X. RAO[1,2,3], A. K. SHETTY[1,2,3]
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Abstract: Gulf war illness (GWI), a chronic multi-symptom heath problem, affects over a third of 700,000 veterans served in the Persian Gulf War-1. Memory problems and depression are the major symptoms related to brain. While the precise etiology is unknown, epidemiological studies imply that exposures to anti nerve gas agent pyridostigmine bromide (PB), pesticides such as DEET and permethrin, and stress during the war underlie GWI in a significant fraction of veterans. This supposition has been supported by studies in animal models. Our studies in a rat model have shown that exposures to these GWI-related chemicals (GWIR-Cs) and mild stress (5 min restraint stress) for 4 weeks is sufficient to cause memory and mood dysfunction associated with decreased hippocampus neurogenesis and increased oxidative stress and inflammation. Here, we investigated the efficacy of treating rats with resveratrol (RESV; a potent antioxidant and anti-inflammatory compound), a month after their exposure to GWIR-Cs and stress for improving memory, mood and dentate neurogenesis, and easing oxidative stress and inflammation. Adult male SD rats were exposed daily to GWIR-Cs DEET (40 mg/kg), permethrin (0.13 mg/kg) and PB (1.3 mg/kg) and 5-minutes of restraint stress for 4 weeks. A month later, a group of these rats received daily RESV (40 mg/kg, SQ) for four weeks while another group received vehicle (VEH). Both groups also received 5'-bromodeoxyuridine (BrdU) during the last 7 days of treatment for analyses of neurogenesis. Cognitive tests done two months after treatment showed improved memory function in RESV treated GWI-rats, in contrast to VEH treated GWI-rats. This comprised improved spatial learning and memory retrieval ability in a water maze test, proficiency for location and recognition memories in object location and novel object recognition tests. Moreover, RESV treated GWI-rats displayed better mood function, which was indicated through reduced latencies to eat food in novelty suppressed feeding test and reduced immobility times in forced swim test. Furthermore, in comparison to VEH-treated GWI-rats, the hippocampus of RESV treated GWI-rats presented greater level of neurogenesis when

quantified through BrdU, BrdU-NeuN and doublecortin assays, reduced expression of genes encoding oxidative stress response, reactive oxygen species metabolism and oxygen transporters, increased expression of antioxidant genes, and reduced numbers of ED-1+ activated microglia. Thus, RESV treatment has promise for reversing brain dysfunction in GWI. It appears that improved neurogenesis, decreased oxidative stress and inflammation mediated by RESV underlie the beneficial effects.

Disclosures: B. Hattiangady: None. **B. Shuai:** None. **G.A. Shetty:** None. **V. Mishra:** None. **M. Kodali:** None. **X. Rao:** None. **A.K. Shetty:** None.

Poster

496. Proliferation: Molecular Mechanisms

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 496.10/B19

Topic: A.02. Neurogenesis and Gliogenesis

Support: North Carolina Biotechnology Center

Title: Choline deficiency decreases proliferation of embryonic neural progenitor cells through inhibiting epidermal growth factor pathway

Authors: *Y. WANG, N. SURZENKO, M. MEHEDINT, K. CORBIN, S. ORENA, W. FRIDAY, S. ZEISEL
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Abstract: Choline deficiency can alter the development of fetal brain. Epidermal growth factor (EGF) is pivotal for neural progenitor cell (NPC) proliferation in the embryo. To address the possibility that EGF signaling pathway plays a functional role in the effect of choline deficiency, we cultured NPCs derived from E14 mouse hippocampus and cortex, then treated them with low choline (5 μM) or medium choline (70 μM). Low choline significantly suppressed the phosphorylation of EGF receptor (EGFR) and activation of downstream effectors ERK1/2 and Akt after stimulation of NPCs with EGF. Moreover, low choline decreased the EGFR protein expression and half-life in NPCs. There were no changes observed on the EGFR mRNA expression and degradation of EGFR protein. However, low choline remarkably inhibited the EGFR nascent protein synthesis. Pregnant Nestin-CFP transgenic mice were fed either a choline-deficient (CD) or control (CT) diet from days 11 to 17 of pregnancy and then E17 fetal brains were studied. In CD fetal cortex and hippocampus, expression of EGFR expression was

decreased. Similar decreased expression was observed in cell sorted CFP-positive cells using fluorescence-activated cell sorting (FACS). Our findings reveal that choline deficiency influences NPC proliferation by suppressing EGFR signaling pathway, which might have long-term effects in the brain.

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Poster

496. Proliferation: Molecular Mechanisms

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Topic: A.02. Neurogenesis and Gliogenesis

Support: DGAPA-UNAM Grant IN209212

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CONACYT Grant 155290

CONACYT Grant 154542

Title: miR-7 promotes cell proliferation and migration by targeting KLF4

Authors: K. F. MEZA-SOSA[1], E. I. PÉREZ-GARCÍA[1], N. CAMACHO-CONCHA[1], O. LÓPEZ-GUTIÉRREZ[1], G. PEDRAZA-ALVA[1], *L. PEREZ-MARTINEZ[2]
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Abstract: Krüppel like factor 4 (KLF4) is a very versatile transcription factor (TF) that can act as an oncogene or as a tumor suppressor due to its ability of regulating the expression of several cell cycle regulators. The role of KLF4 during the central nervous system (CNS) development has been recently uncovered indicating that KLF4 protein levels decrease as the mouse brain develops and accordingly, KLF4 overexpression during early embryonic days affects the differentiation of ependymal cells causing hydrocephalus. Moreover, neural precursor cells (NPCs) derived from transgenic mice that overexpress KLF4 in the CNS, present a reduced proliferative ability and a preferential acquisition of a gliogenic fate over a neurogenic one. In

addition, neurons derived from KLF4-overexpressing NPCs fail to migrate and to differentiate adequately. These data suggest that KLF4 expression must be tightly regulated during the early CNS development. In this sense, microRNAs (miRNAs) are small RNAs (19-21 nt) that bind to the 3' untranslated region (3' UTR) of their target mRNAs and negatively regulate their expression at the post-transcriptional level. Additionally, miRNAs regulatory potential is crucial for differentiation of distinct cell types including neurons. Based on the dynamic KLF4 expression profile during the CNS development and on the KLF4 ability to control the cell cycle, we were interested on determining whether KLF4 expression is regulated by miRNAs during the early stages of neurogenesis. Bioinformatic, functional and molecular analyses revealed that KLF4 is a direct target of miR-7 and that regulation of KLF4 by miR-7 has functional implications in different cellular processes including cell proliferation and cell migration. In part, this result from misregulation of KLF4 target genes involved in cell cycle control such as p21 and Cyclin D. Taken together, these findings indicate that miR-7 can control cell cycle by directly regulating KLF4 expression and indirectly some of its target genes.

Disclosures: K.F. Meza-Sosa: None. **L. Perez-Martinez:** None. **E.I. Pérez-García:** None. **N. Camacho-Concha:** None. **O. López-Gutiérrez:** None. **G. Pedraza-Alva:** None.

Poster

496. Proliferation: Molecular Mechanisms

Location: Halls A-C

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Topic: A.02. Neurogenesis and Gliogenesis

Support: NEI RO1 EY022030-02

Title: Hedgehog signaling is required for and amplifies FGF2-induced formation of Muller Glia derived Progenitor cells in the avian retina

Authors: *L. J. TODD, A. J. FISCHER
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Abstract: The capacity of retinal regeneration varies substantially across different vertebrate species, but consistently involves Müller glia as the cellular source for progenitors. Although retinal regeneration is robust in fish, the regeneration in birds and mammals is limited. After retinal injury, Müller glia in the avian retina can undergo de-differentiation, proliferation, some progeny differentiation as neurons, while most cells remain as undifferentiated progenitor-like

cells. The signaling pathways that influence the formation of Müller glia-derived progenitor cells (MGPCs) are slowly being revealed, whereas the coordination and cross-talk between these pathways remains largely unresolved. The purpose of this study was to investigate whether Hedgehog-signaling is a key node in the signaling network of MGPC formation. In NMDA-damaged retinas, we found that Hedgehog-signaling is dynamically up-regulated in Müller glia/MGPCs, and inhibition of Hedgehog-signaling decreases numbers of MGPCs. By comparison, activation of Hedgehog-signaling increases the formation of MGPCS in moderately damaged retinas, but is not sufficient to induce the formation of MGPCs in the absence of injury. Fibroblast growth factor 2 (FGF2) alone induces the formation of MGPCs in undamaged retina. We found that Hedgehog-signaling is necessary for FGF2-induced MGPCs as inhibition of Hedgehog-signaling with KAAD-cyclopamine, a SMO inhibitor, inhibits progenitor cell formation. Data is provided suggesting that KAAD-cyclopamine attenuates MGPC formation by preventing the accumulation of MAPK effectors in Müller glia. Additionally, co-application of a smoothened-agonist with FGF2 leads to an increase of MGPC formation. We conclude that Hedgehog-signaling stimulates the formation of proliferating MGPCs in acutely damaged retinas. Further, in undamaged retinas, our data indicate that FGF2/MAPK-signaling recruits Hedgehog-signaling to enable the de-differentiation and proliferation of MGPCs.

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

Poster

496. Proliferation: Molecular Mechanisms

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 496.13/B22

Topic: A.02. Neurogenesis and Gliogenesis

Title: Temporal dynamics of human cerebrospinal fluid vesicles

Authors: *D. M. FELICIANO, A. TIETJE, K. L. MARON, Y. WEI
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Abstract: Elaboration of the cerebral cortex requires exquisite orchestration of cellular events through coordinated exchange of information between distally located cells. One mechanism by which intercellular communication is achieved is through the transfer of exosomes. Exosomes are vesicles that carry lipids, nucleic acids, and proteins and are detectable in most biological fluids including cerebrospinal fluid (CSF). Here we report that CSF vesicle concentrations, including that of exosomes, and vesicle content undergo age dependent fluctuations. CSF

vesicles encapsulated miRNAs that contain a conserved recognition sequence. We found that proteins important for miRNA recognition are found in vesicles produced by human choroid plexus epithelial cells and that they decreased with age in CSF. To determine the significance of declines in CSF exosomes in development we performed bioinformatic analysis of CSF vesicle miRNAs and *in vivo* inhibition of exosome release which support the hypothesis that exosomes mediate a developmental switch in signaling pathways. These results provide insight into exosomal exchange of miRNAs within the brain and a framework to understand how changes in exosomes may have an important impact on brain development.

Disclosures: D.M. Feliciano: None. **A. Tietje:** None. **K.L. Maron:** None. **Y. Wei:** None.

Poster

496. Proliferation: Molecular Mechanisms

Location: Halls A-C

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Program#/Poster#: 496.14/B23

Topic: A.02. Neurogenesis and Gliogenesis

Support: CIMO Foundation

HBGP

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Academy of Finland

Title: Laser-induced ATP dependent cortical calcium waves in E13-E15 mouse embryos ex utero

Authors: *M. YURYEV[1], C. PELLEGRINO[2], V. JOKINEN[3], S. KHIRUG[1], S. FRANSSILA[3], L. KHIROUG[1], C. RIVERA[1,2]
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Abstract: Calcium activity has been shown to have an effect on the cell cycle during brain development in *in vitro* and *ex vivo* conditions. The calcium activity in acute slices obtained from the embryonic brain has been shown to occur in different patterns such as singular events, synchronized coupled activity in neighboring cells as well as calcium waves. Calcium waves

have been found in the ventricular zone and shown mostly to be comprised in radial glia cells population, a transient cell type existing mainly in the period of active neurogenesis. In *ex vivo* conditions, it has been shown that calcium waves occur spontaneously and propagate by extracellular ATP rise and involve IP3-mediated intracellular calcium release. The disruption of the calcium waves during the peak of embryonic neurogenesis decreases cell proliferation *in vivo*. However, the properties of the calcium waves *in vivo* are poorly studied so far. Here we present the method for the calcium imaging in live mouse embryos connected to the mother at the age E13-E15. Prior to the imaging the embryos were injected with the acetoxymethyl ester form of different calcium dyes intraventricularly *in utero*. The embryos were positioned in a custom made chamber for stable fixation of the embryos that allows keeping the embryos connected to the anesthetized mother through the umbilical cord where two-photon imaging of the embryonic brains was performed. The physiological conditions of the embryos have been monitored by the blood flow level. Spontaneous singular calcium activity events were detected. To study the properties of the calcium wave propagation we stimulated a small group of cells (3-5) by means of high power laser emission from the same infrared laser as used for imaging. In the control conditions the stimulation of the cells caused calcium waves propagated through 340±240 cells/mm^2 (n=20) in the field of view. The strong stimulation of the cells always caused the calcium increase in the neighboring groups of cells. In case of injection of non-selective ATP receptors blocker suramin together with the calcium dye the calcium level increases were detected either confined in the zone of laser emission (3-5 cells) or recruiting significantly less number of cells (56±51 cells/mm^2; n=16) than under control conditions. On the contrary, the number of cells involved was increased after injection of caffeine (1190±370 cells/mm^2; n=15). Altogether, *in vivo* data demonstrates the possibility of calcium wave induction and propagation in the embryonic brain together with different pharmacological manipulations using the aforementioned method.

Disclosures: **M. Yuryev:** None. **C. Pellegrino:** None. **V. Jokinen:** None. **S. Khirug:** None. **S. Franssila:** None. **L. Khiroug:** None. **C. Rivera:** None.

Poster

496. Proliferation: Molecular Mechanisms

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Topic: A.02. Neurogenesis and Gliogenesis

Support: NIH R01DA024681

NIH R21NS072483

Title: Functional interaction between neural progenitors and the vasculature regulates neocortical interneuron production

Authors: *X. TAN, W. SHI, Z. LI, S. SHI
Mem. Sloan Kettering Inst., New York, NY

Abstract: Neurons in the neocortex can be classified into two primary groups, excitatory neurons and inhibitory interneurons, which are precisely and intricately interconnected to form functional circuits for various behavioral controls. Excitatory neurons in the neocortex are produced by radial glial cells (RGCs) in the ventricular zone of the dorsal telencephalon and they migrate radially along the radial glial processes into the cortical plate. In contrast, most if not all inhibitory interneurons are generated in the ganglionic eminence (GE) of the ventral telencephalon and they migrate tangentially over a long distance to reach the neocortex. Although extensive studies over the past decade have provided a comprehensive view on the production and positioning of excitatory neurons, our understanding of interneuron neurogenesis remains limited. Recently, we have identified the progenitor cells in the medial ganglionic eminence (MGE) responsible for producing more than 60% of neocortical interneurons are RGCs in nature, which typically exhibit a bipolar morphology with a ventricular endfoot and a long basal process. Using retroviral labeling, we found that most RGCs in the MGE possessed a highly diverse basal endfoot structure that was closely associated with nearby vasculature throughout the embryonic development. Further time-lapse imaging experiments indicated that the interaction between RGCs and vasculature was actively maintained and this interaction may be critical for the proliferation and maintenance of the interneuron radial glial progenitors. Amongst the large group of integrin receptors critical for cell-cell/ECM adhesion, integrin beta1 (Itgb1) has been shown to contribute to regulating the proliferation, maintenance and survival of neural stem cells. Our immunostaining results showed that Itgb1 was highly expressed in the RGCs in the MGE. Conditional knockout of Itgb1 specifically in the MGE led to a significant increase of RGCs that failed to interact with nearby vasculature. Furthermore, neocortical interneuron cell number was significantly decreased in Itgb1conditional knockout mice at P21, indicating the interaction between RGCs and vasculature mediated by integrins is important for proper interneuron production. We are continuing to analyze these defects in interneuron production and link these defects to the proliferation and maintenance of progenitors in the MGE.

Disclosures: X. Tan: None. **S. Shi:** None. **W. Shi:** None. **Z. Li:** None.

Poster

496. Proliferation: Molecular Mechanisms

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Topic: A.02. Neurogenesis and Gliogenesis

Support: NIH R01NS045702

P01NS062686

IDDRC P30HD40677

Title: Sirt1 positively regulates the regenerative response of glial progenitors to neonatal brain injury

Authors: *B. JABLONSKA[1], M. GIERDALSKI[2], A. LIACHAUCO[1], J. CABRERA-LUQUE[1], T. HAWLEY[3], V. GALLO[1]
[1]Ctr. for Neurosci. Res., [2]Div. of Cardiac Surgery, Children's Natl. Med. Ctr., WASHINGTON, DC; [3]Flow Cytometry Core Facility, George Washington Univ., WASHINGTON, DC

Abstract: Regenerative processes in brain pathologies require the production of distinct neural cell populations from endogenous progenitor cells. To study how epigenetic factors regulate a regenerative response of parenchymal progenitors in white matter after hypoxia, we have used a mouse model of neonatal hypoxia that reproduces all the landmarks of brain injury observed in premature infants, including DWMI. In this model, we have previously demonstrated that - one week after hypoxia - the regenerative and proliferative response of oligodendrocyte progenitor cells (OPCs) was regulated by the Cdk2-p27Kip1 pathway. Since functional activity of cell cycle proteins is dependent on phosphorylation and acetylation status, we studied the role of Sirt1 (NAD dependent deacetylase) in the regulation of the Cdk2 signaling pathway in OPCs after hypoxia. Immunocytochemical studies demonstrated that hypoxia upregulated total number of Sirt1+ and NG2+Sirt1+, cells, as well as their proliferative potential. A higher expression of Sirt1 mRNA transcript was observed in FACS purified NG2+ cells after hypoxia. Furthermore, knockdown of Sirt1 protein or blockage of Sirt1 activity with sirtinol (Sirt1 inhibitor) caused a significant decrease in OPC proliferation after hypoxia. Finally, inactivation of Sirt1 also promoted OPC differentiation to oligodendrocytes. Molecular studies revealed that hypoxia enhanced Sirt1 levels and Sirt1/Cdk2 complex formation. Sirt1 deacetylated retinoblastoma (Rb) in the Cdk2/Rb/E2F1 complex, leading to dissociation of E2F1 and enhanced OPC proliferation. Blockage of Sirt1 activity in cultured cells enhanced expression of acetyl-lysine for Rb after hypoxia, indicating its lower acetylation status. Our results indicate that Sirt1 is an essential regulator of OPC proliferation and oligodendrocyte regeneration after neonatal brain injury. Therefore, enhancing Sirt1 activity may promote oligodendrocyte recovery after DWMI.

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Poster

496. Proliferation: Molecular Mechanisms

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Topic: A.02. Neurogenesis and Gliogenesis

Support: VA Merit Award to AKS (Dept of Veterans Affairs)

Emerging Technology Funds (State of Texas)

Title: Long-standing effects of exposure to gulf war illness related chemicals and mild stress on the expression of genes encoding oxidative stress and inflammation in the hippocampus

Authors: ***G. SHETTY**[1,2,3], B. HATTIANGADY[1,2,3], B. SHUAI[1,2,3], A. K. SHETTY[1,2,3]
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Abstract: Impairments in cognition and mood are the major CNS related symptoms seen in Gulf war Illness (GWI), a chronic multi-symptom illness afflicting nearly 250,000 veterans who served in the 1991 Gulf War. Epidemiological studies have suggested that synergistic interaction of pyridostigmine bromide (PB, given to troops to shield against nerve gas agents) and pesticides such as DEET and permethrin (used copiously to protect against pest-borne diseases) with or without stress underlie GWI in a significant fraction of GW veterans. Our studies in a rat model have shown that exposures to these GWI-related chemicals (GWIR-Cs) and mild stress (5 min restraint stress) for 4 weeks cause hippocampus-dependent spatial and location memory impairments as well as a greatly declined neurogenesis. To understand mechanisms, we examined whether exposure to GWIR-Cs and mild stress causes enduring alterations in the expression of genes encoding oxidative stress and inflammation in the hippocampus. A group of adult male SD rats were exposed daily to DEET (40 mg/kg), permethrin (0.13 mg/kg) and PB (1.3 mg/kg), and 5-minutes of restraint stress for 4 weeks. Another group received VEH and handling. Six months later, hippocampi were analyzed for the expression of 84 genes relevant for oxidative stress via qRT-PCR array. Expression of multiple genes related to inflammation was also analyzed. Hippocampi from rats exposed to GWIR-Cs and stress displayed upregulation of

multiple genes related to oxidative stress response (Gpx1-3, Gpx5-6. Gclm, Txnip, Park7, Als2, Gsr, Gclc, Prdx1-2, Ercc6, Ctsb, Dhcr24, Txnrd2, Nudt1, Sqstm1, Cat, Hmox1, Nqo1, Txnrd1, Psmb5, Gpx4, Gpx7, Prdx6, Prnp, Sepp1, Apoe, Idh1, Tpo, Ercc2, Duox2, Mpo, Ucp3), ROS metabolism (Cyba, Noxo1, FMo2, Scd1, Ccs, Sod2-3, Ucp2), and oxygen transport (Mb, Cygb, Dnm2, Ift172, Slc38a1, Fancc, Ngb). Presence of increased oxidative stress was also revealed through upregulation of multiple antioxidant genes (Ptgs1, Prdx1-6, Gstk1, Gpx1-7, Sod3, Ehd2, Gstp1, Cat, Txnrd1, Srxn1, Gsr, Apc, Ctsb, Txnrd2, Ptgs1, Lpo). Furthermore, rats exposed to GWIR-Cs and stress showed up-regulation of several genes that promote inflammation (NF-κB, IL-1α, IL6, Csf2, Bcl6). Additionally, a gene encoding blood-brain-barrier leakage (Vegfa) was immensely upregulated and a gene having a role in maintaining Redox balance (Fox03) was severely downregulated. Genes encoding anti-inflammatory proteins (IL4 and IL10) were unaltered however. Thus, oxidative stress and inflammation likely underlie the persistent cognitive dysfunction observed in GWI. From this viewpoint, antioxidant and anti-inflammatory drug therapies seem ideal for treating GWI.

Disclosures: G. Shetty: None. **B. Hattiangady:** None. **B. Shuai:** None. **A.K. Shetty:** None.

Poster

496. Proliferation: Molecular Mechanisms

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 496.18/B27

Topic: A.02. Neurogenesis and Gliogenesis

Support: Swedish Medical Research Council

Västra Götalands LUA/ALF funding

Barncancerfonden

NBCNS

Title: Altered cellular responses in the juvenile and adult subventricular zone induced by epidermal growth factor

Authors: *G. KUHN, O. R. LINDBERG, A. BREDERLAU
Univ. of Gothenburg, Gothenburg, Sweden

Abstract: The ErbB receptor family mediates effects in numerous tissues and cellular contexts. Signaling through the ErbB receptors is often altered in cancers. Increased ErbB signaling, mainly ErbB1 (EGFR) and ErbB2, can lead to increased proliferation and invasiveness of tumor cells. Intracerebroventricular infusion of epidermal growth factor (EGF) induces hyperproliferative polyp-like dysplasia in the subventricular zone (SVZ) of the adult rodent brain. EGF induces a differentiation shift in the subventricular zone towards a highly proliferative glial cell type, while the generation of neuronal progenitor cell is strongly diminished. Interestingly, blood vessels develop in about 30% of the polyps after continuous EGF infusion for 14 days. Structurally, the newly formed vessels are of a disorganized and glomeruloid appearance, sometimes as bundles sprouting from a single SVZ blood vessel, and other times as a highly vascularized ventricle wall. These vessels are to a large extent covered by pericytes strongly expressing NG2. The angiogenic area shows multi-luminal vessels and signs of immaturity based on ultrastructural characteristics, such as a thickened endothelial cell layer. As polyp growths progresses, microglia/macrophages accumulate in the polyp core concurrent with increasing cell death. Both microglia/macrophage accumulation and cell death peak during angiogenesis and decline after polyp vascularization. EGF infusion into the adult rodent brain causes substantially reduced neuroblast production; however, after EGF infusion into the juvenile brain (starting at postnatal day P21) the number of neuroblasts (DCX) and glial progenitor cells (Sox2, Olig2) in the SVZ was not altered. In the juvenile brain the number of DCX was reduced, and the number of Sox-2 and Olig2 cells was increased only if animals had received whole brain irradiation at P9 (8Gy) prior to EGF treatment. Hyperproliferative polyps were formed after EGF infusion at both P21 and P80 in both naive and irradiated animals. This indicates that stem and progenitor cells capable of extensive proliferation are present even in the irradiated SVZ.

Disclosures: **G. Kuhn:** None. **A. Brederlau:** None. **O.R. Lindberg:** None.

Poster

496. Proliferation: Molecular Mechanisms

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 496.19/B28

Topic: A.02. Neurogenesis and Gliogenesis

Support: NIH Grant EY011261

NIH Grant 1K99ES022992-01

Title: Thyroid hormone acts locally to increases the rate of neurogenesis and dendritic arborization in the tadpole visual system

Authors: *C. K. THOMPSON, H. T. CLINE
The Scripps Res. Inst., La Jolla, CA

Abstract: Thyroid hormone is a critical regulator of brain development. For instance, low levels of maternal thyroid hormone during gestation leads to cretinism in humans. Nevertheless, the specific effects of direct action of thyroid hormone on different regions of the brain are not well understood. To address this issue we have developed a method for direct application of thyroid hormone to distinct parts of the Xenopus laevis tadpole visual system. Crystalline triiodothyronine (T3) was diluted into melted coconut oil, which was then injected via micropipette and picospritzer into either the 3rd ventricle near the optic tectum or into the eye. These animals were treated with CldU, a thymdine analogue, in order to label newly-divided cells and then sacrificed 2 days later. We found that T3 placed in the brain increased the rate of proliferation nearly tenfold relative to animals that received T3 in the eye. In contrast, T3 in the eye increased the rate of proliferation in the ciliary region by fivefold, whereas T3 in the brain had no effect on retinal proliferation. In another experiment, we electroporated the tectum with morpholinos against type 3 iodothyronine deiodinase (DIO3), which converts T3 to reverse T3, an inactive form of thyroid hormone. We found that DIO3-knockdown increased the rate of proliferation in the tectum, likely by increasing the local concentration of active T3. Combining DIO3 morpholino treatment with systemic exposure to methimizole, which blocks thyroid synthesis in the thyroid gland, prevented the increased proliferation seen with DIO3 morpholino alone. These experiments suggest that T3 acts locally in the tectal progenitor cells to regulate their proliferation. Last, we sought to examine the effects of local T3 on arborization of tectal neuron dendrites. We electroporated tectal neurons with GFP and after 7 days, when the neurons had already acquired a complex dendritic arbor, we injected T3 coconut oil into the 3rd ventricle. We imaged the GFP-expressing neurons with a 2-photon microscope the day before T3 delivery and each day afterward for 3 days. We found that T3 treatment significantly increased dendrite arbor length and branch tip number by 66% and 73%, respectively. These experiments demonstrate that thyroid hormone can act directly on specific neural circuits and have a substantial impact on brain development.

Disclosures: C.K. Thompson: None. **H.T. Cline:** None.

Poster

496. Proliferation: Molecular Mechanisms

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 496.20/B29

Topic: A.01. Brain Patterning

Support: Medical Research Council

Title: Fate choice in the cranial neural crest

Authors: *R. CARR, A. GRAHAM
MRC Ctr. for Developmental Neurobio., King's Col. London, London, United Kingdom

Abstract: Neural crest cells (NCCs) are a transient embryonic population which diversify into many cell types, including peripheral neurons, pigment cells and the majority of the craniofacial skeleton. The mechanisms by which these fate choices are regulated remain elusive. Cranial NCCs enter the pharyngeal arches and switch to an ectomesenchymal fate, upregulating Dlx2. Those cranial NCCs that do not enter the arches maintain the expression of early crest markers, and will form non-ectomesenchymal derivatives, including those that contribute to the peripheral nervous system. Previous work has shown that this switch to an ectomesenchymal fate at least in part involves FGF signalling. However, NCCs are also exposed to other signalling pathways, including SHH, BMP and WNT, and it is unclear how they respond to these. We find that the presence of Wnt in NCC-explant cultures has clear effects causing huge depletion of NCC numbers; conversely, FGF allows survival and growth of NCC-explants in culture. We also show *in vivo* that although Shh is expressed in the pharyngeal arch epithelia, NCCs do not appear to respond to this signal. We have also shown that NCCs respond to BMP signalling *in vivo*, though a role of BMP in NCC fate switch has yet to be elucidated.

Disclosures: R. Carr: None. **A. Graham:** None.

Poster

497. Neuron–Glia Interactions During Development

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 497.01/B30

Topic: B.01. Neurotransmitters and Signaling Molecules

Title: The role of serotonin in running-induced neuronal activity

Authors: *C. HAINER[1], F. KLEMPIN[1], A. MACMAHON[2], M. BADER[1], N. ALENINA[1]
[1]MDC Berlin, Berlin, Germany; [2]Columbia Univ., New York, NY

Abstract: Serotonin (5-hydroxytryptamine) is a small biogenic amine with functions in the peripheral as well as the central nervous system (CNS). As neurotransmitter, serotonin was recently shown to be necessary for the running-induced increase in adult neurogenesis, the generation of new neurons in the dentate gyrus (DG). The mechanisms coupling physical activity with neuron formation via serotonin are not yet fully understood. This project aims at investigating brain areas that are affected by serotonin signaling during exercise. Mice lacking the enzyme TPH2 with central serotonin deficiency (TPH2 -/- mice) have been generated in the Bader lab and serve as an effective tool for this study. Analysis was done with TPH2 -/- mice in comparison to serotonin competent (TPH2 +/+) animals. Methods used in my study comprise 20 min sessions of forced running on a rodent treadmill. Neuronal firing was detected by labeling expression of the immediate early gene c-Fos on histological brain slices. Surprisingly, no changes in c-Fos expression have been observed in serotonergic neurons of the brain stem raphe nuclei. However, quantification of c-Fos expression in the DG revealed a significant increase in activated neurons in TPH2 +/+ as well as TPH2 -/- animals after running. These data show a condition rather than a genotype effect. Thus, the observed result seems to be serotonin independent and other neurotransmitters may mediate running-induced cell activation in the DG. Further analysis will be done for other brain regions. Studying intracellular signaling mechanisms is of great clinical importance for the development of novel therapeutic approaches.

Disclosures: C. Hainer: None. **F. Klempin:** None. **A. MacMahon:** None. **M. Bader:** None. **N. Alenina:** None.

Poster

497. Neuron–Glia Interactions During Development

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 497.02/B31

Topic: B.11. Glial Mechanisms

Support: MH099554 (to YY)

Title: VGluT1+ neuronal glutamatergic signaling regulates postnatal developmental maturation of cortical protoplasmic astroglia

Authors: *M. TOLMAN, L. MOREL, H. HIGASHIMORI, Y. YANG
Tufts Univ., Boston, MA

Abstract: Functional maturation of astroglia is characterized by the development of a unique, ramified morphology and the induction of several important functional proteins, such as glutamate transporter GLT1. Although pathways regulating the early fate specification of astroglia have been characterized, mechanisms regulating postnatal maturation of astroglia remain essentially unknown. Here we employed a new *in vivo* approach to illustrate and quantitatively analyze developmental arborization of astroglial processes in multiple brain regions. Our analysis found a particularly high increase in the number of VGluT1+ neuronal glutamatergic synapses that are ensheathed by processes from individual developing astroglia from P14 to P26 when astroglia undergo dramatic postnatal maturation. Subsequent silencing of VGluT1+ synaptic activity in VGluT1 knockout (KO) mice significantly reduces astroglial domain growth and the induction of GLT1 in the cortex, but has no effect on astroglia in the hypothalamus where non-VGluT1+ synaptic signaling predominates. Electron microscopy (EM) was used to analyze the localization of GLT1 relative to the synapse showed that significantly fewer perisynaptic astrocytic contacts in the cortex of VGluT1 KO mice. To further determine whether synaptically released glutamate mediates VGluT1+ synaptic signaling, we pharmacologically inhibited and genetically ablated metabotropic glutamate receptors (mGluRs, especially mGluR5) in cortical astroglia and found that the developmental arborization of astroglial processes and expression of functional proteins such as GLT1 is significantly decreased. In summary, our genetic analysis provides new *in vivo* evidence that VGluT1+ glutamatergic signaling, mediated by the astroglial mGluR5 receptor, regulates the functional maturation of cortical astroglia during development. These results elucidate a new mechanism for regulating the developmental formation of functional neuron-glia synaptic units.

Disclosures: **M. Tolman:** None. **L. Morel:** None. **H. Higashimori:** None. **Y. Yang:** None.

Poster

497. Neuron–Glia Interactions During Development

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 497.03/B32

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: NIH Intramural ZIA ES102805 04

Title: New mouse strains for manipulating genetically defined subpopulations of central norepinephrine neurons

Authors: *N. PLUMMER[1], J. DE MARCHENA[1], D. D'AGOSTIN[1], S. D. ROBERTSON[1], C. J. TUCKER[2], P. JENSEN[1]
[1]Lab. of Neurobio., [2]Lab. of Signal Transduction, NIH/NIEHS, Research Triangle Park, NC

Abstract: Central norepinephrine (NE) neurons comprise a small yet diverse population of cells that vary in their anatomical location, connectivity, and response to disease and environmental insult. NE neurons project to virtually all areas of the central nervous system, and they play an essential role in a wide range of behavioral and physiological processes. Recently, we identified four NE neuron subpopulations derived from progenitors located in different rhombomeres of the embryonic hindbrain (Nat Neurosci 16:1016-23). Because they are defined genetically, these NE subpopulations are accessible for experimental manipulation in mice via a genetic intersectional strategy. Here we describe a suite of new mouse strains that will permit these experiments and can also be adapted to studies of other neuronal populations. A triple-recombinase responsive fluorescent indicator allele uses the Dre/rox, Flp/FRT, and cre/loxP recombinase systems to label neuron cell bodies and axons at the intersection of three distinct gene expression domains. This allele can therefore reveal additional diversity within the rhombomere-derived subpopulations, including the subpopulation derived from rhombomere 1 (r1) which encompasses the functionally important locus coeruleus (LoC). A similar strategy was used to generate a triple-recombinase responsive effector allele expressing the hM3Dq DREADD receptor, which will permit us to directly link structural and behavioral consequences of altered NE signaling during development to a specific subpopulation of NE neurons. Furthermore, two new recombinase driver alleles, a Dre recombinase knock-in allele of Engrailed 1 and a Dre-dependent cre knock-in of the dopamine β-hydroxylase gene, will allow any cre-responsive indicator, effector, or conditional knockout allele to be expressed in the r1-derived NE subpopulation, thus granting unprecedented ability to control locus coeruleus development and function.

Disclosures: N. Plummer: None. **J. de Marchena:** None. **D. D'Agostin:** None. **S.D. Robertson:** None. **C.J. Tucker:** None. **P. Jensen:** None.

Poster

497. Neuron–Glia Interactions During Development

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 497.04/B33

Topic: A.02. Neurogenesis and Gliogenesis

Support: NMSS RG4706A4/2

NIH R01 NS056427

NMSS RG3954A1/2 (LJC)

Title: Sox17 promotes hedgehog-mediated oligodendroglial lineage survival in the subcortical white matter

Authors: *B. MCELLIN, M. CATRON, E. HONG, L.-J. CHEW, X. MING, V. GALLO
Neurosci., Children's Natl. Med. Ctr., Washington, DC

Abstract: Successful repair in demyelinating diseases, such as multiple sclerosis, is dependent on the development and maturation of oligodendrocyte progenitor cells (OPCs). A better understanding of molecular mechanisms of oligodendrocyte development through the study of its regulatory factors will provide important clues for potential therapeutic strategies. Our lab has previously identified the SRY-box containing gene 17 (Sox17) as a transiently expressed High Mobility Group-domain factor that positively regulates oligodendrocyte development *in vitro* by promoting cell cycle exit and differentiation of OPCs. To expand upon these studies, a novel CNPase-based conditional Sox17 knockout mouse (Sox17 cko) was used to study the effects of Sox17 ablation on oligodendrocyte development and remyelination in the subcortical white matter (SCWM). In the absence of injury, Sox17 knockout animals show reductions in both RNA and protein levels of myelin proteins, as well as increased numbers of delaminated axons by electron microscopy at P30. In addition, Sox17 cko mice show motor deficits on inclined beam tests relative to controls, suggesting that abnormalities in myelination are functionally significant. To understand how this phenotype arises, cellular analysis of the developing SCWM was performed. Results showed persistent deficits in total numbers of Olig2+ and CC1+ cells in Sox17 cko during development, likely due to apoptotic death in CC1+ cells, as a 4-5 fold increase in cleaved caspase3+/CC1+ positive cells was observed in Sox17 cko at P30. A pro-survival function for Sox17 in oligodendrocytes is supported by recently published data using in a CNP-Sox17 transgenic mouse (Ming et al., J. Neurosci 2013 33(30):12528-12542). In this study, oligodendrocytes with high Sox17 were resistant to lysolecithin-induced cell death, due to increased levels of Gli2 and its downstream effector Bcl2, an anti-apoptotic protein. Consistent with this data, Sox17 cko mice show reduced numbers of oligodendrocytes expressing Gli2 and Bcl2, indicating regulation of the SHH pathway during normal development. Ongoing work with Sox17 cko mice is also testing whether Sox17 has similar functions in remyelination following cuprizone- or lysolecithin-induced demyelination. Preliminary analysis suggests that Sox17 cko mice have fewer surviving, newly-born oligodendrocytes after 3 weeks of cuprizone treatment. Future experiments will determine whether this is due to impaired survival of newly formed oligodendrocytes and/or failure to mobilize OPCs for regeneration.

Disclosures: **B. McEllin:** None. **M. Catron:** None. **E. Hong:** None. **L. Chew:** None. **X. Ming:** None. **V. Gallo:** None.

Poster

497. Neuron–Glia Interactions During Development

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 497.05/B34

Topic: A.02. Neurogenesis and Gliogenesis

Support: NIMH K01MH087845

Brain Behavior Research Foundation NARSAD Young Investigator Award

University of Louisiana Graduate Student Oranization

Title: Glial cells and neurons express fibroblast growth factor receptor 1 (FGFR1) in the cortex and hippocampus in the developing mouse brain

Authors: L. CHOUBEY[1], J. COLLETTE[3], *K. M. SMITH[2]
[1]Biol., [2]Univ. of Louisiana At Lafayette, Lafayette, LA; [3]Biol., Univ. of Louisiana at Lafayette, Lafayette, LA

Abstract: Fibroblast growth factors (Fgfs) and their receptors have various morphological, regulatory and endocrine effects on the developing and adult CNS. Inactivation of Fgfr1 results in reduced hippocampal volume, disruption of corpus callosum and hippocampal commissure due to abnormal midline glia development. A decrease in FGFR1 expression in the dorsal telencephalon leads to postnatal loss of cortical interneurons expressing parvalbumin (PV). Neuronal imbalances between excitatory and inhibitory neurons have been seen in patients with schizophrenia, autism, bipolar disorder, Tourette syndrome and attention-deficit/hyperactivity disorder. A previous study shows that FGFR1 is mostly expressed in neurons. Here, we use a transgenic reporter line, the tgFGFR1-EGFP BAC transgenic line (GENSTAT) where EGFP is regulated under the FGFR1 promoter, to demonstrate FGFR1 is expressed mostly in astrocytes, along with neurons, oligodendrocytes and Sox 2 positive cells. Previous staining of one month tgFGFR1-EGFP mice with antibodies against PV and GFP showed interneurons expressing PV did not colocalize with cells expressing GFP. We report that FGFR1 in E14.5 mice is strongly expressed in the hippocampal hem, choroid plexus and cortical ventricular zone, and weakly expressed in the medial ganglionic eminenece (MGE). FGFR1 is expressed in radial glial cells

undergoing soma translocation in P7 mice. In one month old mice, 57% ± 0.9% of cortical GFP positive cells colocalize with GFAP positive astrocytes and 25% ± .5% of cortical GFP positive cells colocalize with NueN positive neurons. Also SOX2 positive cells colocalize with GFP positive cells in the dentate gyrus of the hippocampus and subventricular zone of one-month old mice. Understanding which cell types express FGFR1 may elucidate its role in neuropsychiatric disorders and brain development.

Disclosures: L. Choubey: None. **J. Collette:** None. **K.M. Smith:** None.

Poster

497. Neuron–Glia Interactions During Development

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 497.06/B35

Topic: A.02. Neurogenesis and Gliogenesis

Support: DFG Grant KU2569/1-1

Christiane Nüsslein-Volhard-Foundation

Title: Patch-clamp study of Schwann cells in mouse sciatic nerve slice: Electrophysiological properties and neurotransmitter receptor expression

Authors: *N. FRÖHLICH, D. EISSLER, M. KUKLEY
Ctr. for Integrative Neurosci. (CIN), Tübingen, Germany

Abstract: Two Schwann cell (SC) types are present in the peripheral nervous system: myelinating and non-myelinating SCs. Both types originate from the same progenitor, the immature SC, which develops from neuronal crest cells. The sequential expression of transcription factors and developmental markers characterize the distinct SC types in the lineage. The electrophysiological properties of these cells are only rarely studied in *ex vivo* slices so far. First hint that SCs could express neurotransmitter receptors for glutamate was shown in invertebrates. Furthermore, neurotransmitters, including glutamate, are able to induce changes of SC membrane potential in the giant squid axons (Lieberman and Sanzenbacher, 1992; Villegas et al., 1987). Moreover, different studies done in cell culture (Liu and Bennett 2003) or with immunohistological methods (Dememes et al. 1995) show that SCs in vertebrates could express different types of glutamate receptors. We investigate the receptor expression on SCs in more natural conditions. In addition, we want to determine and characterize SCs in *ex vivo* slices. We

developed a new preparation of sciatic nerve slices of late embryonic (E16-18) and early postnatal mice (P0-P2). Using patch clamp recording and immunohistological analyses, we could (dependent on the age) characterize two (E16-E18) or three (P0-P2) electrophysiologically different SC types. These SC types differ in their expression of voltage-dependent sodium and potassium channels. First analyses show that they express tetraethylammonium (TEA) and 4-aminopyridine (4-AP) sensitive delayed rectifying potassium channels. During patch-clamp recordings we included a fluorescent dye into the pipette solution, so we could combine the electrophysiological properties of the different SC types with their morphological features. These analyses show that SCs before birth display higher number of processes and branches whereas SCs after birth have longer but a lower number of processes and less branches. Using the fast pressure-application system, we want to investigate whether SCs in acute slices express functional ionotropic glutamate receptors and whether SC types differ in their expression. Application of 1mM glutamate induced an inward current in at least two SC types and in both age groups. The evoked current is sensitive to the AMPA/kainate blocker GYKI53655, indicating that functional glutamate receptors of the AMPA/kainate type are expressed.

Disclosures: **N. Fröhlich:** None. **D. Eissler:** None. **M. Kukley:** None.

Poster

497. Neuron–Glia Interactions During Development

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 497.07/B36

Topic: B.11. Glial Mechanisms

Support: NIH ROI NIDA grant 031833

Ruth K. Broad Foundation

Title: How does glia-secreted sparc block synaptic development?

Authors: ***H. DINGSDALE**, A. PAMUKCU, S. SINGH, C. EROGLU
Duke Univ., Durham, NC

Abstract: Precise control of the formation, maintenance and turnover of synapses is critical for the correct functioning of the nervous system. Aberrant synaptogenesis, either congenital or due to injury, can underlie epilepsy, autism and other neurological diseases. We have previously shown that hevin is an astrocyte-secreted molecule that promotes the formation of synapses by

bridging pre- and post-synaptic molecules. Astrocytes and microglia secrete a close homolog of hevin, called SPARC (secreted protein, acidic and rich in cysteine). SPARC is not synaptogenic; on the contrary SPARC specifically blocks hevin-induced synapse formation, whilst having no effect on the pro-synaptogenic activities of other known promoters such as thrombospondins. SPARC has also been shown to downregulate AMPA receptors at the synaptic cleft, and reduce the size of the presynaptic vesicular pool at cholinergic synapses. Taken together, these findings indicate the SPARC is a pro-immaturity factor that blocks the formation and maturation of synapses in the CNS. This function of SPARC may be important for keeping synaptic connections plastic during critical developmental periods. The mechanism by which SPARC specifically inhibits the prosynaptogenic function of hevin has not yet been elucidated. Therefore, here we investigated three possible mechanisms by which SPARC may inhibit hevin-induced synaptogenesis: 1. SPARC binds to and sequesters hevin; 2. SPARC competes with hevin for binding of its synaptic receptor; 3. SPARC blocks hevin's synaptogenic signaling by activating an inhibitory signaling pathway within neurons. Determining the mechanism by which SPARC inhibits hevin during synaptogenesis will further our understanding of this critical process.

Disclosures: H. Dingsdale: None. **A. Pamukcu:** None. **S. Singh:** None. **C. Eroglu:** None.

Poster

497. Neuron–Glia Interactions During Development

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 497.08/B37

Topic: A.02. Neurogenesis and Gliogenesis

Support: Creighton University Grant

NIGMS 8P20GM103427

National Center for Research Resources 5P20RR016469

Title: Cortical neuronal damage stimulates a microRNA and epigenetic-associated transition in activated microglia that enhances neuronal differentiation and survival

Authors: A. K. JOHNSON[1], N. W. MATHY[1], M. TAPPATA[1], *A. SHIBATA[2]
[1]Biol., [2]Dept Biol, Creighton Univ., Omaha, NE

Abstract: Contribution of microglia to neurogenesis under different pathological conditions is unclear. Both pro and anti-neurogenic effects reflect the heterogeneity of microglial activation. We have utilized an *in vitro* model system to co-culture microglia with mechanically damaged or undamaged primary neurons. Accelerated and increased expression of Nestin, α-internexin, β-tubulin III, and NeuN was observed in damaged neurons co-cultured with activated microglia. Western blot analysis revealed an increase of 118.5 ± 21.3% (p=.064) in Nestin expression in damaged neurons co-cultured with activated microglia as compared to controls. Alpha-internexin expression increased 67.0 ± 27.1% (p=.009) and immunofluorescence increased 109 ± 2.1% (p=.0001) in neurons one day after damage and co-culture with activated microglia. β-tubulin III immunofluorescence increased 96.1 ± 1.9% (p=.0001) after one day of co-culture and 623.8 ± 7.22% (p=.0001) at four days of co-culture with activated microglia. By seven days of co-culture, GFAP expression decreased by 26.2 ± 8.4% (p=.028) and NeuN expression increased 40.2 ± 26.2% (p=.0069) in damaged neurons suggesting that neurogenesis and neuronal survival was enhanced in the absence of significant astrogliogensis. Transition to a neurogenic microglial phenotype may involve regulation of protein expression via non-coding RNAs and epigenetic modification. RT-RCR analysis of microRNA (miR) in microglia co-cultured with undamaged and damaged neurons revealed significant increases in let-7c, miR-124, and miR-9 expression. Co-culture of microglia with undamaged neurons increased let-7c expression by 8.5 fold ± 2.008 (p=.038) and miR-124 expression by 9.8 ± 1.29 (p=.002) as compared to control microglia. Co-culture of microglia with damaged neurons increased let-7c expression by 12.2 fold ± 3.94 (p=.009) and miR-124 expression by 9.9 ± 0.86 9(p=.0001) as compared to control microglia. MiR-9 expression is specifically increased (4.6± .64 fold) in microglia co-cultured with damaged neurons as compared to microglia co-cultured with undamaged neurons (p=.037) suggesting a specific regulatory role for miR-9 in neurogenic microglia. A 1.74 ± .02 fold increase (p=0.031) in H3K9me1 levels was seen in activated microglia co-cultured with damaged neurons. MiR9 and H3K9 methylation may regulate protein expression in microglia. RT-PCR and ELISA analysis confirmed significant decreases in Ccl3/MIP1-α and TNF-α mRNA and protein in co-cultures of activated microglia and damaged neurons. Our data suggests that microglia possess modifiable properties to become potential targets for neuroprotective therapies.

Disclosures: **A.K. Johnson:** None. **A. Shibata:** None. **N.W. Mathy:** None. **M. Tappata:** None.

Poster

497. Neuron–Glia Interactions During Development

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 497.09/B38

Topic: B.11. Glial Mechanisms

Support: NIH Grant DA031833

Title: Bidirectional signaling between neurons and astrocytes via neuroligin-1 and hevin

Authors: *J. A. STOGSDILL[1], S. K. SINGH[1], L.-J. PILAZ[2], D. L. SILVER[2], C. EROGLU[1]
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Abstract: The dynamic communication between astrocytes and neurons is critical for synapse formation and maturation within the CNS. Defects in this bi-directional communication may underlie the pathology of neurological diseases such as autism, depression, and epilepsy. Hevin, an astrocyte-secreted synaptogenic protein, regulates the maturation of both pre- and post-synaptic specializations of excitatory synapses. Previously, we identified the post-synaptic cell adhesion molecule neuroligin (NL) as a post-synaptic receptor for hevin. NLs (1-3) are required for hevin-induced synapse formation of retinal ganglion cells *in vitro*; however, if NL/hevin interaction is important for synapse formation and maturation *in vivo* was not known. We found that, *in vivo*, hevin and NL1 co-localize in the mouse visual cortex. To determine if hevin-NL1 interactions control synapse formation in the visual cortex, we knocked down NL1 with shRNA in layer 2/3 cortical neurons by *in utero* electroporation (IUE) in wild type and hevin KO animals. We found that knockdown of NL1 in wild type brains reduces the density of all dendritic spines, in particular mature mushroom spines and increased the length of all spines types in layer 2/3 pyramidal neurons. In contrast, knockdown of NL1 in the hevin KO background resulted in no changes to dendritic spine length or density. These findings show that hevin and neuroligin-1 are required for proper dendritic spine formation and maturation within the visual cortex. Additionally, we find that hevin protein levels are profoundly elevated in wild type brains with reduced NL1. These data suggest that hevin is required for NL1-abundance dependent competitive synaptogenesis and may be regulated via activity-dependent mechanisms. In summary, our findings identify a mechanism whereby neurons and astrocytes communicate to establish the synaptic connectivity within the brain. Both hevin and NLs are linked to neurological disease, therefore, these data may give novel insight into neurodevelopmental disorders wherein the balance of neuroligin and/or hevin is altered such as in autism.

Disclosures: J.A. Stogsdill: None. **S.K. Singh:** None. **L. Pilaz:** None. **D.L. Silver:** None. **C. Eroglu:** None.

Poster

497. Neuron–Glia Interactions During Development

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 497.10/B39

Topic: A.02. Neurogenesis and Gliogenesis

Support: JSPS KAKENHI Grant 25861289

Title: PACAP induces differentiation of neural progenitor cells into glial lineage in cerebral cortex development

Authors: *J. WATANABE[1,2], H. OHTAKI[2], T. NAKAMACHI[2,3], Z. XU[2], K. SUGIYAMA[2], S. SASAKI[2], S. ARATA[1], S. SHIODA[2]
[1]Ctr. for Biotech., [2]Dept. of Anatomy, Sch. of Med., Showa Univ., Tokyo, Japan; [3]Lab. of Regulatory Biology, Grad. Sch. of Sci. and Engin., Univ. of Toyama, Toyama, Japan

Abstract: Neural development is controlled by region-specific factors that regulate cell proliferation, migration and differentiation. Pituitary adenylate cyclase-activating polypeptide (PACAP) is a neuropeptide that exerts a wide range of effects on different cell types in the brain as early as the fetal stage. The distribution of PACAP and its specific receptor, PAC1-R mRNA during development suggests that PACAP is associated with differentiation or proliferation of neural progenitor cells (NPCs)1. However, little is known about the role of PACAP in NPCs. Previously, we reported that PACAP induced differentiation of cultured mouse embryonic NPCs into astrocytes2. Here, we report that the effect of PACAP *in vivo* on the glial differentiation. We studied the localization of PAC1-R during neural development by use of immunohistochemistry. PAC1-R immunopositve cells were observed in the sub-ventricular zone in E14 mouse embryo. Double-immunostaining showed that immunoreactivity for PAC1-R was co-localized with cell proliferation marker, Ki67 and radial glia marker, vimentin. Astrocyte marker, GFAP-positive cells appeared around the ventricle from E16. GFAP-immunopositive cells appeared in the region where PAC1-R immunoreactivity was strongly expressed. These data suggest that endogenous PACAP leads NPCs to differentiate into radial glia then astrocytes. Addition of PACAP into primary cultured-NPCs increased the expression of another radial grail marker, GLAST after 4 days. The expression of GFAP was up-regulated 6 days after PACAP administration. Furthermore, intracerebroventricular injection of PACAP into the ventricle of telencephalon of E13 embryos *in utero* increased the number of GLAST immunopositive cells and decreased the number of nestin immunopositive cells. These data suggest that PACAP plays crucial roles in the differentiation of NPCs into astrocytes via radial grail lineage. 1 Watanabe J et al. Peptides. 2007; 28(9):1713-9. 2 Watanabe J et al. J Neurosci Res. 2006; 84(8):1645-55.

Disclosures: J. Watanabe: None. **H. Ohtaki:** None. **T. Nakamachi:** None. **Z. Xu:** None. **K. Sugiyama:** None. **S. Sasaki:** None. **S. Arata:** None. **S. Shioda:** None.

Poster

497. Neuron–Glia Interactions During Development

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 497.11/B40

Topic: B.11. Glial Mechanisms

Support: NIH grant DA031833

Title: Astrocyte-secreted hevin promotes synapse formation and maturation by trans-synapticallybridging neurexin 1-alpha and neuroligins

Authors: *S. SINGH[1], J. A. STOGSDILL[1], L.-J. PILAZ[2], H. DINGSDALE[1], A. PAMUKCU[1], D. L. SILVER[2], C. EROGLU[1]
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Abstract: Establishment of synaptic adhesions is the critical first step in the construction of neuronal circuits. Interactions between presynaptic Neurexins (Nrxs), and postsynaptic Neuroligins (NLs) coordinate the formation of synaptic adhesions. An isoform/splice variant-dependent code determines the compatibility of Nrxs and NLs for interactions, and thereby contributes to the target recognition between axons and dendrites. However, the presence of synaptic linkers that can bridge incompatible Nrx-NL pairs across the synaptic cleft, thus strengthen the synaptic adhesions was unknown. Using a combination of *in vitro* and *in vivo* approaches we found that an astrocyte-secreted synaptogenic protein, hevin organizes pre- and post-synaptic specializations by interacting with Nrx1-alpha and NLs respectively. Mechanistically, hevin links Nrx1α and NL1 to each other transcellularly by bridging these otherwise incompatible isoforms. These findings show that astrocyte-secreted hevin is a synaptic linker protein that links Nrx1-alpha and NLs trans-synaptically and thereby control synapse formation and maturation.

Disclosures: S. Singh: None. **J.A. Stogsdill:** None. **L. Pilaz:** None. **H. Dingsdale:** None. **A. Pamukcu:** None. **D.L. Silver:** None. **C. Eroglu:** None.

Poster

497. Neuron–Glia Interactions During Development

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 497.12/B41

Topic: A.02. Neurogenesis and Gliogenesis

Support: FAPERJ

CNPq

SR2 - Uerj

Title: Perinatal protein malnutrition during the 10 first lactation days alters the vimentin and cell proliferation pattern of the dentate gyrus

Authors: *A. C. B. BARBOSA, P. L. GUEDES, M. L. M. ROCHA, P. BARRADAS, F. TENORIO
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Abstract: Perinatal malnutrition can lead to permanent damage in brain morphology, physiology and neurochemistry. The hippocampus is a structure particularly sensitive to nutritional alterations during development. Dentate gyrus is characterized as the most proliferative hippocampal region. As hippocampus is a structure responsible for the mechanism of learning and memory, alterations in its astrocitary populations may affect this process. Besides their role in neuronal nutrition and support in ionic concentrations, other important function of astrocytes is related to the secretion of neurotrophic factors, that may be associated to neurogenesis. In this study, we evaluated the effect of protein malnutrition restricted to the 10 first lactation days on cell proliferation and precursor glial cells distribution in dentate gyrus. This study was approved by our University Ethics Committee (CEA/055/2009). Wistar male rats on postnatal day 5 (P5), 10 (P10), and 15 (P15) were used. The animals were settled in two groups: control group (CG) and malnourished group (MG). CG dams were fed ad libitum with a normoprotein diet (22% protein) during gestation. During the first 10 days of lactation MG received a 0% protein diet. On each age studied animals were anesthetized and perfused with saline solution 0,9%, paraformaldehyde (PF) 4% and PF 4% plus sucrose 10%. Sections of 20μm were immunostained using anti-vimentin and anti-Ki-67 antibodies, revealed with a secondary antibody conjugated with Alexa 555 and Alexa 488, respectively and observed at a fluorescence microscope. Our analyses show that during the development, vimentin positive cells tend to concentrate in the polimorfic and molecular layers. But, in MG, these cells can be seen through all the dentate gyrus and at age (P15), they are more visible in the polimorfic layer (PL) next to the granule layer, while the immunostainings of CG seem to be equally distribuited in this layer. The Ki-67+ cells are found disperse in both groups, but MG presents a much more difused distribuition than CG, that is more uniform and contains more imunostainned cells (CG - 99.80 ± 9.272 N=3; MG - 35.53 ± 9.327 N=3; $p<0,05$). At P10, with apparently lesser positive cells than the previous age,

it is possible to observe that the less stained layer is the molecular one in MG and the granular layer in CG. At P15, the densest area with Ki-67+ cells is the PL next to the granular layer to MG and the center of PL to CG. The present study demonstrates that protein restriction may modify the pattern of vimentin and cell proliferation in the dentate gyrus.

Disclosures: A.C.B. Barbosa: None. **P.L. Guedes:** None. **M.L.M. Rocha:** None. **P. Barradas:** None. **F. Tenorio:** None.

Poster

497. Neuron–Glia Interactions During Development

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 497.13/B42

Topic: A.02. Neurogenesis and Gliogenesis

Support: NSERC

Title: Sustained learning and memory programmes modulate OPC proliferation and differentiation

Authors: *J. BOULANGER[1], C. MESSIER[2]
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Abstract: Environmental enrichment and physical activity have been shown to upregulate OPC proliferation rates (Ehninger et al., 2011; Okuda et al., 2009; Simon et al., 2011). However, not all agree on the fate of these newly divided OPCs. We looked at rates of OPC proliferation and differentiation in response to a sustained programme of learning and conditioning that targeted multiple neural networks in the hopes of maximising the possible changes in OPC dynamics. BrdU was administered through the mice's drinking water. When looking strictly at OPC proliferation, 3-5 month old CD-1 mice were exposed to a programme consisting in a radial arm maze task, which utilizes reference and working memory, an operant conditioning task, and the Morris Water Maze, which draws on spatial memory. The training programme had to be modified for the NG2Cre/R26S-EYFP transgenic mice used to study differentiation as they showed increased levels of toxicity to BrdU. The programme administered in the differentiation phase of the study consisted in the Barnes Maze, which measures short- and long-term memory as well as spatial memory, and an operant condition task. Preliminary results show that a sustained programme of learning and conditioning increases the proportion of newly proliferated

OPCs in the cortex and that in response to such a programme, OPCs either remain undifferentiated or adopt an oligodendroglial phenotype.

Disclosures: J. Boulanger: None. **C. Messier:** None.

Poster

497. Neuron–Glia Interactions During Development

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 497.14/B43

Topic: A.02. Neurogenesis and Gliogenesis

Support: Japan MEXT Grant C 24500437

Title: HMGN family proteins promote astrocyte differentiation of neural precursor cells

Authors: *M. NAGAO[1], D. LANJAKORNSIRIPAN[2], Y. ITOH[2], Y. KISHI[2], Y. GOTOH[2], T. OGATA[1]
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Abstract: Astrocytes are the most abundant cell type in the mammalian brain and are important for the functions of the central nervous system (CNS). Although previous studies have shown that the STAT signaling pathway or its regulators promote the generation of astrocytes from multipotent neural precursor cells (NPCs) in the developing mammalian brain, the molecular mechanisms that regulate the astrocytic fate decision have still remained largely unclear. Here, we show that the high mobility group nucleosome-binding (HMGN) family proteins, HMGN1, 2 and 3, promote astrocyte differentiation of mouse NPCs during brain development. HMGN proteins were expressed in NPCs, Sox9+ glial progenitors and GFAP+ astrocytes in perinatal and adult mouse brains. Forced expression of either HMGN1, 2 or 3 in mouse NPCs in cultures or in the late embryonic neocortex increased the generation of astrocytes at the expense of neurons. Conversely, knockdown of either HMGN1, 2 or 3 in mouse NPCs suppressed astrocyte differentiation and promoted neuronal differentiation. Importantly, overexpression of HMGN proteins did not induce the phosphorylation of STAT3 or activate STAT reporter genes. In addition, HMGN family proteins did not enhance DNA demethylation and acetylation of histone H3 around the STAT-binding site of the gfap promoter. Moreover, knockdown of HMGN family proteins significantly reduced astrocyte differentiation induced by gliogenic signal ciliary

neurotrophic factor (CNTF), which activates the JAK-STAT pathway. Therefore, we propose that HMGN family proteins are novel chromatin regulatory factors that control astrocyte fate decision/differentiation in parallel with or downstream of the JAK-STAT pathway through modulation of the responsiveness to gliogenic signals.

Disclosures: M. Nagao: None. **T. Ogata:** None. **D. Lanjakornsiripan:** None. **Y. Gotoh:** None. **Y. Itoh:** None. **Y. Kishi:** None.

Poster

497. Neuron–Glia Interactions During Development

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 497.15/B44

Topic: B.11. Glial Mechanisms

Support: CIHR

NSERC Banting Fellowship

Title: D-Serine influences the maturation of glutamatergic synapses and axonal refinement in the developing visual system of the Xenopus tadpole

Authors: *M. VAN HORN[1], L. POLLEGIONI[2], E. RUTHAZER[1]
[1]Neurol. and Neurosurg., McGill Univ., Montreal, QC, Canada; [2]Dept. of Biotech. and Life Sci., Univ. of Insubria, Varese, Italy

Abstract: D-Serine is an endogenous co-agonist for synaptic N-methyl-D-aspartate receptors (NMDAR). Here we examined the neurophysiological significance of D-serine, a known gliotransmitter, in regulating synaptic transmission and axonal remodeling in the developing visual system. Acute D-serine (100 μM) wash-on enhances NMDAR currents of optic tectal neurons. Conversely, degradation of D-serine by RgDAAO reduces evoked NMDAR currents. To investigate the pathways involved in modulating D-serine release in the optic tectum, we used D-serine-sensitive amperometric biosensors and found that α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) application results in an increase in D-serine release, presumably by activating non-NMDARs on glial cells. These results indicate that in the retinotectal system, the NMDAR glycine site is not saturated under normal physiological conditions *in vivo*, D-serine is an endogenous agonist of the NMDAR and its release is modulated by AMPA receptor activation. We next tested whether chronically elevating D-serine

levels could influence the maturation of glutamatergic synapses. We find that tadpoles raised in elevated levels of D-serine (100 μM) for 2 days have higher frequencies of miniature excitatory postsynaptic AMPAR currents and higher retinotectal synaptic AMPA/NMDA ratios compared to control animals. To examine the effects of D-serine on axonal development, retinal ganglion cells were electroporated to express EGFP and 2-photon images were collected daily over 4 days. We found that increasing available D-serine results in less complex retinal axons arbors. These findings are consistent with the hypothesis that D-serine enhancement of NMDAR currents promotes synaptic maturation and leads to stabilization of axonal branches. Taken together, these results suggest that D-serine levels are modulated by glutamatergic neurotransmission *in vivo* and can influence the maturation of retinotectal synapses and circuit refinement.

Disclosures: M. Van Horn: None. **L. Pollegioni:** None. **E. Ruthazer:** None.

Poster

497. Neuron–Glia Interactions During Development

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Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 497.16/B45

Topic: A.02. Neurogenesis and Gliogenesis

Support: FRC

IDEX-Paris Cité Sorbonne

ENP

ARSEP

Title: Unitary synaptic connections between GABAergic interneurons and NG2 cells in the developing somatosensory cortex

Authors: D. ORDUZ[1], P. P. MALDONADO[1], M. BALIA[1], M. VÉLEZ-FORT[1], V. DE SAARS[2], Y. YANAGAWA[3], V. EMILIANI[2], *M. C. ANGULO[1]
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Abstract: NG2-expressing cells, the main pool of progenitors in the CNS, have the primary role of generating myelinating oligodendrocytes in the developing brain. These progenitors are

unique non-neuronal cells that receive bona fide synaptic inputs from neurons. However, our understanding of synaptic physiology of NG2 cells is still limited because it derives only from studies based on averaged synaptic currents generated by the stimulation of unidentified neurons. In the developing somatosensory cortex, NG2 cells receive a major GABAergic synaptic input from interneurons, but how these progenitors are wired and integrate interneuronal networks remains unknown. By using paired recordings and holographic photolysis, we investigated the dynamics of synaptic transmission at the level of unitary interneuron-NG2 cell connections in acute somatosensory cortical slices of vGAT-Venus;NG2-DsRed transgenic mice during the second postnatal week. Among the heterogeneous cortical population of interneurons, we identified fast-spiking interneurons (FSIs) as a preferential presynaptic input of NG2 cells. Quantal analysis revealed that interneurons innervate NG2 cells through single or double release sites. Both fast-spiking (FSI) and non-fast-spiking cells (NFSI) contact NG2 cells, but these two neuronal populations target distinct anatomically segregated subcellular domains that differ on the postsynaptic receptor subunit composition. In addition, holographic photolysis was very effective in photo-activating interneurons at a single cell resolution in order to build connectivity maps of interneurons innervating NG2 cells. Using this technique, we found that the spatial arrangement of interneuron-NG2 cells connections is more local than those of interneuron-pyramidal cell connections, forming a specific subnetwork characterized by a local microarchitecture. Our findings on unitary interneuron-NG2 cell connections uncover specific rules of synaptic connectivity for these progenitors that differ from those of classical neuronal GABAergic synapses in the neocortex. Also suggest that FSIs, beyond their role in neocortical networks, play important roles in controlling NG2 cell function.

Disclosures: D. Orduz: None. **P.P. Maldonado:** None. **M.C. Angulo:** None. **M. Balia:** None. **M. Vélez-Fort:** None. **V. Emiliani:** None. **V. De Saars:** None. **Y. Yanagawa:** None.

Poster

497. Neuron–Glia Interactions During Development

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Topic: B.01. Neurotransmitters and Signaling Molecules

Support: NIEHS ZIA ES102805 04

Title: Hoxb1 is required for the development of a subset of pontine norepinephrine neurons

Authors: *S. D. ROBERTSON, P. JENSEN
Natl. Inst. of Envrn. Hlth. Sci., Durham, NC

Abstract: Norepinephrine (NE) neurons in the central nervous system support a range of essential behaviors, including attention, stress, memory and appetite. The molecular mechanisms that contribute to this divergence of adult NE neuron function are unknown. Recently, we classified mature NE neurons based on differences in developmental gene expression history. We defined four subpopulations that differ in their anatomical location, efferent projection pattern and axon morphology. One of these subpopulations, defined by the expression of the homeobox gene Hoxb1, consists of NE neurons that are distributed across the pons and medulla. Interestingly, this Hoxb1-derived NE subpopulation has a propensity to project to key components of the central autonomic nervous system. However, it is unclear if Hoxb1 expression is required for the development of these NE neurons and therefore important for central autonomic function. A previous study by Gaufo et al 2004, suggested that Hoxb1 is required for the specification of medullary NE neurons based on the absence of Dbh mRNA within rhombomere 4 (r4) of the Hoxb1-null embryonic hindbrain. In the current study, we employed a recombinase-based intersectional genetic strategy to selectively label Hoxb1-derived NE neurons with eGFP, and all other NE neurons with mCherry, thus enabling us to trace the long-term fate of all NE neurons in the context of the Hoxb1-null mutation. We mapped these populations onto the mature anatomically defined pontine (Subcoeruleus (SubC), A5) and medullary (A1 and A2) NE nuclei and counted the number of mCherry and eGFP positive NE neurons. In the pontine SubC and A5 nuclei, we observed a striking absence of Hoxb1-derived NE neurons. In contrast, mapping of Hoxb1-null NE neurons onto the medullary A1 and A2 nuclei revealed a significant increase in the number of Hoxb1-derived NE neurons. This increase in the number of eGFP-positive NE neurons is likely due to the ectopic expression of cre which has been reported in Hoxb1-null embryos caudal to r4. In summary, we have demonstrated a requirement for Hoxb1 in the proper development of a subset of Hoxb1-derived NE neurons. Studies are ongoing to examine the effects of the Hoxb1-null mutation on NE neuron efferents throughout the brain, particularly in regions important for the regulation of central autonomic function. Results from these studies promise new insight into how Hoxb1 contributes to the generation of functionally divergent subsets of NE neurons

Disclosures: S.D. Robertson: None. **P. Jensen:** None.

Poster

497. Neuron–Glia Interactions During Development

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Topic: A.02. Neurogenesis and Gliogenesis

Support: Fapesp Grant 2010/17519-0

Fapesp Grant 2011/19747-3

Title: Tridimensional *in situ* description of axonal branches contacting multi and uni-ciliated cells lying on the lateral wall of lateral ventricle, in adult Long-Evans rats

Authors: *C. S. HAEMMERLE, M. I. NOGUEIRA, I.-S. WATANABE
Anat., Univ. of Sao Paulo; Inst. of Biomed. S, Sao Paulo, Brazil

Abstract: Introduction: the canonical definition for the lining of brain ventricles introduce us to the single layer of multiciliated cells named ependyma; however, new findings specially at lateral ventricle has yielded information about additional and distinct cell elements further the multiciliated surface, influencing the cell proliferation in this neurogenic niche. In this field, the axonal signaling occurring on the lining epithelia seems to make an important contribution to the regulation of neurogenesis. Using a high-resolution and ultrastructural analysis provided by High-Resolution Scanning Electron Microscopy (HRSEM) and Transmission Electron Microscopy (TEM), we demonstrate the proper anatomical details about the innervation of different ciliated cells present on surface of neurogenic niche of lateral ventricle. Methods: we used adult male Long-Evans rats (n=10). For HRSEM, following a whole mounting dissection of lateral ventricle, the samples were post-fixed in OsO_4 1%, dehydrated, dried and covered with gold ions and examined in field emission scanning electron microscope. For TEM, the brains were perfused, reduced and immersed in modified-Karnovsky solution. After sectioning, post-fixation was done in OsO_4 1%, followed by dehydration and embedding in Spurr resin. Semi-thin sections confirmed the lining surface region and ultrathin sections were examined in TEM microscope. Results: we observed a heterogeneous pattern for cilia distribution throughout the different poles of the lateral ventricle surface. Besides the canonical definition about the multiciliated cells lying on the ventricular surface, we corroborate that the lateral ventricle lining also contains single/primary cilium and biciliated cells, with its peculiar lenghts; we also demonstrated axonal bundles and varicose axons on ependymal surface surrounding and contacting these cells, but not in its totality. The axonal contacts making synapses were also confirmed by TEM. Therefore, we provide a unique ultrastructural methodology that describes the tridimensional *in situ* organization of lateral ventricles lining, regarding to the discrete innervation on the surface elements, in a way to corroborate the available functional findings about the heterogeneity of factors regulating this neurogenic niche.

Disclosures: C.S. Haemmerle: None. **M.I. Nogueira:** None. **I. Watanabe:** None.

Poster

497. Neuron–Glia Interactions During Development

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 497.19/B48

Topic: B.11. Glial Mechanisms

Title: Constitutive expression of cannabinoid CB2 receptors in cultures of microglial and astroglial cells: Immunocytochemical, biochemcial and pharmacological evidence

Authors: *A. C. SHIVACHAR, D. N. JACKSON, J. JOSEPH
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Abstract: While the localization and function of cannabinoid CB1 receptors in astroglia, and CB2R receptors in microglia are well established, the CB2 receptors expression and function in astroglia remains controversial. Primary mixed cultures of astrocytes and microglial cells were grown from 1-day old rat brain cortices. Once confluent, microglial cells grown on the bed of astrocytes were separated by mechanical shaking and were grown separately. The resulting astroglial and microglial cells were used for immunocytochemical, biochemical and pharmacological studies. Immunocytochemical double-labeling studies showed that cells expressing microglial specific marker CD11b were stained positive for CB2 receptors. Whereas, the astroglial cultures, expressing the specific marker, glial fibrillary acidic protein (GFAP) were also stained positive for the cannabinoid CB2 receptors besides CB1 receptors. Quantitative western blot analyses showed a single immunoreactive band at 62 KDa that increased with increase in cell lysate protein when probed with an anti-CB1R antibody. The cell lysate, when probed with an anti-CB2R antibody, showed a smaller, but prominent immunoreactive protein band at 40 KDa, the immunoreactivity of which was blocked by a CB2R blocking peptide, thus confirming it was indeed a CB2R protein band. To determine if the CB2R in astroglial cells is functional, the agonist-induced cAMP inhibition was measured. The mixed agonist WIN 55212-2 (1μM) inhibited about 61% of forskolin-induced cAMP accumulation (80.87±5.08 nmol/well) which was attenuated either by the CB1 receptor-selective antagonist /inverse agonist, AM 251(25 nM), or by the CB2 receptor-selective antagonist/inverse agonist, AM 630 (100nM). However the inhibition was not additive when both of these antagonists were added, suggesting an integrated signaling response. Our findings suggest that CB2 receptors are constitutively expressed in rat cortical astrocytes and microglial cells. Astroglial cells, by expressing both CB1 and CB2 receptors may integrate their signaling pathways when simultaneously activated by a CB1/CB2 agonist.

Disclosures: A.C. Shivachar: None. **D.N. Jackson:** None. **J. Joseph:** None.

Poster

497. Neuron–Glia Interactions During Development

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 497.20/B49

Topic: B.11. Glial Mechanisms

Title: Ultrastructural distribution of glycogen in hippocampal astrocytic processes

Authors: *C. CALI[1], J. BAGHABRA[1], H. LEHVASLAIHO[1], G. KNOTT[2], I. ALLAMAN[3], P. J. MAGISTRETTI[1]
[1]BESE, KAUST, Thuwal, Saudi Arabia; [2]CIME, [3]BMI, EPFL, Lausanne, Switzerland

Abstract: Glycogen is a major energy store in astrocytes that provides energy support and signals for plasticity to neurons under the form of lactate. While the biochemistry of glycogen is well known, the spatial distribution of glycogen granules within astrocytes remains largely unknown. To this purpose, we imaged a 220 μm^3 volume of adult mouse hippocampus neuropil using a FIB-SEM microscope. This technique allowed us to automatically acquire very thin serial section micrographs (5 nm) at very high resolution (5 nm). Perfect isotropy of resulting voxels allowed iLastik, a state of art semi-automated software for EM stacks segmentation, to rapidly reconstruct every axon, dendrite, and the astrocytic processes present within the volume. Glycogen granules inside an astrocyte were recognized by morphology, and manually reconstructed using TrakEM2, a widely used tool for manual segmentation of serial section electron micrographs. We then used Blender, a three-dimensional modeling software, to visualize the entire 3D model in the fully immersive virtual reality CAVE system. This detailed walk-in made it very clear that the apparently random cloud of glycogen granules in fact appeared in clusters. The DBSCAN algorithm, optimized with the help of Silhuette coefficient, determined the extent and number of glycogen clusters. Compared to other clustering algorithms, DBSCAN allows extraction of irregularly shaped clusters, and is much more sensitive to noise and outliers, making it a perfect choice for our case. We also used the Silhuette coefficient that allowed the optimal initialization parameters for DBSCAN, thus validating the clustering. In order to determine weather the clusters have a spatial relationship with the pre- or post- synaptic elements, we extracted all spines and boutons, as well as their size, using Neuromorph, a scientific package available as addons for Blender, designed for detailed morphological analysis of the neuropil. Preliminary results indicate a regular 3D organization of glycogen granules, and a slight preference for the glycogen granules in astrocytic processes close to presynaptic boutons compared to postsynaptic spines (75% vs 25%, respectively). We are aiming to repeat the

approach using samples from animals undergoing learning protocols in order to asses the plasticity of such an arrangement of glycogen granules within perisynaptic astrocytes.

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Poster

497. Neuron–Glia Interactions During Development

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Topic: B.11. Glial Mechanisms

Support: NIH Grant 2R01MH083728-03

Title: Neuron-astrocyte interplay: role of astrocytic DISC1 in neurodevelopment

Authors: *M. XIA[1,2], A. SHEVELKIN[1], C. YANG[1], M. V. PLETNIKOV[1]
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Abstract: Astrocyte dysfunction has been implicated in the pathogenesis of psychiatric disorders. The role of candidate psychiatric genetic risk factors in astrocytes dysfunction remains poorly understood. Here, we studied the effect of dominant-negative mutant Disrupted-In-Schizophrenia-1 (DISC1) selectively expressed in astrocytes on neuronal maturation and differentiation using a co-culture approach. Primary embryonic hippocampal neurons and astrocytes were co-cultured using a cell insert system that allows for indirect co-culture of distinct cell populations in the absence of direct cell contacts. Neurons from wild-type embryos were cultured for 5 days and then were combined with primary control astrocytes or astrocytes that express mutant DISC1 . We found that neurons co-cultured with mutant DISC1 astrocytes exhibited significantly less elaborated dendritic tree as evidenced by decreased dendrites branch level and dendritic branch point after 5 days of co-culturing. Analysis of expression of pre-and postsynaptic markers of glutamatergic and GABA-ergic neurons as well as identification of putative astrocyte-secreted factors to affect neurodevelopment are in progress. We suggest that astrocytic DISC1 impacts neuronal maturation and contributes to risk of major mental illness.

Disclosures: M. Xia: None. **A. Shevelkin:** None. **C. Yang:** None. **M.V. Pletnikov:** None.

Poster

497. Neuron–Glia Interactions During Development

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 497.22/B51

Topic: B.11. Glial Mechanisms

Title: Myelination compensates for visual deprivation and diminished neurotransmission as a form of functional plasticity

Authors: *A. ETXEBERRIA[1], X.-L. CHOU[2], K. HOKANSON[3], S. R. MAYORAL[1], L. I. ZHANG[2], E. M. ULLIAN[3], H. W. TAO[2], J. R. CHAN[1]
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Abstract: Underlying the remarkable versatility of the mammalian nervous system are numerous mechanisms for functional plasticity. Myelination of axons increase action potential conduction velocity, modulating network function; however current mechanisms of plasticity do not consider myelination of axons as a factor for functional plasticity. We demonstrate that after long-term monocular deprivation (MD) the latency of response in the deprived visual pathway is shortened and that this decrease correlates with an enhancement in the differentiation of oligodendrocytes and myelination of the optic tract. Our results suggest that oligodendrocyte differentiation and myelination are triggered by a reduction in sensory stimuli. Generating a conditional knockout of the vesicular glutamate transporter 2 (VGlut2) in retinal ganglion cells significantly decreases glutamate neurotransmission along the retinogeniculate pathway and phenocopies the changes in myelination detected after MD. Together our results indicate the existence of a feedback mechanism between neurons and oligodendroglial cells that tailors myelination to functional requirements.

Disclosures: **A. Etxeberria:** None. **X. Chou:** None. **K. Hokanson:** None. **S.R. Mayoral:** None. **L.I. Zhang:** None. **E.M. Ullian:** None. **H.W. Tao:** None. **J.R. Chan:** None.

Poster

498. Development of Motor, Sensory, and Limbic Systems

Location: Halls A-C

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Program#/Poster#: 498.01/B52

Topic: A.07. Development of Motor, Sensory, and Limbic Systems

Support: NIH-MH091451

NIH-DC009910

Title: Short and long term consequences of maternal presence on *in vivo* physiology of the developing amygdala

Authors: *D. A. WILSON[1,2], E. C. SARRO[1,2], R. M. SULLIVAN[1,2]
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Abstract: The survival of altricial infants depends upon the quality of caregiving, exerting both immediate and enduring neurobehavioral changes that determine individual differences, with maternal maltreatment of pups initiating the pathway to pathology. The mechanism for this neurobehavioral effect is incompletely understood. Here we explore one well-documented influence on brain development, brain oscillation and assess how the quality of maternal care influenced this neural activity. Here we addressed this by recording spontaneous neocortical local field potential (LFP) activity for 7 consecutive days in freely behaving infants (~postnatal days 12-19) in their natural environment during interactions with their mother. We have previously shown that infant cortical activity can be directly influenced by maternal presence and her behaviors in the nest, however it is unknown whether prior early-life trauma can modulate this maternal influence on infant brain activity. We implanted infant rat pups (PN12) with bipolar stainless steel electrodes, targeting the amygdaloid complex and connected to a wireless transmitter (DSI) implanted subcutaneously. Prior to implantation, one group of infants was reared from PN8-12 with a naturally abusive mother and another group were odor-shock conditioned at PN8-12. Both paradigms lead to long-term behavioral deficits. We recorded spontaneous amygdala LFP activity in freely behaving infants, specifically assessing 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). Fast Fourier Transform analysis (2.4 Hz bins) was used to quantify LFP oscillatory power in theta (5-15Hz), beta (15-35Hz) and gamma (35-80Hz) bands. We were particularly interested in whether recent trauma leads to modifications in the amygdala activity during interaction with the mother and littermates. In a separate experiment, we examined the long-term consequences of a learned abusive odor (naturalistic maternal odor or artificial conditioned odor) on amygdala LFP activity by presenting this odor in adulthood to animals from the same conditions. We show odor-specific enhancements in LFP power in response to the learned abusive odor, supporting the long-term behavioral influence that the learned maternal odor can impose on adult behavior. Given the important role of neural activity in shaping circuit development and refinement during

development, this suggests a powerful direct and long-term influence of maternal care on brain development. Key words: maternal care, development, local field potential, amygdala, telemetry

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

Poster

498. Development of Motor, Sensory, and Limbic Systems

Location: Halls A-C

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Program#/Poster#: 498.02/B53

Topic: A.07. Development of Motor, Sensory, and Limbic Systems

Support: NIH-MH091451

NIH-DC009910

T32MH096331

Title: Neurobehavioral development of fear and aggression following early life abuse

Authors: ***R. E. PERRY**[1,2,3,4], R. M. SULLIVAN[1,2,3]
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Abstract: Early life abuse produces increased sensitivity to threat and a heightened prevalence of aggressive and violent behaviors emerging by adolescence in humans. Here we use a rodent model of abuse to assess the neurobehavioral development of fear and aggression following early life abuse using a Resident Intruder Test (RIT), as well as a novel Threat Response Selection Test (TRST), which allows for the assay of active (i.e. fighting, exploration) and passive (i.e. freezing, hiding) defensive responses. Furthermore, we assess environmental enrichment as a possible way to attenuate abuse-induced deficits in response to threat. Rat pups were reared with a normal or abusive mother (postnatal days 8-12), and tested in the TRST at post-weaning (PN26-30), adolescence (PN40-45), and adulthood. In this test, individuals were placed in a center "start" chamber of a three chamber arena and given the option to approach a predator odor (fox urine), freeze, or hide in a shelter. The rat was allowed free roam of the apparatus for 5 minutes, and active and passive fear responses were assessed. In a separate group of animals, aggressive behaviors were assessed in the RIT. In this test, animals were single housed for one week before introducing a smaller, same-sex intruder into the home cage for 10 minutes.

Offensive and defensive aggressive behaviors were scored. At all ages, following the TRST or RIT, brains were removed for c-Fos immunohistochemistry analyses within the amygdala, prefrontal cortex, and hypothalamus. Lastly, the effects of housing animals in a socially and physically enriched environment (weaning until time of testing) were assessed on the adult response to threat (TRST) after abuse. In the TRST, post-weaning rats that were abused in infancy showed heightened avoidance of the predator odor, as well as increased hiding in the provided shelter. However, by adulthood, abused rats showed decreased time hiding, and increased approach and interaction with the predator odor, relative to control rats. Furthermore, early life abused animals show increased offensive behaviors in the RIT, including attacking, boxing, pinning, and chasing the intruder. Group differences were found in c-Fos expression following the TRST and RIT. Environmental enrichment prevents previously abused adult animals from approaching the predator odor in the TRST. Infant abuse alters later life responses to threatening stimuli throughout development, making it more likely for one to place itself in harm's way. Understanding the neurobiology of threat responding and its modulation by infant experience will provide insight for treatment of fear and aggression-related disorders that occur after abuse.

Disclosures: R.E. Perry: None. **R.M. Sullivan:** None.

Poster

498. Development of Motor, Sensory, and Limbic Systems

Location: Halls A-C

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Topic: A.07. Development of Motor, Sensory, and Limbic Systems

Support: NSF GRFP DGE-1137475 to MRC

NIH-MH091451 to RMS

NIH-DC009910 to RMS

Title: Developmental disruption of rat social behavior following early life abuse is mediated by the amygdala and rescued by environmental enrichment

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Abstract: Disrupted social behavior is a hallmark feature of numerous psychiatric disorders, including depression, anxiety and autism (Kennedy and Adolphs, 2012). Moreover, many of these disorders have origins in early life and may be exacerbated by infant experiences such as early life abuse (Rutter, 1984). Early life abuse negatively affects the development of the amygdala- a critical brain area for emotion, learning, and social behavior in both humans and other mammals (Phelps and LeDoux, 2005; Rincón-Cortés and Sullivan, 2014). Importantly, abnormal amygdala function is a common component of psychiatric disorders and has been implicated in the pathophysiology of depression (Anand and Shekhar, 2003; Whalen et al., 2002). Here we use a naturalistic rodent model of early life abuse associated with mother rats roughly handling pups when they are provided with insufficient bedding for nest building. We have previously shown that the effects of infant abuse include but are not limited to: disrupted attachment to the mother amygdala dysfunction and social behavior deficits (Raineki et al., 2012). However, while the amygdala has been implicated in social behavior (Félix-Ortiz and Tye, 2014), its exact contribution to social behavior deficits following infant abuse is currently unknown. Here, we explore the role of amygdala as a mediator of social behavior differences following abuse or normal rearing. To this end, infant rat pups were reared with either an abusive or normal caregiver from postnatal (PN) days 8-12, received age-appropriate social behavior tests throughout early development (infancy, preweaning, adolescence; n=6-8 p/g) and were assessed for c-FOS immunoreactivity within the amygdala following social behavior testing. Pups reared with an abusive mother from PN 8-12 demonstrated social behavior deficits, as indexed by reductions in social interaction time ($p<0.05$), and atypical amygdala activity compared to normally reared pups. However, these changes were only observable at preweaning (PN20) and adolescence (PN40-48), suggesting a developmental delay in the expression of these deficits. Thus, early life abuse produces long-lasting and persistent changes in social behavior and the amygdala that may contribute to the expression of depressive-like behavior during later life. However, an enriched social and physical environment during the postweaning period attenuates the neurobehavioral consequences of early life abuse, as animals receiving environmental enrichment treatment did not show social behavior deficits during adolescence.

Disclosures: M. Rincón Cortés: None. **R.M. Sullivan:** None.

Poster

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Program#/Poster#: 498.04/B55

Topic: A.07. Development of Motor, Sensory, and Limbic Systems

Support: NS-23805

FRSQ

Title: Reorganization of the axon terminations of accumbens neurons projecting to the ventral tegmental area between adolescence and adulthood

Authors: *L. YETNIKOFF, K. P. PARSELY, D. S. ZAHM
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Abstract: The development of the mesocorticolimbic system has been studied extensively, but almost exclusively with regard to dopaminergic output. By comparison, the ontogeny of inputs to the ventral tegmental area (VTA), the source of mesocorticolimbic dopamine, is less studied. This is not a trivial oversight, as the activity of VTA neurons, which reflects the transmission of information about salient events, is sensitively modulated by their afferent connections. We recently compared the numbers of retrogradely labeled neurons in adolescent and adult rats following injections of the retrograde tracer, cholera toxin β subunit, into the VTA and found significantly fewer in the prefrontal cortex and ventral striatopallidum, including the nucleus accumbens (Acb), in adolescent as compared to adult brains (JCN 522: 1031-47, 2014). Here, we evaluated whether the protracted maturation of Acb inputs to the VTA is reflected in differences in the organization of the axonal terminations of Acb neurons in the VTA. Groups of four adolescent (postnatal day 29) and seven adult (> postnatal day 60) rats received injections of the anterograde tracer, Phaseolus vulgaris-leucoagglutinin (PHA-L), into the Acb shell. Ten days later, the rats were perfused and the brains sectioned at 50 μm. Three and five adjacent series of sections from the adolescent and adult brains, respectively, were collected. One series from each brain was immunoprocessed to show PHA-L, mounted in rostrocaudal sequence on glass slides and coverslipped. Numbers of varicosities in the VTA 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 VTA innervated by Acb fibers. No difference in numbers or density of varicosities in the VTA between adult and adolescent rats was observed. Because overall numbers of Acb neurons do not differ in the adolescent and adult brain (JCN, cited above) and, as noted above, fewer Acb neurons project to the VTA during adolescence as compared to adulthood, this finding suggests that Acb neurons that do innervate the VTA during adolescence have more varicosities than do adult VTA-projecting Acb neurons. It follows that VTA neurons may be influenced differently by Acb inputs during adolescence and adulthood.

Disclosures: L. Yetnikoff: None. **K.P. Parsely:** None. **D.S. Zahm:** None.

Poster

498. Development of Motor, Sensory, and Limbic Systems

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Program#/Poster#: 498.05/B56

Topic: A.07. Development of Motor, Sensory, and Limbic Systems

Support: University of Vermont

Title: Gestational stress produces hyperlocomotion and attenuated weight gain in adult male offspring

Authors: H. BAUERLE[1], *D. J. TOUFEXIS[2]
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Abstract: Stress during the second trimester of pregnancy has been associated with the development of both schizophrenia and autistic spectrum disorder. This study examines the possible relationship between prenatal stress and schizophrenia by exposing pregnant Sprague-Dawley rats to three iterations of 45 minutes of restraint stress, from days 15 to 20 of pregnancy, and then examining behaviors similar to those commonly observed with schizophrenia in humans. Prenatal stress was associated with hyperlocomotion and attenuated weight gain in 90 day old male, but not 90 day old female, offspring of dams that were stressed during gestation. Baseline startle and prepulse inhibition were equivalent across all groups at this age. Cross-fostering male pups from gestation-stressed to non gestation-stressed dams on day 3 postpartum mitigated the hyperlocomotion and the attenuated weight gain observed in prenatally-stressed pups.The maternal behavior of gestation-stressed dams had no significant effect on pups cross-fostered from non gestation-stress dams. These data suggests that certain behavioral and physical changes that have been associated with schizophrenia are also observed in the adult male offspring birthed and raised by dams exposed to stress during gestation.

Disclosures: H. Bauerle: None. **D.J. Toufexis:** None.

Poster

498. Development of Motor, Sensory, and Limbic Systems

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 498.06/B57

Topic: A.07. Development of Motor, Sensory, and Limbic Systems

Title: Volumetry of the human memory circuit: Differential effect of age on hippocampal subfields and associated white matter volumes

Authors: *R. S. AMARAL, JR[1], M. T. M. PARK[1], J. PRUESSNER[2], J. PIPITONE[1], J. WINTERBURN[1,3], S. CHAVEZ[4,1], M. SCHIRA[7], N. LOBAUGH[1,5], J. P. LERCH[8,6], A. VOINESKOS[1,4], M. CHAKRAVARTY[1,3,4]
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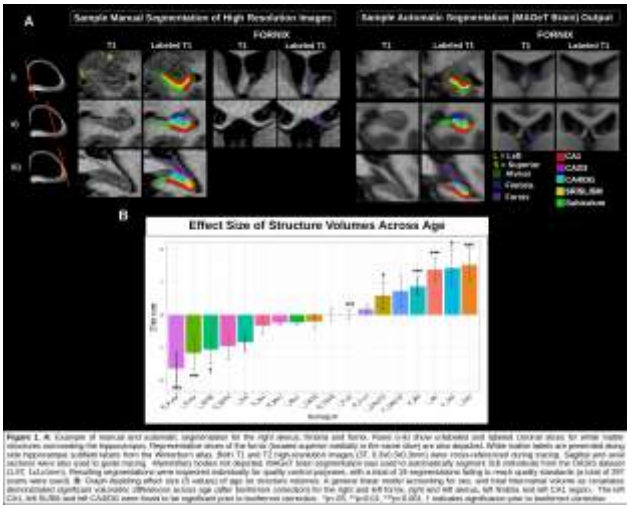
Abstract: INTRODUCTION The human memory circuit includes thin white matter projections (alveus and fimbria), which emanate from inside the hippocampus (HC) and lead out of the medial temporal lobe via the fornix. The volumetric trajectory of the HC subfields and, in particular, the above-mentioned white matter structures (WM) have received little attention with respect to healthy aging. Using precise manual and automatic segmentation methods, our aim was to identify if any volumetric changes in constituents of this circuit are associated with healthy aging. METHODS High-resolution T1 and T2-weighted MRI images were acquired for 5 healthy controls (mean age=37 years). A novel tracing protocol that identified the alveus, fimbria, fornix, and mammillary bodies was created and used in conjunction with the Winterburn HC subfield atlas (Fig A). MAGeT Brain segmentation, previously validated for use in medial temporal lobe, was used to automatically segment WM and HC subfields using the high-resolution atlases on 297 healthy individuals aged 19-94 (mean age=44.85 ± 23.43) from the OASIS dataset (See Fig A for sample output segmentation). RESULTS A general linear model accounting for sex, and intracranial volume indicated a significant decrease in left and right fornix volume with age (respectively; $t=-3.27$, $p<0.001$; $t=-4.11$, $p<0.001$). The left and right alveus (respectively; $t=7.16$, $p<0.001$; $t=6.58$, $p<0.001$) and left CA1 subfield ($t=4.75$, $p<0.001$) demonstrated significant increases in volume across age, maintaining the largest effect sizes (Fig B). No significant differences were observed in the fimbria, whole-HC or combined WM volumes. CONCLUSION Here we present evidence of bilateral increases in CA1 and alveus volumes and decreased fornix volume during healthy aging. Previous studies have demonstrated preservation of the CA1 subfield in healthy aging; however, the volumetric trajectory of HC WM remains relatively elusive. Our results suggest preservation of such structures may be linked to a decreased likelihood of developing neuropsychiatric disorders (e.g. Alzheimer's Disease).



Disclosures: **R.S. Amaral:** None. **M.T.M. Park:** None. **J. Pruessner:** None. **J. Pipitone:** None. **J. Winterburn:** None. **S. Chavez:** None. **M. Schira:** None. **N. Lobaugh:** None. **J.P. Lerch:** None. **A. Voineskos:** None. **M. Chakravarty:** None.

Poster

498. Development of Motor, Sensory, and Limbic Systems

Location: Halls A-C

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Program#/Poster#: 498.07/B58

Topic: A.07. Development of Motor, Sensory, and Limbic Systems

Title: Environmental enrichment differentially affects the perinatal and adult mouse brain

Authors: *R. ALLEMANG-GRAND[1,2], J. SCHOLZ[1], E. LANGILLE[1], D. FERNANDES[1,2], J. P. LERCH[1,2]
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Abstract: Introduction: Physically and cognitively stimulating environments affect the nervous system throughout the lifespan. During perinatal life, housing in an enriched environment (EE) accelerates the maturation of cellular and behavioural measures of murine brain development. Additionally, exposing adult mice to EE triggers neurogenesis and neuroplasticity. In this study, *in vivo* magnetic resonance imaging (MRI) is used to compare the neuroanatomical changes that occur following exposure to EE during perinatal life and in adulthood. Methods: Pregnant CD-1 dams were housed in enriched environments (multi-level maze and running wheel in a large rat

cage) or standard housing (no maze or wheel) conditions. Pups lived in the cage until weaning, at which point they were assigned into the same environment they were raised. Pups were longitudinally scanned at 2.5, 3.5 and 5 weeks of age with a manganese-enhanced MRI (MEMRI) protocol to acquire high-resolution images of the brain. Images acquired at each time point were registered and deformed to generate a consensus average. A mixed effects model was computed at each voxel relating the volume to the fixed effects of age and group. Multiple comparisons controlled for with a false discovery rate (FDR). In the second part of the study, 7 week old C57Bl6 mice were exposed to EE for 3 weeks and scanned with MEMRI. Results: At 2.5 weeks of age, enriched pups had volume increases in regions of the hippocampus and cerebellum as well as volume reductions in the cortex (10% FDR). Interestingly, the trajectory of these differences between enriched and control mice did not change by 3.5 and 5 weeks of age. Separately in adults, a significant interaction (time point by group) was found in the hippocampus and retrosplenial cortex between the two imaging time points in mice exposed to EE. We are currently attempting to replicate these findings in adult CD-1 mice for comparative purposes with the cohort of perinatal EE CD-1 mice. Future work: We are currently scanning 1 week old pups to determine whether the early life neuroanatomical changes are caused by exploration in the enriched cage, or developmentally programmed via an epigenetic mechanism. Our findings suggest that perinatal EE increases the volume of the hippocampus and decreases the volume of the cortex, while adult EE leads to localized volume increases in the hippocampus and retrosplenial cortex.

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Poster

498. Development of Motor, Sensory, and Limbic Systems

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Topic: A.07. Development of Motor, Sensory, and Limbic Systems

Support: NSERC Grant

NSERC Graduate scholarship

Title: Maternal programming of mice offspring by prenatal exposure to predator odor

Authors: *S. ST-CYR, P. O. MCGOWAN
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Abstract: In rodents, maternal programming of offspring phenotype using classical stressors (e.g. restraint, handling) or of combinations of multiple stressors (chronic variable stress) has been extensively documented. Imposing these stressors during development of early life typically leads to offspring that show an increase in anxiety-like behavior and alterations in related neural pathways. However, the precise somatosensory impact or ecological relevance of these stressors is not always clear. In this study, we used a prenatal stressor that is evolutionarily and ecologically relevant to mice, uniform in its intensity, psychological and unconditioned: predator odor. Pregnant mice dams were exposed to 3 different predator odors daily or distilled water (control) over the second half of their pregnancy when the fetal stress axis is developing. Subsequently, levels of maternal behavior in dams were measured. Furthermore, anxiety-like, sociality and anti-predatory behaviors as well as endocrine stress axis reactivity and gene expression levels were assessed in stress-related brain areas in adult male and female offspring. Predator odor exposure lead to an increase in maternal nest quality while offspring in adulthood showed an increase in anxiety-like behavior in a standardized test (EPM), increased social investigation and affiliation, increased anti-predator behavior and stress axis reactivity in response to predator odor exposure. A number of genes were also found to differ in their expression in key limbic structures, the hippocampus and amygdala. Some genes which showed altered expression are documented targets of early-postnatal stress while others constitute novel markers potentially specific to prenatal predator odor exposure. Consequently, prenatal exposure to a potent ecologically relevant stressor leads to an altered integrative phenotype on a behavioral, physiological and functional genetic level. Future work will examine potential epigenetic mechanisms mediating the persistent effects of prenatal predator odor exposure on the adult phenotype.

Disclosures: S. St-Cyr: None. **P.O. McGowan:** None.

Poster

498. Development of Motor, Sensory, and Limbic Systems

Location: Halls A-C

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Topic: D.10. Spinal Cord Injury and Plasticity

Support: Pfizer Neuropathic Pain Research Award

Title: Interactions of kinins and TRPV1 in a model of neonatal mouse spinal cord inflammation

Authors: *S. MANDADI[1], P. HONG[3], H. LEDUC-PESSAH[2], J. EJDRYGIEWICZ[2], T. TRANG[2], P. J. WHELAN[2]
[1]Hotchkiss Brain Inst., [2]Comparative Biol. & Exptl. Med., Univ. of Calgary, Calgary, AB, Canada; [3]Med., Univ. of Alberta, Edmonton, AB, Canada

Abstract: The impact of spinal cord inflammation on the operation of locomotor networks during development is poorly understood. We show that the kinin-induced changes to central sensitization of spinal nociceptive networks occur perinatally. Furthermore, conditions of the plasticity of kinin receptors induced functional changes in spinal locomotor networks. We investigated the role of kinins (bradykinin (BK) and des-Arg9-bradykinin (DABK)) and their receptors (Bradykinin-2 receptor (B2R) and Bradykinin-1 receptor (B1R)), a neurotrophin (glial cell-derived neurotrophic factor (GDNF)) and transient receptor potential vanilloid 1 (TRPV1) in altering sensorimotor function. Immunohistochemical and pharmacological evidence showed expression of B2R, but not B1R, in the dorsal and ventral horns of naïve animals. Basal B2R expression was downregulated following either an intrathecal or an *in vitro* spinal cord application of BK (B2R agonist). Basal B1R expression was upregulated by an intrathecal injection of GDNF. In hind limb attached to spinal cord (HL) preparations, application of a noxious heat stimulus (> 42 °C) to the hind paw with simultaneous activation of B2R by BK in the spinal cord resulted in a transient disruption of the locomotor rhythm. Conversely, application of noxious heat to the hind paw in the presence of DABK, a B1R agonist, caused a sustained disruption of the locomotor rhythm in spinal cords with upregulated B1R. The BK- or DABK-induced hypersensitivity of locomotor networks to noxious heat was abolished in the HL preparations from trpv1-/- mice. B2R- or B1R-induced hypersensitivity was inhibited in the presence of the protein kinase C blocker, chelerythrine. Using a neonatal model of spinal cord injury, we are characterizing the changes in the expression of GDNF, kinin receptors and activation of glia. These results suggest that early injury and plasticity in the sensorimotor networks may impact normal development of sensorimotor function.

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Poster

498. Development of Motor, Sensory, and Limbic Systems

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Topic: A.07. Development of Motor, Sensory, and Limbic Systems

Support: NIMH Grant RC2MH089921

Allen Institute for Brain Science

Title: Molecular architecture of the developing and adult human hippocampal formation

Authors: *S.-L. DING, J. J. ROYALL, A. BONGAARTS, R. DALLEY, P. D. PARKER, M. HAWRYLYCZ, A. R. JONES, E. S. LEIN, J. G. HOHMANN
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Abstract: The hippocampal formation (HF) including the dentate gyrus (DG), hippocampus proper (CA1-4), and subicular complex [prosubiculum (ProS), subiculum (Sub), presubiculum/postsubiculum (PrS/PoS) and parasubiculum (PaS)] is well established for its importance in learning and memory, and in the processing of emotion and anxiety. The HF is also implicated in many major neurological and psychiatric diseases such as Alzheimer's disease, epilepsy and schizophrenia. Although intensive studies have been carried out in normal and abnormal conditions for various species, there remains very limited information on the molecular architecture of the different components in the developing and adult human HF. To gain insight into molecular cell types across sub-regions, layers, anterior-posterior axis and developmental stages of human HF, we have investigated and compared gene expression patterns of mid-gestational, postnatal and adult human HF using *in situ* hybridization data from the Allen Human Brain Atlas and Brainspan Atlas of the Developing Human Brain. In mid-gestation (post-conception week15-21), many genes are expressed in the cortical plate of HF including FADS2, FOXG1, FOXP1, ENC1, NRGN, LMO4, GAP43, ETV1 and FEZF2, although region-specific expression is not obvious. In adult, conspicuous region-specific gene expression was observed in the DG (NPY1R, CALB1, PDYN, PCP4, BDNF, CD8, TRHR, GABRD, GRM1 and GRM7), CA3 (SLC1A1, CNR1, GRIN2A, BDNF, GRIK5 and HTR2C), CA2 (CALB1, PCP4 and RGS4), CA1 (HTR2A and CARTPT), ProS (TH and NTS), Sub (GFRA1, RGS4, ABAT, CDH11, HTR2C, GRIK1 and GRIK2), PrS/PoS (PDYN, PENK, GRIA4, NEUROD6 and HTR2A,) and PaS (MAOB and HTR2C). Sub-layer enriched gene expression was also detected in the principal layers of CA1 (HTR1A and HTR2C in the upper part, and PCP4 in the deep part) and Sub (NEUROD6 in the upper part, and PCP4 & CHRM2 in the deep part). Some genes (e.g. PDYN, CARTPT, HTR1A and GABRG1) were expressed preferentially in the most anterior (uncal) portion of HF while others were enriched in more posterior HF. During postnatal development (3 months -17years), expression patterns of certain genes (CALB1 and PDYN) were similar to that in adult, while others (PENK and HTR2C) differed between the developing and adult HF. All data for this study is publicly available at www.brain-map.org.

Disclosures: **S. Ding:** None. **J.J. Royall:** None. **A. Bongaarts:** None. **R. Dalley:** None. **P.D. Parker:** None. **M. Hawrylycz:** None. **A.R. Jones:** None. **E.S. Lein:** None. **J.G. Hohmann:** None.

Poster

498. Development of Motor, Sensory, and Limbic Systems

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 498.11/C2

Topic: A.07. Development of Motor, Sensory, and Limbic Systems

Support: NIH RO1 NIDA201040

NIH RO1 NIDCD12020

Title: Dbx1 links embryonic development of the medial amygdala to innate behavior

Authors: S. ESUMI[1], K. SOKOLOWSKI[2], Y. KAMAL[3], J. LISCHINSKY[4], D. FELDMAN[4], P. LI[4], A. PIERANI[5], N. TAMAMAKI[1], N. SHAH[6], M. HUNTSMAN[7], *J. CORBIN[4]
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Abstract: Innate behaviors in mammals are mediated by a connected circuit of limbic system structures, which most prominently includes the olfactory system, bed nucleus of stria terminalis (BNST), medial amygdala (MeA) and hypothalamus. As innate behaviors are inborn, there must be embryonic genetic mechanisms that encode establishment of innate circuits. However, how these circuits are established from development remains unknown. Our previous work (Hirata et al., Nature Neuroscience, 2009; Carney et al., Neural Development, 2010) revealed a one to one relationship between a specialized ventral telencephalic progenitor niche marked by expression of the developmentally regulated homeodomain transcription factor Dbx1, and the generation of an electrophysiologically homogenous population of neurons in the MeA. Here we further demonstrate that Dbx1-derived neurons express a unique profile of genes implicated in sexually dimorphic behaviors, and in a complementary pattern to genes expressed by neighboring FoxP2+ MeA neurons. Furthermore, consistent with their restricted electrophysiological and molecular

profiles, Dbx1-derived neurons are selectively activated by restricted subsets of innate behaviors. Thus, parcellation of MeA subpopulations based on developmental genetics predicts electrophysiological, molecular and behavioral specificity.

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Poster

498. Development of Motor, Sensory, and Limbic Systems

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 498.12/C3

Topic: A.07. Development of Motor, Sensory, and Limbic Systems

Support: Spanish MINECO grant BFU2012-33029 to L.M.

MCINN predoctoral fellowship BES-2010-038400 to A.V.

Title: Identification of the central extended amygdala of chicken based on gene expression patterns and fate mapping analysis

Authors: A. VICARIO, A. ABELLÁN, *L. MEDINA
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Abstract: In mammals, the central extended amygdala is a cell corridor of the basal telencephalon involved in control of fear responses. Developmental data in mouse have demonstrated that it shows a mosaic-like organization with different neurons coming from distinct embryonic domains and showing a different genetic profile (Waclaw et al., 2010; Bupesh et al., 2011). In the central amygdala neurons coming from the dorsal lateral ganglionic eminence (dLGE) express Pax6 and primarily populate the capsular subdivision, overlapping with pre-proenkephalinergic (ppENK) neurons; those coming from the ventral lateral ganglionic eminence (vLGE) express Islet1 and populate lateral and medial subdivisions, partially overlapping with different peptidergic neurons, including those expressing corticotropin-releasing factor (CRF); neurons coming from the medial ganglionic eminence (MGE) express Nkx2.1 and somatostatin (SOM), and primarily concentrate in the medial subdivision. The avian central extended amygdala is poorly known, and a central amygdalar nucleus has not been found yet. Our aim was to identify the components of the central extended amygdala in the developing

brain of chicken by analyzing the expression of cPax6, cIslet1, cppENK, cCRF and cSOM, and by experimental fate mapping. We observed two distinct cell masses at caudal telencephalic levels expressing either Pax6 or Islet1 that appeared to derive from the dLGE-like or vLGE-like striatal domains, respectively. We called these two chicken subdivisions the central capsule (CeC, rich in Pax6) and the oval central nucleus (Ce-ov, rich in Islet1). Both subdivisions include neuron subpopulations expressing either cppENK or cCRF. Another cell mass, the perioval zone, appeared to extend from the pallidal domain into the central amygdala, and became interposed between the CeC and the Ce-ov. This area contains many cppENK- and some cSOM-expressing cells. Our *in vitro* migration assays combined with immunofluorescence showed that the dorsal striatal domain produces cPax6-expressing neurons that mainly populate the CeC, whereas the ventral striatal domain produces cIslet1-expressing cells, populating the Ce-ov. Regarding the chicken lateral bed nucleus of the stria terminalis (BSTL), like that of mammals, it also contains neuron subpopulations coming from the pallidal or the striatal subdivisions (both dLGE- and vLGE-like), although in different proportions and locations. In conclusion, we identified the central amygdala and the BSTL in chicken, having subcomponents similar to those present in mammals based on genetic profile and embryonic origin.

Disclosures: **A. Vicario:** None. **L. Medina:** None. **A. Abellán:** None.

Poster

498. Development of Motor, Sensory, and Limbic Systems

Location: Halls A-C

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Program#/Poster#: 498.13/C4

Topic: A.07. Development of Motor, Sensory, and Limbic Systems

Support: Badgley Grant (MMC)

Title: Developmental differences in psychophysiological responses to music

Authors: J. ANCELLE, R. TIETZE, S. WEINBERGER-LITMAN, *D. A. HUNTER
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Abstract: The use of music as a therapeutic tool can be traced to ancient preliterate cultures. However, it was not until recently that music therapy was recognized as an effective treatment for various neurological disorders such as non-fluent aphasia, memory and communication deficits associated with dementia and limited emotional processing in autism. Research in the field has been steadily growing since, but much still needs to be understood regarding the effects

of music on the brain and body. During early adolescence, the rapidly maturing limbic system parallels the increasing capacity to create emotions. The prefrontal cortex, responsible for the ability to make rational judgments is one of the last regions to mature. Therefore, the regulation of emotions is less restrained and often contributes to long lasting and robust emotional relationships made during this time. The objective of this study was to determine which time period (adolescence or adulthood) evokes the strongest emotions to self-selected music using psychophysiological measures (heart rate-HR and electrodermal responses -EDR) as a measure of quantifying emotions. To this end, male or female participants ranged in age from 18-72 years of age (N=16) Participants were asked to complete a background questionnaire that consisted of demographic questions and a request for a list of 4 songs that they developed a strong connection to during adolescence (12 to 17 years of age) and adulthood (>18). Two songs were randomly selected from each category and played to the participants in a randomized order while simultaneous recordings of psychophysiological responses were recorded. Psychophysiological responses were compared with a self-report questionnaire accessing subjective evaluations of felt emotions to music selections. Preliminary results show that music altered HR and EDR relative to baseline and there was trend reflecting stronger EDR responses to music that corresponded to the participant's adolescence. A better understanding of psychophysiological responses to self-selected music should help design better therapeutic approaches to treating individuals using music therapy to help alleviate deficits incurred via neurological damage.

Disclosures: J. Ancelle: None. **R. Tietze:** None. **S. Weinberger-Litman:** None. **D.A. Hunter:** None.

Poster

498. Development of Motor, Sensory, and Limbic Systems

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 498.14/C5

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

Support: NIH Grant NS057690 to NCS and DD

Title: Peptide-signalling and miRNA regulation of transmitter specification affecting kinship recognition

Authors: *D. DULCIS[1,2], G. LIPPI[1,2], L. H. DO[1], D. K. BERG[1,2], N. C. SPITZER[1,2]
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Abstract: Activation of sensory circuits regulates the number of interneurons that express specific neurotransmitters (NT) in the brain, as shown in the hypothalamus following stimulation of the retino-hypothalamic projection and more recently in accessory olfactory bulb (AOB) interneurons after activation of the olfactory nervous system. Frogs use olfactory-mediated kin recognition to distinguish siblings from non-siblings via kinship cues; 4-day old Xenopus larvae display attraction/aversion behavior to water-borne kin (K)/nonkin (NK) odorants, respectively. AOB interneurons were identified by immunocytochemical markers for dopamine (tyrosine hydroxylase, TH) and GABA. Following 48 hr exposure to K (sibling condition) or NK (non-sibling condition) odorants, the extent of TH and GABA expression in the AOB changed dramatically, compared to odorant deprivation (orphan condition, O). We found that altering the ratio of dopamine/GABA coexpression in AOB interneurons, as well as blocking their receptors, reverses kinship recognition behavior. To identify the signalling molecules that mediate kinship recognition we performed comparative mass spectrometry analysis of K- and NK-conditioned water samples. We identified two vitellogenin-derived peptides (PP1, AVILNGFPESGLS; PP2, VVVNPHEAQAS) that were uniquely present in kinship-conditioned samples of one genotype. Behavioral tests showed that PP1 is sufficient to elicit aversion behavior in NK larvae and that its signaling specificity is lost when the amino acid sequence is scrambled or swapped. MicroRNA (miR) regulates many aspects of development. To test miR involvement in regulating NT switching we sequenced RNA from AOB tissue of animals exposed to K, NK, and O conditions and identified 183 miRs based on their predicted targets, 20 of which were differentially regulated across conditions. The miRs showing a substantial fold change matching the up- or down-regulation expected according to the kinship-dependent NT phenotype switch were selected for qPCR validation. To screen validated miR candidates for their role in NT respecification *in vivo*, locked nucleic acid (LNA) miR inhibitors were delivered via electroporation in the AOB during the sensory-induced changes in NT expression. Our results show that miR-375 is a potent inhibitory regulator of the dopaminergic phenotype. We are currently testing miR candidates expected to regulate the GABA phenotype. Understanding the molecular machinery regulating this form of chemosensory-induced neuroplasticity affecting behavior may be relevant to behavioral states associated with neurological disorders that involve abnormal expression of NTs.

Disclosures: D. Dulcis: None. **G. Lippi:** None. **L.H. Do:** None. **D.K. Berg:** None. **N.C. Spitzer:** None.

Poster

498. Development of Motor, Sensory, and Limbic Systems

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 498.15/C6

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

Support: UCSD/Salk biology graduate program to DM

Ellison Medical Foundation and WM Keck Foundation to NCS and DD

Title: Neurotransmitter switching in single neurons in the adult rat brain

Authors: *D. MENG[1,2], D. DULCIS[1,2], S. LEUTGEB[1,2], K. DEISSEROTH[3], N. C. SPITZER[1,2]
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Abstract: The nervous system responds to changes in endogenous activity and the external environment by modulating its function through various forms of neuroplasticity, many of which occur at synapses. However, it has been believed that neurotransmitters are fixed and invariant in the brain. Recent work has demonstrated activity-dependent transmitter respecification in neuronal populations both in the developing and adult nervous system. For example, the numbers of dopaminergic (DA) neurons and somatostatin (SST) neurons in the hypothalamus of the adult rat change in response to altered photoperiods. Long days (19hr:5hr L:D) decrease the number of DA neurons and increase the number of SST neurons by the same amount, which leads to depression-like behaviors. Short days (5L:19D) elicit the opposite effects. Can single neurons switch their transmitter? Despite evidence demonstrating transmitter switching in neuronal populations, it remains unclear whether this form of plasticity takes place by single neuron reprogramming in the adult brain. We have taken a genetic approach to permanently label DA neurons, by injecting the hypothalamic paraventricular nuclei (PaVN) of tyrosine hydroxylase (TH)::Cre rats with a Cre-dependent AAV virus expressing EYFP. The originally inverted viral EYFP sequence is flipped in neurons expressing TH, and EYFP expression is driven by an internal promoter independent of Cre. Since EYFP expression persists during long-day exposure when PaVN DA neurons lose TH expression, formerly DA neurons can be identified. Following viral injection and long-day exposure, brains were stained for TH and SST and compared to controls (12L:12D). If transmitter switching takes place only within single neurons, the formerly DA neurons (EYFP+) will lose TH and express SST instead. In contrast, if transmitter switching occurs only at the population level, the formerly DA population and the newly acquired SST population will remain separate. Our results indicate that EYFP+SST+TH- neurons are present following 19L:5D exposure, indicating that these formerly DA neurons have acquired the SST phenotype. Additionally, EYFP+SST-TH- neurons and EYFP-SST+TH- neurons are also present, consistent with switching at the population level. We are quantifying these results with multichannel fluorescence stereology. These findings identify an unexpected flexibility of the hardwired connectome. Since transmitter respecification can involve a switch between excitatory

and inhibitory transmitters that alters behavior, this activity-dependent plasticity may explain a variety of normal behaviors and neurological disorders.

Disclosures: D. Meng: None. **D. Dulcis:** None. **S. Leutgeb:** None. **K. Deisseroth:** None. **N.C. Spitzer:** None.

Poster

499. Evolution of Developmental Mechanisms

Location: Halls A-C

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Program#/Poster#: 499.01/C7

Topic: A.09. Evolution of Developmental Mechanisms

Support: JST.PRESTO

Grant-in-Aid for Challenging Exploratory Research (24657158)

Title: Convergent evolution of pallial basal progenitors in amniotes

Authors: *T. NOMURA[1,2], W. YAMASHITA[3], F. CALEGARI[4], Y. MURAKAMI[5], H. GOTOH[1], K. ONO[1]
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Abstract: The emergence of bigger brains with enormous numbers of neurons is an evolutionary innovation in amniotes, particularly mammals and birds. However, the corresponding changes in cortical developmental programs during amniote evolution are poorly understood. In the developing mammalian neocortex, neural stem/progenitors in the ventricular zone give rise to basal progenitors (BPs) including Tbr2-positive intermediate progenitors and Pax6-positive outer radial glial cells (oRGs), which contribute to enhanced neurogenesis and encephalization. Here we show that various progenitor populations also exist in the developing avian pallium. Interestingly, avian BPs exhibit mammalian oRGs characteristics, which are distiguished from Tbr2-positive cell population. Manipulation of regulatory genes for progenitor dynamics such as CDK4/CyclinD1 provided distinct outcomes in the mammalian and avian pallium. Furthermore, we identified basal mitotic cells in non-avian reptiles and also amphibians, which implies the origins of progenitor diversities. Our results suggest that specific BPs have evolved

independently in mammalian and avian lineages, by modifying ancestral regulatory mechanisms for neural stem/progenitor maintenance during amniote evolution.

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Poster

499. Evolution of Developmental Mechanisms

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

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Topic: D.19. Comparative Anatomy and Evolution

Support: James S. McDonnell Foundation Grant 22002078

James S. McDonnell Foundation Grant 220020293

National Science Foundation Grant BCS-0827531

South African National Research Foundation Grant FA2005033100004

Danish Cardiovascular Research Program

Colorado College

Title: Comparative neuronal morphology of the cerebellar cortex in afrotherians, carnivores, cetartiodactyls, and primates

Authors: *B. G. JACOBS[1], N. L. JOHNSON[1], D. WAHL[1], C. B. JOHNSON[1], D. MOHR[1], B. KOPEC[1], M. SCHALL[1], B. C. MASEKO[2], A. H. LEWANDOWSKI[3], M. A. RAGHANTI[4], B. WICINSKI[5], C. BUTTI[5], W. D. HOPKINS[6], M. F. BERTELSEN[7], T. WALSH[8], J. R. ROBERTS[8], R. L. REEP[9], P. R. HOF[5], C. C. SHERWOOD[10], P. R. MANGER[2]
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Abstract: Although the basic morphological characteristics of neurons in the cerebellar cortex have been documented in several species, virtually nothing is known about the quantitative morphological characteristics of these neurons across different taxa. To that end, the present study investigated cerebellar neuronal morphology among eight different, large-brained mammalian species comprising a broad phylogenetic range: afrotherians (African elephant, Florida manatee), carnivores (Siberian tiger, clouded leopard), cetartiodactyls (humpback whale, giraffe) and primates (human, common chimpanzee). Specifically, several neuron types (e.g., stellate, basket, Lugaro, Golgi, and granule neurons; N = 317) of the cerebellar cortex were stained with a modified rapid Golgi technique and quantified on a computer-assisted microscopy system. There was a 64-fold variation in brain mass across species in our sample (from clouded leopard to the elephant) and a 103-fold variation in cerebellar volume. Most dendritic measures tended to increase with cerebellar volume. The cerebellar cortex in these species exhibited the trilaminate pattern common to all mammals. Morphologically, neuron types in the cerebellar cortex were generally consistent with those described in primates (Fox et al., 1967) and rodents (Palay and Chan-Palay, 1974), although there was substantial quantitative variation across species. In particular, Lugaro neurons in the elephant appeared to be disproportionately larger than those in other species. To explore potential quantitative differences in dendritic measures across species, MARSplines analyses were used to evaluate whether species could be differentiated from each other based on dendritic characteristics alone. Results of these analyses indicated that there were significant differences among all species in dendritic measures.

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Poster

499. Evolution of Developmental Mechanisms

Location: Halls A-C

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Program#/Poster#: 499.03/C9

Topic: A.09. Evolution of Developmental Mechanisms

Support: Eunice Shriver Kennedy NICHD Grant R01HD078561

Eunice Shriver Kennedy NICHD Grant R21HD069001

James S. McDonnell Foundation grant 220020293

Title: Evo-devo and the cortical connectome highlights systematic changes in frontal connections across primates

Authors: *C. J. CHARVET[1], A. VAN DER KOUWE[2], W. D. HOPKINS[3], P. R. HOF[4], C. C. SHERWOOD[5], E. TAKAHASHI[1]
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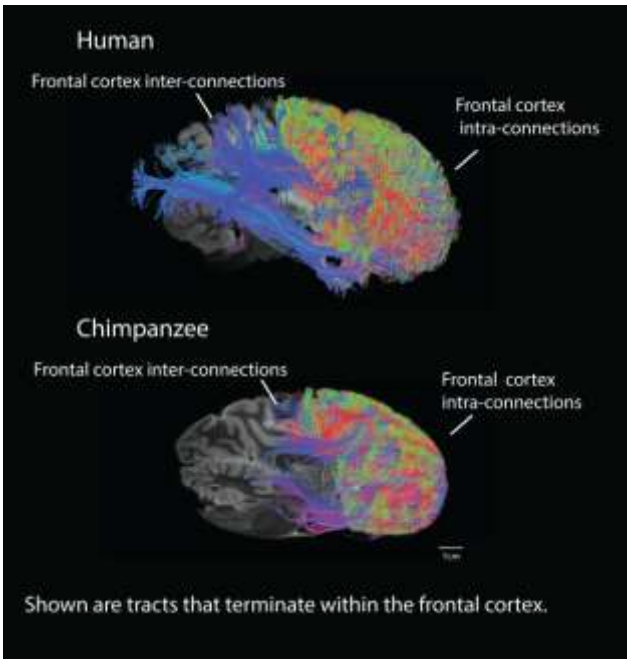
Abstract: Gradients in developmental maturation across the rostro-caudal axis of the isocortex align with gradients in adult anatomical features as well as a hierarchy in cognitive processing. The maturation (e.g., myelination) of the frontal cortex is protracted compared with more caudal regions (e.g., occipital cortex). By adulthood, neurons have more elaborate dendritic branches towards the frontal cortex and it mediates more abstract processing than more caudal regions. However, very little is known about how evolutionary changes across these developmental gradients account for systematic changes in connections across the isocortex. To that end, we used HARDI (high-angular resolution diffusion MRI) tractography to compare the strength of connections across the isocortex of several primate species (e.g., marmoset, *Callithrix jacchus*; chimpanzee, *Pan troglodytes*; orangutan, *Pongo pygmaeus*; human, *Homo sapiens*). These species were chosen because they vary in their brain size and length of developmental schedules. We placed regions of interest through the frontal, temporal, parietal and occipital lobes and estimated the density of connections within and between each of these lobes. We found that as brains expand and overall developmental schedules lengthen, proportionally more tracts arise and terminate within the frontal cortex than in more caudally situated regions (e.g., occipital lobe). Lengthened developmental schedules may promote increased time for the formation of within-frontal cortical connections, resulting in increased frontal lobe within-connectivity in adulthood. More generally, these findings highlight that, as primate brains expand, the isocortex increases the integration of information within the frontal cortex.



Disclosures: C.J. Charvet: None. **A. van der Kouwe:** None. **W.D. Hopkins:** None. **P.R. Hof:** None. **C.C. Sherwood:** None. **E. Takahashi:** None.

Poster

499. Evolution of Developmental Mechanisms

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Program#/Poster#: 499.04/C10

Topic: D.19. Comparative Anatomy and Evolution

Support: Grant BCS-0827531

NS-042867

Title: Uncovering the genetic differences of hemispheric lateralization in humans

Authors: *G. MUNTANÉ[1,2], G. SANTPERE[1], A. VERENDEEV[2], A. BAUERNFEIND[3], A. NAVARRO[1], W. D. HOPKINS[4], C. C. SHERWOOD[2]
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Abstract: A prominent asymmetrical brain function in humans is preferential handedness, with more than 90% of the population showing more skillful usage of the right hand, which is controlled by the left hemisphere. The left hemisphere is also dominant for language, whereas the right hemisphere excels in spatial recognition. Even though genetic models of human handedness and language have been proposed, the actual gene that underlie these functions in humans have not yet been identified. In the current project we used microarrays in both humans and macaque monkeys to examine gene expression profiles in three cortical regions involved in language or handedness: inferior frontal cortex and posterior superior temporal cortex (which in humans correspond to Broca's area and Wernicke's area, respectively) and primary motor cortex. As follow up, levels of candidate genes were confirmed using other techniques such as RT-PCR and immunohistochemistry. The results demonstrated that the overall pattern of gene expression is very similar between hemispheres in both humans and macaques. Therefore, weighted gene correlation network analysis (WGCNA) was used to find clusters (modules) of highly correlated genes. WGCNA showed that human inferior frontal cortex and posterior superior temporal cortex present weaker module preservation between hemispheres. The method also facilitated the identification of hub genes in these modules. In sum, bioinformatic analyses of these data revealed that the human and macaque monkey's interhemispheric expression profiles are very similar, although subtle differences appear in human Wernicke's and Broca's areas at the level of gene co-expression networks.

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Poster

499. Evolution of Developmental Mechanisms

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Program#/Poster#: 499.05/C11

Topic: D.19. Comparative Anatomy and Evolution

Support: NSF BCS-BCS-0827531

NSF DGE-0801634

James S. McDonnell Foundation 22002078

James S. McDonnell Foundation 220020293

Title: Asymmetry in the inferior parietal lobe in chimpanzees and its relationship with handedness

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Abstract: The inferior parietal lobe (IPL) is associated with tool-use in humans and non-human primates and can be divided into rostral and caudal regions. The rostral portion is involved in the execution and observation of grasping, and the caudal portion that is active during visually guided arm and hand movements. Previous studies have indicated that areas associated with tool-use such as the planum temporale (PT) and parietal operculum (PO) have population-wide leftward asymmetry in chimpanzees and humans, with an increased magnitude of leftward PT asymmetry for right-handed individuals. This study examines if the chimpanzee IPL and its rostral and caudal parts show a similar pattern of asymmetry and link with handedness. The sample consisted of T1-weighted structural MRI scans from 30 chimpanzees from Yerkes National Primate Research Center (23F, 7M, age = 22.1 +/- 2.7 yrs). Volume was calculated for the whole IPL, rostral IPL, and caudal IPL bilaterally. IPL regions of interest were based on Bailey et al. (1950)'s map of histologically parcellated areas in the chimpanzee brain. Asymmetry quotients (AQ) were calculated for each measure using the formula (R-L)/[(R+L)/0.5]. Handedness was based on hand preference for a simulated termite fishing task. Chimpanzees exhibit population-wide leftward asymmetry for the entire IPL and caudal IPL, and rightward asymmetry for the rostral IPL. IPL asymmetries were not correlated with handedness, and there was no relationship between magnitude of IPL asymmetry and handedness. The IPL and PO appear to follow a similar pattern of population-wide asymmetry that is not correlated with handedness. Although tool-use relies on the use of the hand and arm, the processing of sensory and motor information is highly integrative and relies on interconnectivity with other brain regions. This interconnectivity may have resulted in hemispheric specialization in tool-use circuits independent of handedness.

Disclosures: L.D. Reyes: None. **C.C. Sherwood:** None. **W.D. Hopkins:** None.

Poster

499. Evolution of Developmental Mechanisms

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 499.06/C12

Topic: D.19. Comparative Anatomy and Evolution

Support: NSF IGERT DGE-0801634

NSF HOMINID BCS-08-27552

Wenner-Gren Foundation

Title: High spatial resolution proteomic comparison of the brain in humans and chimpanzees

Authors: *A. L. BAUERNFEIND[1], M. L. REYZER[2], R. M. CAPRIOLI[2], J. J. ELY[3], C. C. BABBITT[4], G. A. WRAY[5], P. R. HOF[6], C. C. SHERWOOD[1]
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Abstract: We performed high-throughput mass spectrometry in individual regions (anterior cingulate, primary motor, primary sensory, and primary visual) and layers (III, IV, and V) of the neocortex, in the cerebellum (granule cell layer), as well as in the caudate nucleus in humans and chimpanzees. A total of 39 mass spectrometry peaks were matched with probable protein identifications in both species, allowing for a direct interspecific comparison. We explored how the pattern of expression of these proteins varies across regions and cortical layers to provide insights into the differences in biological function of these neural structures between species. Molecular signatures in the expression of proteins differed principally in a regional and laminar-specific pattern, while the expression of proteins that differentiate species were less pronounced. Specifically, human and chimpanzee brains are fundamentally similar in their distribution of proteins related to the regulation of transcription and enzyme activity but differ more markedly in their expression of proteins supporting aerobic metabolism. While most work of molecular expression differences in the brains of primates has been performed on gene transcripts, our unique dataset extends current understanding of differential molecular expression patterns that may underlie human cognitive specializations.

Disclosures: A.L. Bauernfeind: None. **M.L. Reyzer:** None. **R.M. Caprioli:** None. **J.J. Ely:** None. **C.C. Babbitt:** None. **G.A. Wray:** None. **P.R. Hof:** None. **C.C. Sherwood:** None.

Poster

499. Evolution of Developmental Mechanisms

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 499.07/C13

Topic: D.19. Comparative Anatomy and Evolution

Support: James S. McDonnell Foundation (220020293)

Title: Differences in serotonin transporter expression in the amygdala of bonobos (Pan paniscus) and chimpanzees (Pan troglodytes): Implications for behavior

Authors: *C. D. STIMPSON[1], W. D. HOPKINS[2,3], J. P. TAGLIALATELA[4,3], N. BARGER[5], P. R. HOF[6], C. C. SHERWOOD[7]
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Abstract: Human's closest living relatives are the bonobo (Pan paniscus) and chimpanzee (Pan troglodytes), sharing over 98% of our genetic material with the genus Pan. Bonobos and chimpanzees themselves are over 99% genetically similar, but despite this, differ greatly in behavior and social structure. Bonobos are considered much more tolerant, bond strongly with one another and often mediate conflicts through sexual interactions. Chimpanzees, on the other hand, form hunting parties, patrol territories and resolve conflicts between and within troops through aggression. Recent studies have identified volumetric and neuronal differences between the two species in the amygdala, an area of the brain related to social cognition. The amygdala serves a role in decision making, memory, attention and emotional responses. In the current study, we sought to determine whether the amygdala shows a measureable difference in serotonin transporter (SERT) expression between bonobos (N=6) and chimpanzees (N=6). SERT is known to regulate the responsiveness of the amygdala to stimuli that provoke fear and aggression, with lower levels of expression associated with increased aggression. We labeled SERT-containing axons using immunohistochemistry and employed stereological methods to estimate their length density. We found that bonobos express a significantly greater density of SERT-immunoreactive axons across the entire amygdala, at levels twice those observed in chimpanzees (Mann-Whitney U, $p = 0.008$). These findings suggest that variation in serotonin levels in the amygdala mediate, in part, the remarkable differences in social behavior exhibited by bonobos and chimpanzees.

Disclosures: C.D. Stimpson: None. **W.D. Hopkins:** None. **J.P. Taglialatela:** None. **N. Barger:** None. **P.R. Hof:** None. **C.C. Sherwood:** None.

Poster

499. Evolution of Developmental Mechanisms

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 499.08/C14

Topic: D.19. Comparative Anatomy and Evolution

Support: Kent State University

NSF BCS-1316829

Title: Age-related neural changes in the chimpanzee hippocampus

Authors: *E. L. MUNGER[1], M. K. EDLER[2], P. R. HOF[3], W. D. HOPKINS[4], J. M. ERWIN[5], C. C. SHERWOOD[5], M. A. RAGHANTI[1,2]
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Abstract: Humans are uniquely susceptible to age-related neurodegenerative pathologies, such as Alzheimer's disease. This vulnerability is likely the result of evolutionary changes related to increased brain size and longevity compared to other primates. In humans, healthy aging is marked by varying degrees of neural deterioration and cognitive impairment. One region particularly affected by aging is the hippocampus, which exhibits a decrease in total volume and an increase in glial cell population and activation. Prior studies of great apes, including chimpanzees, did not find an age-associated decrease in hippocampus volume or a substantial neuronal loss in the CA1 subregion. These reports suggest the great ape brain may age differently than the normal human brain. The objective of the present analysis was to examine the effect of age on neuronal and glial densities in the hippocampus of chimpanzees, our closest living relatives. Using Nissl-stained sections and stereological methods, we quantified neuron and glial density in the CA1 and CA3 fields of the hippocampus in a sample of 16 chimpanzees (ages 12-58 years). Overall, CA3 possessed higher neuron and glial densities relative to CA1 (Mann-Whitney, $p < 0.01$). Variation in neuron densities was associated with age in both hippocampal subregions (CA1: $p = 0.02$, $r2 = 0.32$; CA3: $p < 0.01$, $r2 = 0.49$), while variation in glial densities was only correlated with age in the CA3 field ($p = 0.030$, $r2 = 0.296$). The ratio of glia to neurons in CA1 and CA3 was not altered with age in chimpanzees. Our preliminary results demonstrate a moderate, age-related decline of both neuron and glial densities for CA3 and neuron densities for CA1. Future analyses will reveal if this association remains with an increased sample size and is related to neurodegenerative pathologies in these individuals.

Disclosures: **E.L. Munger:** None. **M.K. Edler:** None. **P.R. Hof:** None. **W.D. Hopkins:** None. **J.M. Erwin:** None. **C.C. Sherwood:** None. **M.A. Raghanti:** None.

Poster

499. Evolution of Developmental Mechanisms

Location: Halls A-C

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Topic: D.19. Comparative Anatomy and Evolution

Support: NSF Grant BCS-0824531

NIH Grant NS-42867

James S. McDonnell Foundation Grant 22002078

James S. McDonnell Foundation Grant 220020293

Title: Heritable anatomical variation in chimpanzee brains. Implications for human evolution

Authors: *A. GOMEZ-ROBLES[1], W. D. HOPKINS[2,3], C. C. SHERWOOD[1]
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Abstract: The brain phenotype of the last common ancestor of chimpanzees and humans is unknown, but it is usually assumed that humans have undergone a much more dramatic divergence from the ancestral condition. Therefore, chimpanzee brains provide the closest available model of the expected phenotype from which human brains evolved. The evolutionary modifications experienced during human evolution must have been determined by genetic variation influencing different brain regions. We address this question by combining morphometric analyses with quantitative genetics in order to evaluate the proportion of phenotypic variation in different brain areas that is heritable and, therefore, available to modification by natural selection. Quantitative genetics uses phenotypic affinities in pedigreed populations to estimate the genetic component of phenotypic variation from similarities between kin-related individuals. We used a sample of more than 200 chimpanzees for which a well-documented pedigree is available. Brain anatomy was characterized by means of a set of homologous landmarks and some linear distances defined between them. Preliminary results show that variables related to primary sensory and motor cortical areas show higher heritabilities,

whereas cortical association areas, especially inferior frontal regions, show the lowest heritability values. Our results underscore the importance that non-genetic, plastic variation in inferior frontal association areas may have had in primate brain evolution and development. Interestingly, parietal areas, which have been proposed to show increased levels of plasticity in modern human brains, show higher heritability than inferior frontal areas in chimpanzees, perhaps pointing to increased plasticity in parietal regions as a late acquisition in human brain evolution.

Disclosures: A. Gomez-Robles: None. **W.D. Hopkins:** None. **C.C. Sherwood:** None.

Poster

499. Evolution of Developmental Mechanisms

Location: Halls A-C

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Topic: D.19. Comparative Anatomy and Evolution

Support: Eunice Shriver Kennedy NICHD Grant R01HD078561, R21HD069001

James S. McDonnell Foundation grant 220020293

Title: Evo-devo of cortical association pathways: Allometric and systematic variation across primates

Authors: *C. SHERWOOD[1], E. TAKAHASHI[2], A. VAN DER KOUWE[3], W. D. HOPKINS[4], P. R. HOF[5], C. J. CHARVET[2]
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Abstract: Several cortical association pathways differ in humans compared with other primates. In particular, the finding that the arcuate fasciculus is more prominent in humans than in chimpanzees and macaques has been used to argue that its expansion is tied to the emergence of language. However, it is not clear whether the arcuate fasciculus expanded in isolation or in concert with other cortical association pathways in primate evolution. Moreover, the developmental mechanisms accounting for the expansion of the arcuate fasciculus in humans

relative to other primates have gone unexplored. To that end, we used HARDI (high-angular resolution diffusion MRI) tractography of brains to compare cortical association pathways (e.g., arcuate fasciculus, inferior fronto-occipital tract, inferior longitudinal fasciculus,) in several adult and developing primates (e.g., marmoset, Callithrix jacchus; chimpanzee, Pan troglodytes; orangutan, Pongo pygmaeus; human, Homo sapiens). We found that each cortical association pathway enlarges with a different allometry as developmental schedules lengthen and brains expand. The arcuate fasciculus becomes disproportionately enlarged as overall brain size increases and overall developmental schedules lengthen. In development, the studied cortical association pathways follow a similar sequence of maturation in chimpanzees and in humans. For instance, the maturation of the arcuate fasciculus is protracted compared with many other association pathways. The lengthened developmental time course of brain maturation in humans may give rise to the particularly disproportionate expansion of tracts that mature late in development. Taken together, these findings demonstrate that the disproportionate expansion of the arcuate fasciculus may be a by-product of lengthened developmental schedules.

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Poster

499. Evolution of Developmental Mechanisms

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 499.11/C17

Topic: A.09. Evolution of Developmental Mechanisms

Support: Swartz Foundation grant

Title: Statistical model of evolution of brain regions

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

Abstract: We study the distribution of brain and cortical area sizes (parcellation units (PUs)) obtained for three species: mouse, macaque, and human. We find that in all these animals the observed distribution of PU sizes is close to log normal. We analyze the model of evolution of brain PUs through splitting or fragmentation. We define splitting statistically, by assuming that each existing PU has a probability to be split into two more specialized regions that depends on PU size only. We show that such a splitting process defined statistically can explain the

distributions of PU sizes observed in three species, including lognormality and the scale of standard deviations. Similar statistical models have been analyzed previously to describe the process of explosive shell fracturing and rock grinding, however, in these models fracturing occurs independently for different fragments. In contrast, the model that we describe for PU fragmentation does not consider PU splitting to be independent for different regions. We find that the model that best captures the observed PU distributions includes fracturing that is independent on the PU size. This finding suggests that, in the course of brain evolution, PUs have approximately similar probability to be split into more specialized regions. The evolutionary pressure to specialize, described statistically, applies equally to large and small brain regions.

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

Poster

499. Evolution of Developmental Mechanisms

Location: Halls A-C

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Program#/Poster#: 499.12/C18

Topic: A.09. Evolution of Developmental Mechanisms

Support: CIHR

Title: Are the foundations for embodied activity already embedded in heterogeneous laminar networks?

Authors: *E. L. OHAYON, A. K. LEE, A. LAM
Neurolinx Res. Inst., The Green Neurosci. Lab., La Jolla, CA

Abstract: What are the neural foundations necessary for movement in the environment? In this study we use embodied computational models to examine the relation of network structure to movement in artificial autonomous agents. Previous studies by members of our group have found that random networks tend to settle to a fixed-point state or limit cycle, oscillatory, behavior reminiscent of seizures. These behaviors could be quickly evolved into embodied movement by applying genetic algorithms to the connectivity. In another set of studies, spatial, laminar networks were shown to support persistent activity in cases where density was varied and connectivity made heterogeneous. However, the question of how the spatial features of laminar network architecture would contribute to interactions with the environment remained open. As such, we connected the laminar networks to a chassis and observed the behavior. RoHS

compliant materials and open source platforms were used in building the embodied agents. Specifically, the Arduino platform was used to interface the neural networks running on an external computer. The chassis was equipped with servo motors for output motion. Sensory input included photoresistors and contact switches. The spatial networks were multi-layered and consisted of up to 100,000 spiking units. Simulations included both inhibitory and excitatory units connected in columnar geometries. Network density was varied by adding or removing units and their connections at 10% increments. Neural activity was initiated with a single initial contact to a touch receptor. Unlike the results in random networks, many of the embodied spatial networks immediately began to show movement, requiring no learning or evolutionary interventions. Specifically, in heterogeneous networks (e.g., density of 60%), embodied forward and turning movements correlated to the persistent propagating activity seen in the isolated networks. These initial observations suggest that the heterogeneous laminar structure is sufficient to generate movements that are reminiscent of nascent embodied motion seen in early development and evolution. The results of this study may thus help illuminate why (i) laminar architectures and (ii) changes in density are important neural features that appear repeatedly in evolution and development.

Disclosures: **E.L. Ohayon:** None. **A.K. Lee:** None. **A. Lam:** None.

Poster

499. Evolution of Developmental Mechanisms

Location: Halls A-C

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Program#/Poster#: 499.13/C19

Topic: A.09. Evolution of Developmental Mechanisms

Support: NSF GRFP

ICBS Research Grant

Title: Decoupled schedules of neural and somatic development during embryogenesis across species

Authors: *A. HALLEY
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Abstract: Significant evolutionary differences exist across species in terms of relative brain structure size, functional architecture, connectivity, and degrees of encephalization, among other

forms of variation (Streidter 2006). While investigations into brain evolution have traditionally examined species differences in adult brains, recent research has increasingly aimed to describe the developmental mechanisms responsible for lineage-unique adult neuroanatomy. Embryonic development, when progenitor cell pools are established and neurogenesis begins, is of primary interest for comparative neurological investigations. Comparative embryology generally utilizes staging techniques (e.g. the Carnegie system; O'Rahilly & Muller 1999) based primarily on the appearance of somatic features to establish morphological equivalence in embryonic development. However, staging obscures differences across species in the relative rate of neural and somatic development, which is central to understanding early alterations to brain development across species. Here we describe interspecies differences in the decoupling of neural and somatic development, based on three approaches: (1) our own meta-analysis of embryonic literature on the appearance of embryonic traits, combined with those described by recent work in Barbara Finlay's lab (Clancy et al. 2001; Workman et al. 2013; www.translatingtime.org); (2) comparative brain morphology at equivalent stages; and (3) allometric measurements of brain/body growth during embryonic development. Comparison across available species shows that a variety of events in embryonic brain development - e.g. neurogenesis, tract formation, etc. - occur significantly earlier in rodent species relative to somatic events when compared against non-rodent mammals. Corresponding differences in both embryonic brain morphology and brain/body allometry are also examined. These findings are discussed in relation to developmental shifts in corticogenesis observed in glires (Workman 2013), as well as potential evolutionary pressures and developmental mechanisms underlying the developmental decoupling of somatic and neural events.

Disclosures: A. Halley: None.

Poster

499. Evolution of Developmental Mechanisms

Location: Halls A-C

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Topic: A.09. Evolution of Developmental Mechanisms

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

Title: FGF2-induced gyrification in the mouse brain: An evolutionary perspective from mice to humans

Authors: *S. TOMASI[1], G. COPPOLA[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: During the course of evolution, selective expansion of different cortical areas of the cortex is a major requirement to increase the complexity of information processing. Indeed, gyrification, the folding of the cortical mantle which generates sulci and convolutions, represents an optimal evolutionary strategy to increase neuronal populations without increasing head size, and is one of the most distinctive morphological features of the brain of primates and humans. Understanding the molecular underpinnings of gyrencephaly represents a major challenge in neurobiology, and may potentially increase our understanding of brain growth under normal and pathological conditions. We previously reported that a single microinjection of Fibroblast Growth Factor 2 (FGF2), but not FGF8, into lateral ventricles of mouse embryos before the onset of neurogenesis (E11.5) resulted in the appearance of rostrolateral, fully-layered gyri in the normally non-convoluted adult mouse brain, which was reminiscent of morphological features of gyrencephalic species. In order to investigate the mechanisms of FGF2-induced gyrification, we set out to analyze gene expression profiles at different time points after FGF2 injections by means of *in situ* hybridization, real-time quantitative PCR and next-generation RNA sequencing, and correlated these data with morphological findings and proliferative dynamics in the region undergoing the cortical enlargement. Interestingly, we found that FGF2 did not seem to alter the cell cycle or cell survival, but modulated the expression profiles of several proneural genes (including Ngn1, Ngn2, NeuroD2, NeuroD4 and NeuroD6) as early as 48 hours after injections. As FGF signaling is a known modulator of other signaling pathways, including Wnt and Notch, we carried out a series of experiments aimed at understanding the nature of this crosstalk. Strikingly, we found that few hours after injection, FGF2 regulated the expression gradients of Notch ligands, and that altered expression corresponded to the region undergoing the cortical expansion, suggesting that FGF2 can shift the timing of neurogenesis and the determination of VZ-SVZ cell identities. Consistently, several FGFs and Notch-related genes are co-expressed in a spatially and temporally-restricted manner during early stages of neurogenesis in humans.

Disclosures: S. Tomasi: None. **G. Coppola:** None. **F.M. Vaccarino:** None.

Poster

499. Evolution of Developmental Mechanisms

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Topic: A.09. Evolution of Developmental Mechanisms

Support: NIH Grant EY01234

NER Foundation

Title: A new dimension of neurobiology in a novel cell type

Authors: *P. H. FREDERIKSE, A. NANDANOOR, C. KASINATHAN
Rutgers SDM BHS, Newark, NJ

Abstract: Extensive similarities shown between neurons and elongated fiber cells in eye lens include the ultrastructure of their microtubule vesicle transport machinery and the membrane protrusions/dendritic spines along their lateral surfaces. These membrane structures have similar size and shape, and share expression of a number of key gene products and associated molecular regulatory mechanisms first shown to work as a unit in neurons at dendritic spines. We showed that AMPA and NMDA glutamate receptors and a core set of associated molecular mechanisms that coordinate their regulation at the DNA, RNA and protein levels also occur in lens. Others showed both membrane structures express clathrin and AP-2 on their surface and contain F-actin, which forms a scaffold in dendritic spines. Lens AMPARs include its pivotal GluA2 subunit which also undergoes Q/R RNA editing, analogous to neurons. We showed NMDAR NR1, NR2A, and NR2B in lenses, and identified p-Tyr GluA2 and p-Tyr 1472 NR2B that are phosphorylated at clathrin/AP-2 interaction sites which block endocytosis. Conversely, we showed STEP Tyr-phosphatase in fiber cells that promotes receptor internalization. Greater STEP and excess AMPAR/ NMDAR endocytosis are key factors in primary neural disorders and important drug targets. Extracellular GluA2 domain is a principal binding partner for GAPDH moonlighting protein at dendritic spine surfaces, and GAPDH internalization has a critical role in neuronal cell death mechanisms. IF *in situ* studies demonstrated overlapping focal distributions of STEP, GAPDH, p-Tyr-GluA2, clathrin, actin, and AP-2 at the perimeter of lens fiber cells at membrane spines in lenses of healthy adult rabbits. In disease, we measured greater STEP61 and decreased p-Tyr-GluA2 in lenses of adult diabetic rabbits together with disrupted fiber cell morphology typical in the rabbit model. We speculate that AMPA and NMDA glutamate receptors actions in lens focus on the remarkably precise nature of its morphogenesis required to produce an optically useful organ. Detailed similarities between lens fiber cells and neurons may also relate to observations first made in the 1970's that neurons can trans-differentiate into lens. In light of speculations first made by W. Gehring (CH) that sensory organs which include the eye and lens preceded the evolution of a brain, these findings in lens provide further evidence of a unique relationship in the evolution and development of lens fiber cells and neurons, and support the further hypothesis that AMPAR/NMDAR functions and regulation in lens may have been antecedents of more dynamic activities that define neuronal functions.

Disclosures: P.H. Frederikse: None. **A. Nandanoor:** None. **C. Kasinathan:** None.

Poster

(Unable to Attend)

499. Evolution of Developmental Mechanisms

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 499.16/C22

Topic: A.09. Evolution of Developmental Mechanisms

Support: Presidential Fellowship for Summer Research at the University of Scranton

Title: Social isolation and brain development in the eusocial ant species camponotus floridanus

Authors: *E. JUNGE[1], M. A. SEID[2]
[2]Biol. Dept., [1]The Univ. of Scranton, Scranton, PA

Abstract: Social interaction plays a key role in the healthy development of social animals. This effect can be seen throughout the mammalian family and is most pronounced in species with complex social networks. When developing offspring do not receive proper social interaction they show developmental impairments. This effect is well documented in mammalian species but controversial in social insects. It has been hypothesized that the enlarged mushroom bodies, responsible for learning, memory and sensory integration, observed in social insect species are needed for maintaining the large social networks or due to other confounding factors such as the demands of parasitism or task related behavior. This study examines the impact of social isolation on the development of mushroom bodies of the eusocial ant species Camponotus floridanus. Ants raised in isolation were shown to exhibit impairment in the growth of the mushroom bodies as well as behavioral deficits when compared to ants raised in small social groups beyond the factors that have been previously shown to influence mushroom body growth during development. These results indicate that social interaction is necessary for the proper development of C. floridanus mushroom bodies.

Disclosures: **E. Junge:** A. Employment/Salary (full or part-time):; The University of Scranton. **M.A. Seid:** A. Employment/Salary (full or part-time):; The University of Scranton.

Poster

499. Evolution of Developmental Mechanisms

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 499.17/C23

Topic: A.09. Evolution of Developmental Mechanisms

Support: NIH, NEI intramural research programs

Title: The rod photoreceptors originate from S-opsin expressing cone precursor cells in the mammalian retina

Authors: *J.-W. KIM, H.-J. YANG, M. J. BROOKS, A. SWAROOP
NIH, Natl. Eye Inst. (NEI), Bethesda, MD

Abstract: Cone and rod photoreceptors in the retina are specialized light-sensing neurons for day and night vision, respectively. Developmental defects or death of photoreceptors can lead to retinal neurodegeneration and consequently blindness. Loss of *Nrl*, a transcription factor essential for rod cell fate determination, results in a mouse retina with only S-(blue or uv sensitive) cone photoreceptors instead of rods. Based on multiple lines of evidence, we proposed that rod photoreceptors are generated from S-cone precursor cells that have developmental plasticity and generate rods when NRL is dominant and active. To test this hypothesis and find possible 'footprints of S-cone precursors', we purified the rod cells using fluorescence activated cell sorter (FACS) from *Nrl*-GFP transgenic mouse retina (from P2 to P28; peak of rod birth to matured rod) that expresses GFP specifically in rod photoreceptors. We performed global transcriptome analysis of flow-sorted developing and mature rods using RNAseq. The RNAseq data revealed no expression of genes specific for non-photoreceptor neurons; however, cone-specific genes, such as *Opn1sw* and *Gnb3*, are highly expressed in rods from early stages of differentiation and their expression decreased as the rods matured. We then examined the epigenetic landscape of rod photoreceptors by reduced-representation bisulfite sequencing (RRBS) for DNA methylation and ChIPseq for histone methylation patterns (H3K4me3 and H3K27me3). The epigenome data demonstrated active epigenetic marks (low levels of DNA methylation, high H3K4me3, low H3K27me3) on cone-specific genes at early development stages but the patterns reversed in mature rod photoreceptors. To further test our hypothesis, we performed lineage tracing by marking S-cone cell specific lineage using *Opn1sw*-Cre;ROSA26-AP (Alkaline phosphatase). Surprisingly, rod photoreceptors also exhibited high AP activity along with S-cones. Taken together, our studies provide strong evidence in support of evolution of rods from S-cone photoreceptors.

Disclosures: **J. Kim:** None. **H. Yang:** None. **M.J. Brooks:** None. **A. Swaroop:** None.

Poster

499. Evolution of Developmental Mechanisms

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Topic: A.09. Evolution of Developmental Mechanisms

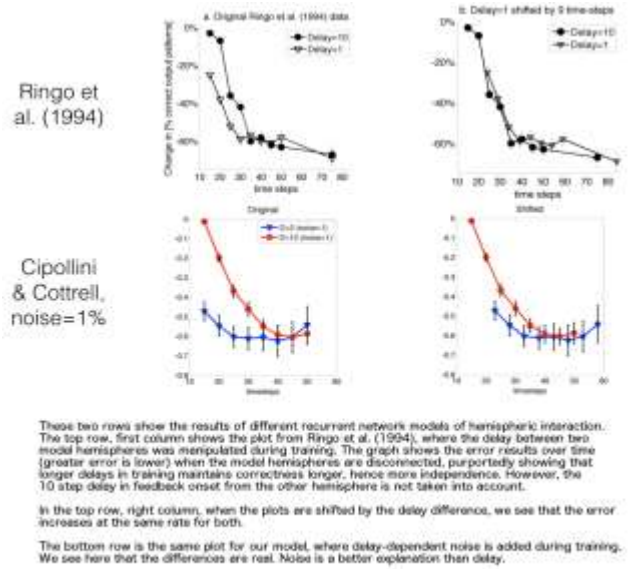
Support: CARTA Fellowship

NSF SMA 1041755

Title: A developmental approach to long-distance connectivity and interhemispheric collaboration

Authors: *B. N. CIPOLLINI, G. COTTRELL
Cognitive Sci., UC San Diego, La Jolla, CA

Abstract: Cerebral lateralization is intertwined with virtually every cognitive function that we think makes us human, yet a clear dichotomy in a leading theory about its origins remains unexplained. Though lateralized processing has been suggested to be due to independent, local development of neural circuits (Ringo et al., 1994; Rilling & Insel, 1999a), these intrahemispheric circuits tend to be complementary (not independent) and show extremely strong functional coupling, suggest robust interhemispheric interactions in the lateralized human brain. Here, we review literature and present modeling evidence suggesting that: (1) previous modeling work that concluded that conduction delay leads to hemispheric independence is overstated and misinterpreted, and is inconsistent with experimental data on the development of lateralization and the corpus callosum. (2) Variability in conduction delays, present in early development due to thin, unmyelinated white matter fibers (Faisal et al., 2008), can bias infant learning towards the use of shorter, local connections, and this effect can drive hemispheric independence. (3) This early bias towards local circuits suppresses lateralization of perceptual processing and enhances lateralization in unilateral motor production (e.g. handedness). These results are consistent with developmental data showing that lateralization increases during development, where communication migrates from local circuits to long-distance circuits. We argue that this effect is greatest in humans, due to a trade-off between maximizing adult brain size while minimizing brain size at birth as indicated by the uniquely human post-natal growth trajectory (Martin, 1983). This suggests that, counter to previous proposals, the physiology of interhemispheric communication in humans may not be a pure function of brain size shared across mammals, but instead is related to the uniquely human problem of birthing a large-brained fetus through a narrow walking pelvis.



These two rows show the results of different recurrent network models of hemispheric interaction. The top row, first column shows the plot from Ringo et al. (1994), where the delay between two model hemispheres was manipulated during training. The graph shows the error results over time (greater error is lower) when the model hemispheres are disconnected, purportedly showing that longer delays in training maintains correctness longer, hence more independence. However, the 10 step delay in feedback onset from the other hemisphere is not taken into account.

In the top row, right column, when the plots are shifted by the delay difference, we see that the error increases at the same rate for both.

The bottom row is the same plot for our model, where delay-dependent noise is added during training. We see here that the differences are real. Noise is a better explanation than delay.

Disclosures: **B.N. Cipollini:** None. **G. Cottrell:** None.

Poster

499. Evolution of Developmental Mechanisms

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 499.19/C25

Topic: A.09. Evolution of Developmental Mechanisms

Support: The UCSF Program for Breakthrough Biomedical Research, which is funded in part by the Sandler Foundation

Title: Comparing human and macaque cortical development through differential gene coexpression analysis

Authors: *M. C. OLDHAM
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Abstract: Unique aspects of human brain development underlie our species' remarkable cognitive abilities and our vulnerability to malformations of brain circuitry, which likely contribute to many neuropsychiatric disorders. However, our understanding of the cellular and

molecular bases of brain development is predominantly based on studies of model organisms. This situation has begun to change with the advent of techniques such as laser micro-dissection (LMD) and transcriptional profiling, which can be combined to generate highly multivariate, quantitative, and precise information about the molecular composition of a single tissue specimen. This approach has recently been used in conjunction with stereotyped sampling strategies to generate atlases of gene expression in the developing brains of humans and rhesus macaque monkeys, providing an unprecedented opportunity to test the hypothesis that changes in the regulation of gene expression were central to the evolution of many unique human qualities, including our expanded neocortex. The present work describes a multivariate analytical strategy for comparing transcriptome organization between humans and macaques during prenatal cortical development. Application of this strategy to LMD samples from cingulate cortex and primary visual cortex of both species reveals conserved and distinct patterns of gene activity, many of which are driven by specific cell types. These results provide an integrated functional context for studying genes that have been implicated in cortical development, while simultaneously identifying novel candidate genes whose activity may distinguish cortical development in humans from other primate species.

Disclosures: M.C. Oldham: None.

Poster

499. Evolution of Developmental Mechanisms

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

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Topic: A.09. Evolution of Developmental Mechanisms

Support: Fondation pour la Recherche Médicale (Programme “équipe FRM”)

Agence Nationale de la Recherche (ANR-08-MNP-030)

LABEX LIFESENSES (ANR-11-IDEX-0004-02)

Région ile de France (DIM Cerveau et Pensée)

École des Neurosciences de Paris

Bristol-Myers Squibb Postdoctoral Fellowship

Wellcome Trust

Title: Positive selection and signaling switch of the axon guidance receptor Robo3 during vertebrate evolution

Authors: F. FRIOCOURT[1], P. ZELINA[1], H. BLOCKUS[1], Y. ZAGAR[1], A. PÉRES[2], Z. WU[3], N. RAMA[1], C. FOUQUET[4], E. HOHENESTER[5], J. SCHWEITZER[6], M. TESSIER-LAVIGNE[3], H. ROEST CROLLIUS[2], *A. C. CHEDOTAL[7]
[1]Inst. de la Vision, Paris, France; [2]Inst. de Biologie de l'ENS, Paris, France; [3]The Rockefeller Univ., New York, NY; [4]CNRS UMR 7102, Paris, France; [5]Dept. of Life Sci., Imperial Col. London, London, United Kingdom; [6]Inst. Biol. I, Univ. of Freiburg, Freiburg, Germany; [7]Vision Institute, INSERM U968, Paris, France

Abstract: Commissural axons exist in all Bilateria, but the development of novel commissural circuits or the modification of existing ones has accompanied the emergence of key neurobiological features in vertebrate evolution, such as depth perception, hearing, lung-based breathing and limb-derived locomotion. In most Bilateria, specific sets of cells occupy the midline and express axon guidance molecules, which regulate crossing. Two ligand/receptor molecular partners play a major role in this process: Netrin-1/DCC (Deleted in Colorectal Cancer) which mediates the attraction of commissural axons towards the midline and Slit/Robo (Roundabout) which mediate repulsion of post-crossing axons away from the midline and prevent ipsilaterally projecting neurons from crossing it. In vertebrates, a key role in midline guidance is played by the divergent Robo family member Robo3. Robo3 is expressed by commissural axons of the mouse spinal cord and hindbrain before and during crossing of the midline floor plate, and many commissures fail to develop in mice and humans lacking Robo3. We provide evidence for positive selection of the Robo3 receptor during mammalian evolution that subverted its mechanism of action. Unlike other Robo receptors, including Robo3 in non-mammalian vertebrates, mammalian Robo3 is not a high affinity receptor for Slits, a difference attributable to a few substituted residues. Moreover, Robo3 regulates responses to the chemoattractant Netrin-1, as Netrin-1 selectively triggers phosphorylation of mammalian Robo3, pontine neurons lacking Robo3 fail to migrate towards a Netrin-1 source, and loss of Robo3 attenuates Netrin-1-induced spinal commissural axon outgrowth. Midline pontine neuron attraction is restored in Robo3 knockout mice expressing mammalian but not non-mammalian Robo3. We propose that Robo3 evolution was key to sculpting mammalian motor circuits by converting a receptor for Slit repulsion into one that both represses Slit repulsion and potentiates Netrin attraction.

Disclosures: F. Friocourt: None. **P. Zelina:** None. **H. Blockus:** None. **Y. Zagar:** None. **A. Péres:** None. **A.C. Chedotal:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Grant from Sanofi. **Z. Wu:** None. **N. Rama:** None. **C. Fouquet:** None. **E. Hohenester:** None. **J. Schweitzer:** None. **M. Tessier-Lavigne:** None. **H. Roest Crollius:** None.

Poster

499. Evolution of Developmental Mechanisms

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 499.21/C27

Topic: A.09. Evolution of Developmental Mechanisms

Support: Duke Institute for Brain Sciences

Title: A human accelerated enhancer of FZD8 regulates corticogenesis

Authors: *D. L. SILVER[1], G. WRAY[2], S. SKOVE[1], L. BOYD[1]
[1]Mol. Genet. and Microbiology, Duke Univ. Med. Ctr., Durham, NC; [2]Biol., Duke Univ., Durham, NC

Abstract: The human neocortex differs from that of other great apes in several notable regards including altered cell cycle, prolonged corticogenesis, and massively increased overall size. While these evolutionary changes likely contributed to the origin of distinctively human cognitive faculties, their genetic basis remains almost entirely unknown. Highly conserved non-coding regions that show rapid sequence changes along the human lineage are candidate loci for the development and evolution of uniquely human traits. Here we report the discovery of a human-accelerated regulatory enhancer (*HARE5*) of *FZD8,* a member of the frizzled family of WNT receptors implicated in brain development and size. Although *Fzd8* is expressed throughout the developing embryo, *HARE5* drives expression primarily in neural stem cells and neurons of the neocortex. Using transgenic mice, we demonstrate striking differences in human and chimpanzee *HARE5* activity, with human *HARE5* driving precocious and more robust expression at the onset of corticogenesis. Chromosome conformation capture assays reveal mouse *HARE5* physically and specifically contacts the core *Fzd8* promoter in embryonic neocortices. To assess the phenotypic consequences of *HARE5* activity, we generated transgenic mice in which *Fzd8* expression is under control of either human or chimpanzee *HARE5*. In comparison to chimpanzee *HARE5*, human *HARE5*-driven expression of *Fzd8* induced a larger fraction of neural stem cells in S and G2/M phases of the cell cycle. Changes in *HARE5* function that are unique to humans thus alter cell cycle state of a critical population of stem cells during corticogenesis, and may therefore underlie some of the distinctive anatomical features of the human brain.

Disclosures: D.L. Silver: None. **G. Wray:** None. **S. Skove:** None. **L. Boyd:** None.

Poster

500. Structure and Function of Nicotinic Receptors and Asics

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 500.01/C28

Topic: B.02. Ligand-Gated Ion Channels

Support: NIH Grant R01GM085237

NIH Grant U19 MH085193

Title: Differing residues in the highly conserved transmembrane domain of β2 and β4 nAChR subunits contribute to differences in function and agonist efficacy of α4β2 and α4β4 receptors

Authors: *M. NEWHOFF[1], C. LI[2], J. B. EATON[1], R. J. LUKAS[1], A. P. KOZIKOWSKI[3], Y. CHANG[1]
[1]Barrow Neurolog. Inst., Phoenix, AZ; [2]Xinxiang Med. Univ., Henan, China; [3]Univ. of Illinois at Chicago, Chicago, IL

Abstract: Neuronal heteromeric nicotinic acetylcholine receptors (nAChRs) composed of α4 and β2 subunits form pentameric ligand-gated cation channels in high-sensitivity (HS) (α4)2(β2)3 and low-sensitivity (LS) (α4)3(β2)2 isoforms. A4 can also form functional HS and LS receptors in conjunction with β4, a lesser-studied nAChR subunit whose presence has been detected in primate brain. Despite a high sequence homology between β2 and β4 subunits, α4β2 and a4β4 nAChRs differ markedly in total function, as well as in the potency and efficacy of agonists including Sazetidine-A (AMOP). Using chimeras of the β2 subunit with the C-terminal domain or the M3-M4 intracellular loop swapped with the corresponding region in β4 and two-electrode voltage-clamp in the oocyte expression system, we demonstrated that the structural determinant for these differences is located in the transmembrane domain (C-terminal domain, excluding the large intracellular loop), suggesting they are potentially due to a gating effect. In comparing sequences of the channel lining domain M2 and the coupling region of β2 and β4 subunits, we found two residues which differ between the two subunits: one in the 13' position of the M2 domain, and one at the end of M2-M3 linker near the "hinge". Point mutations interchanging these two residues in the wild-type subunits altered efficacies for the agonist AMOP and other related compounds towards those of the alternate wild type receptor. Receptor function was also affected: while our WT α4β2 receptors had lower function than our WT α4β4 receptors, we found that the V253F and V272I mutations in β2 increased function dramatically, such that the mutant receptor function more closely resembled that of WT α4β4 than of WT α4β2. Our results suggest that differences in gating and coupling efficiency between β2 and β4

subunits can influence agonist efficacy. (Supported by NIH Grant R01GM085237 to YC and U19 MH085193 to APK, RJL, and YC)

Disclosures: **M. Newhoff:** None. **C. Li:** None. **J.B. Eaton:** None. **R.J. Lukas:** None. **A.P. Kozikowski:** None. **Y. Chang:** None.

Poster

500. Structure and Function of Nicotinic Receptors and Asics

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 500.02/C29

Topic: B.02. Ligand-Gated Ion Channels

Support: University of Sciences, Philadelphia college pharmacy

Title: MTS modification of substituted cysteines in the β2 nachr subunit reduces dFBr potentiation of α4β2 receptors

Authors: ***Y. HUANG**[1], R. M. CERCHIO, Jr[1], R. A. GLENNON[2], M. K. SCHULTE[1]
[1]pharmacology and Toxicology, Univ. of Sci., Philadelphia, PA; [2]Dept. of Medicinal Chem., Virginia Commonwealth Univ., Richmond, VA

Abstract: Neuronal nicotinic acetylcholine receptors (nAChRs) are members of the Cys-loop super family of ligand gated ion channels (LGIC) and play a critical role in synaptic transmission. Changes in receptor expression resulting from neurodegenerative and epigenetic processes produce a range of neurological disorders including Autism, Alzheimer's and Parkinson's disease. Unfortunately, few drugs are available for treatment of these neurological disorders. Desformylflustrabromine (dFBr) is a natural product originally purified from the marine bryozoan Flustra foliacea. Using a synthetic form of dFBr, we previously determined that dFBr is a selective allosteric modulator of the nicotinic α4β2 receptor. When co-applied with acetylcholine on these receptors, dFBr produces a 265% increase in efficacy compared to acetylcholine alone. The EC50 for potentiation by dFBr is 1μM; however, at dFBr concentrations greater than 3μM, acetylcholine induced responses are inhibited in the presence of dFBr, producing a "bell shaped" dose response curve. The precise binding site for dFBr and the mechanism of potentiation remain unknown. In this study we use substituted cysteine accessibility method (SCAM) to determine if dFBr binds at the β2+/α4- interface of the α4β2 receptor. To prevent modification of the two endogenous cysteines located on the intracellular M1-M2 loop by MTS we mutated these residues on both the α4 and β2 subunits to serine and

glycine (C262S and C269G). We have previously shown that these mutations produce no effect on acetylcholine responses on mutant α4β2 receptors (pseudo wt). While these mutations did block potentiation by calcium, no effect on dFBr potentiation was observed, thus calcium and dFBr potentiation appear to involve different mechanisms. Using these "pseudo wt" receptors, we introduced single point cysteine mutations in the B and C loops of the β+ subunits. Mutation of β2D217 of loop C to cysteine produced no effect on either acetylcholine responses or dFBr's ability to potentiate acetylcholine responses. Modification of D217C by MTS also produced no observable effect on the acetylcholine response but reduced the potentiation produced on co-application of dFBr by 80%. As MTS modification of the cysteine introduced at position 217 would block dFBr access to β2+/α4- interface, these data support our hypothesis that dFBr binds at this position to produce potentiation.

Disclosures: Y. Huang: None. **M.K. Schulte:** None. **R.M. Cerchio:** None. **R.A. Glennon:** None.

Poster

500. Structure and Function of Nicotinic Receptors and Asics

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Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 500.03/C30

Topic: B.02. Ligand-Gated Ion Channels

Support: Texas A&M University Health Science Center

Title: Properties of extracellular domain α3β2 nicotinic acetylcholine receptors with a protease site inserted upstream of M1

Authors: *G. B. WELLS, V. L. FRANKOVICH, A. M. PERSON
Mol. & Cell. Med., Texas A&M Univ. Hlth. Sci. Ctr., COLLEGE STATION, TX

Abstract: Background: Water-soluble extracellular domain human nicotinic acetylcholine receptors (ECD nAChRs) would be useful for understanding how human nAChR work. Their production, however, remains elusive and appears to need initial expression with the first transmembrane domain (M1). Because of that requirement, post-expression proteolysis to remove M1 is a potential path to water-soluble ECD nAChRs. The path requires that ECD nAChRs tolerate an inserted protease site upstream of M1. Indeed, α7 ECD nAChR and α4β2 ECD nAChR with inserted protease sites upstream of M1 have high structural fidelity to full length receptors. Thus, a proteolysis strategy might have general utility for many or even all

nAChRs. **Goal and Objective**: The goal is to determine whether human α3-containing ECD nAChRs, like α7 and α4β2 ECD nAChRs, are suitable for post-expression proteolysis to produce water soluble receptors. The objective is to measure properties of human α3-containing ECD nAChRs containing inserted amino acid residues, including a site-specific protease site, upstream of M1. **Methods**: Human α3 and β2 cDNAs were truncated after M1 (α3M1 and β2M1). Mutagenesis placed a heterologous 5-residue sequence (adding two restriction sites; α3M1RE and β2M1RE) or a 7-residue TEV protease site (α3M1TEV) at the ECD-M1 interface. Subunits were expressed in *Xenopus* oocytes. Immunoblotting and affinity and yield of [^{3}H]epibatidine binding sites assessed effects of inserted residues on expression and structural fidelity. **Results**: Immunoblotting showed expression of α3M1, β2M1, α3M1RE, β2M1RE, and α3M1TEV. The dissociation constant of [^{3}H]epibatidine with α3M1/β2M1 ECD nAChR was similar to the dissociation constant with full length α3β2 nAChR. With heterologous sequences inserted at the ECD-M1 interface, α3M1RE/β2M1RE and α3M1TEV/β2M1RE produced yields of high affinity [^{3}H]epibatidine binding sites comparable to the yield of α3M1/β2M1 ECD nAChR. **Conclusions:** Based on dissociation constants with [^{3}H]epibatidine, the α3 nicotinic subunit forms α3M1/β2M1 ECD nAChR with high structural fidelity to full length α3β2 nAChR. Based on yield of high affinity [^{3}H]epibatidine binding sites, the α3M1 subunit tolerates heterologous residues, including a TEV protease site, inserted immediately upstream of M1. These results suggest that α3β2 ECD nAChR, like α7 and α4β2 ECD nAChRs, might be suitable for post-expression proteolysis to produce water soluble receptors.

Disclosures: G.B. Wells: A. Employment/Salary (full or part-time):; Texas A&M University. **V.L. Frankovich:** None. **A.M. Person:** A. Employment/Salary (full or part-time):; Texas A&M University.

Poster

500. Structure and Function of Nicotinic Receptors and Asics

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 500.04/C31

Topic: B.02. Ligand-Gated Ion Channels

Support: NIH GM48677

NIH GM103801

BFU2012-30997

Marie Curie International Postdoctoral Fellowship

Title: Biophysical and pharmacological properties of native human α6β4 nicotinic acetylcholine receptors

Authors: *A. J. HONE[1], J. MCINTOSH[3,4], J. PASSAS[5], A. ALBILLOS[2]
[1]Neurosci., [2]Pharmaoclogy and Therapeut., Univ. Autonoma De Madrid, Madrid, Spain; [3]Biol., Univ. of Utah, Salt Lake City, UT; [4]Psychiatry, George E. Wahlen Veterans Affairs Med. Ctr., Salt Lake City, UT; [5]Urology, Hosp. Doce de Octubre, Madrid, Spain

Abstract: Human adrenal chromaffin cells express nicotinic acetylcholine receptors (nAChR) that facilitate the release of catecholamines when activated. The two main receptor subtypes expressed by these cells are α6β4 and α7. The α7 subtype has been widely studied in other cells and expression systems but much less is known about the α6β4 subtype in part because of its sparse expression in the nervous system and relatively poor expression in heterologous systems. Consequently, very little is known about the pharmacological and biophysical properties of this subtype. We performed whole-cell voltage-clamp electrophysiology on isolated human chromaffin cells obtained from organ donors to elucidate these properties. Several agonists were tested to determine their activities on α6β4 nAChRs including acetylcholine, nicotine, varenicline, DMPP, and 5-I-A-85380. The rank order potency of these ligands was 5-I-A-85380 = DMPP = varenicline > nicotine = acetylcholine and all had EC50 values in the low micromolar range. The relative efficacies of these agonists also differed. Varenicline and 5-I-A-85380 were both full agonists whereas nicotine and DMPP were partial agonists relative to acetylcholine. Desensitization experiments with acetylcholine and nicotine revealed that chromaffin cell α6β4 nAChRs rapidly desensitized during continuous exposure to EC50 concentrations of either agonist. Furthermore, these receptors recovered relatively rapidly after complete desensitization by nicotine. The pharmacological properties observed were not due to the presence of receptors that contained a β2 binding site because the acetylcholine-evoked currents were relatively insensitive to inhibition by a β2-selective antagonist, an analog of α-conotoxin LvIA, and could be completely abolished by the β4-selective antagonist α-conotoxin BuIA(T5A,P6O). Additional experiments were performed to examine the current-voltage relationship and calcium permeability of α6β4 nAChRs. These results provide valuable information on the properties of native human α6β4 nAChRs.

Disclosures: **A.J. Hone:** None. **J. McIntosh:** None. **A. Albillos:** None. **J. Passas:** None.

Poster

500. Structure and Function of Nicotinic Receptors and Asics

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Topic: B.02. Ligand-Gated Ion Channels

Support: NIH Grant DA012844

NIH Grant DA015389

Title: Roles for N-terminal domains of nicotinic acetylcholine receptor (nAChR) β3 subunits in enhanced functional expression of mouse α6β2β3- and α6β4β3-nAChRs

Authors: *B. DASH[1], R. J. LUKAS[2], M. D. LI[1]
[1]Psychiatry and Neurobehavioral Sci., Univ. of Virginia, Charlottesville, VA; [2]Neurobio., Barrow Neurolog. Inst., Phoenix, AZ

Abstract: Functional heterologous expression of naturally-expressed mouse α6*-nicotinic acetylcholine receptors (mα6*-nAChRs; where '*' indicates presence of additional subunits) has been difficult. Here we expressed and characterized wild-type (WT), gain-of-function, chimeric or gain-of-function chimeric nAChRs, sometimes as hybrid nAChRs containing both human (h) and mouse (m) subunits, in Xenopus oocytes. Hybrid mα6mβ4hβ3- (~5-8 fold) or WT mα6mβ4mβ3-nAChRs (~2 fold) yield higher function than mα6mβ4-nAChRs. Function is not detected when mα6 and mβ2 subunits are expressed together or in the additional presence of hβ3 or mβ3 subunits. However, function emerges upon expression of mα6mβ2mβ3(V9'S)-nAChRs containing β3 subunits having gain-of-function, V9'S [valine(V)-to-serine(S)] mutations in their transmembrane segment II 9' residues and is ~89% lower than those of mα6mβ2hβ3(V9'S)-nAChRs. Studies involving WT or gain-of-function chimeric mouse/human β3 subunits indicate involvement of N-terminal and other segments/domains of β3 subunits in their differential effects on function of mα6*- nAChRs. Using hβ3 subunits as templates, site directed mutagenesis studies indicate that substitution with mβ3 subunit residues in putative "β2-β3" (Q94 and E101), "β5-β6" (Loop E: S144 and S148) or "β9-β10" (loop C: E221 and F223) loops increases function of mα6mβ2*- (~2-3 fold) or mα6mβ4*- (~2-4 fold) nAChRs. EC50 values for nicotine at mα6mβ4hβ3-, mα6mβ4mβ3-, mα6mβ4mβ3(S144V+S148V)-, mα6mβ4mβ3(E221D)- and mα6mβ4mβ3(E221D+F223V)- nAChRs are same ($p > 0.05$). Thus, amino acid residues located on the putative primary (loop C) or complementary (β2-β3 or E loops) faces of β3 subunits are some of the molecular impediments for functional expression of mα6mβ2mβ3- or mα6mβ4mβ3-nAChRs.

Disclosures: B. Dash: None. **R.J. Lukas:** None. **M.D. Li:** None.

Poster

500. Structure and Function of Nicotinic Receptors and Asics

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Program#/Poster#: 500.06/C33

Topic: B.02. Ligand-Gated Ion Channels

Support: NIH grant R01 GM103801

NIH grant R01 GM48677

Title: Molecular interaction of α-conotoxin RgIA with α9α10 nAChR

Authors: *L. AZAM[1], J. MCINTOSH[2]
[1]Biol., Univ. of Utah, SALT LAKE CITY, UT; [2]Dept. of Pyschiatry & Biol., Veterans Affairs Med. Ctr. & Univ. of Utah, Salt Lake City, UT

Abstract: The α9α10 nAChR was first identified in the auditory system, where it mediates synaptic transmission between efferent olivocochlear cholinergic fibers and hair cells of the cochlea. This receptor has gained further recent attention due to its potential role in chronic pain, breast and lung cancers. We previously showed that α-conotoxin RgIA, one of the few α9α10 selective ligands identified to date, is 300-fold less potent on human vs. rat α9α10 nAChR. This species difference was conferred by only one residue in the (-) complementary, rather than (+) principal, binding region of the α9 subunit. In light of this unexpected discovery, we sought to determine other residues that interact with α-conotoxin RgIA. A recent molecular modeling study concluded that RgIA interacts with Glu194, Pro197 and Asp198 on the (+) face of the α9 subunit and Glu58 and Asp114 of (-) face of the α10 subunit (1). However, mutations of these nAChR subunit residues had little or no effect on toxin block of α9α10 nAChR function. In contrast, mutation of homologous residues in the opposing nAChR subunits resulted in loss of toxin activity ranging from 19 to ≥1700-fold. The results of the present study demonstrate interaction of α-conotoxin RgIA with an α10/α9 rather than α9/α10 nAChR subunit interface. These findings may help guide the development of other selective ligands of therapeutic potential. 1 Pérez EG, Cassels BK, Zapata-Torres G. Molecular modeling of the alpha9alpha10 nicotinic acetylcholine receptor subtype. Bioorg Med Chem Lett. 2009 Jan 1;19(1):251-4.

Disclosures: L. Azam: None. **J. McIntosh:** None.

Poster

500. Structure and Function of Nicotinic Receptors and Asics

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Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 500.07/C34

Topic: B.02. Ligand-Gated Ion Channels

Title: What non-natural conotoxin can tell us about α7 nAChRs

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Abstract: The finding that the α-conotoxin ImI binds with high affinity the α7 nAChRs opened new possibilities to investigate the structure function of these nicotinic acetylcholine receptors. As expected for a complex protein-protein interaction, binding affinity was found to depend upon the tridimentional structure of the conotoxin (Ulens et al., 2006). Examples, obtained with the α-conotoxin PnIA have shown that exchange of a single amino acid residue at position 10 in the conotoxin was sufficient to switch its activity from antagonist at the α7L'9T to agonist indicating the exquisite structural sensitivity required to stabilize the active state (Hogg et al., 2003). Based on these initial observations we postulated that even small structural changes of the α conotoxin might modify their functional effects. Taking advantage of the automated HiClamp system, which allows electrophysiological recordings in small volumes we examined the effects of a series of small conformational changes on the pharmacological properties of non-natural α conotoxins ImI. Determination of the dose-response activity revealed that inhibition of ACh evoked current strongly depends upon the α-conotoxin structure and that as much as a ten fold increase in affinity at α7 nAChRs can be obtained with an IC50 down to 50 nM. Further characterization of the most relevant conformations provides additional information about the conserved selectivity of these α7 conotoxin on the different nAChRs subtypes. In conclusion this work provides new insights for the understanding of the binding site of nAChRs, the importance of conserved residues in the N-terminal domain of the receptor opening new possibilities to engineer small protein of interest for drug development. References Hogg RC, Hopping G, Alewood PF, Adams DJ, Bertrand D (2003) Alpha-conotoxins PnIA and [A10L]PnIA stabilize different states of the alpha7-L247T nicotinic acetylcholine receptor. J Biol Chem 278:26908-26914. Ulens C, Hogg RC, Celie PH, Bertrand D, Tsetlin V, Smit AB, Sixma TK (2006) Structural determinants of selective alpha-conotoxin binding to a nicotinic acetylcholine receptor homolog AChBP. Proc Natl Acad Sci U S A 103:3615-3620.

Disclosures: **F. Marger:** None. **T. Schaer:** None. **C. Heinis:** None. **D. Bertrand:** None.

Poster

500. Structure and Function of Nicotinic Receptors and Asics

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 500.08/C35

Topic: B.02. Ligand-Gated Ion Channels

Support: MRC Grant G1001602

Title: Allosteric modulation of α7 nicotinic acetylcholine receptors is altered by transmembrane mutations

Authors: *A. CHATZIDAKI[1], J. M. D'OYLEY[2], T. D. SHEPPARD[2], N. S. MILLAR[1]
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Abstract: Acetylcholine activates nicotinic acetylcholine receptors (nAChRs) by binding at an extracellular orthosteric site, located at the interface between two adjacent subunits. In the case of homomeric α7 nAChRs, activation by acetylcholine, or by other orthosteric agonists, results in rapid desensitisation. Previous studies have described a series of positive allosteric modulators (PAMs) that are selective for homomeric α7 nAChRs. These include Type I PAMs, that exert little or no effect on the rate of receptor desensitisation after activation by an orthosteric agonist, and Type II PAMs that cause a dramatic loss of agonist-induced desensitisation. A binding site for both Type I and Type II PAMs has been identified within the transmembrane domain of α7 nAChRs. In addition, there is evidence that nAChRs can be activated by ligands (allosteric agonists) binding to a similar allosteric transmembrane site. In the case of α7 nAChRs, activation by allosteric agonists has been found to induce a much lower level of desensitisation than that which occurs after activation by orthosteric agonists. Here we report evidence that transmembrane mutations in α7 nAChRs have interesting and diverse effects on receptor activation and desensitisation by allosteric ligands. It has been reported previously that the L247T mutation (located in the second transmembrane domain) confers increased potency to acetylcholine and reduced levels of desensitisation. In contrast, the M260L mutation (located higher up in the second transmembrane domain) does not show any difference in the acetylcholine potency and desensitisation profile compared to wild-type receptors. We have found that in receptors containing the L247T mutation, both Type I PAMs and Type II PAMs are converted into non-desensitising agonists. In contrast, in receptors containing the M260L mutation, this effect is seen only with Type II PAMs. These findings, indicating that the M260L mutation has a selective effect on Type II PAMs, have been confirmed both with a series of previously described PAMs and also with a series of novel α7-selective PAMs. The novel PAMs examined in this study have close chemical similarity but diverse pharmacological properties.

For example, they include compounds displaying effects on receptor desensitisation that are typical of classical Type I and Type II PAMs but, in addition, they include compounds with intermediate properties. It is hoped that studies examining the effects of a series of transmembrane mutations on PAMs with diverse pharmacological effects will help to provide an insight into the mechanism of action of nAChR allosteric modulators.

Disclosures: A. Chatzidaki: None. **N.S. Millar:** None. **J.M. D'Oyley:** None. **T.D. Sheppard:** None.

Poster

500. Structure and Function of Nicotinic Receptors and Asics

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 500.09/C36

Topic: B.02. Ligand-Gated Ion Channels

Support: NIH Grant GM1836

ALSAM Foundation

Title: Chimeric acetylcholine binding proteins provide insight to the structure and function of human nicotinic receptors

Authors: *T. T. TALLEY[1,2], J. LINDSTROM[3], J. LUO[3], J. BOBANGO[1], M. WILSON[1], K. GALLEGOS[1], P. TAYLOR[2]
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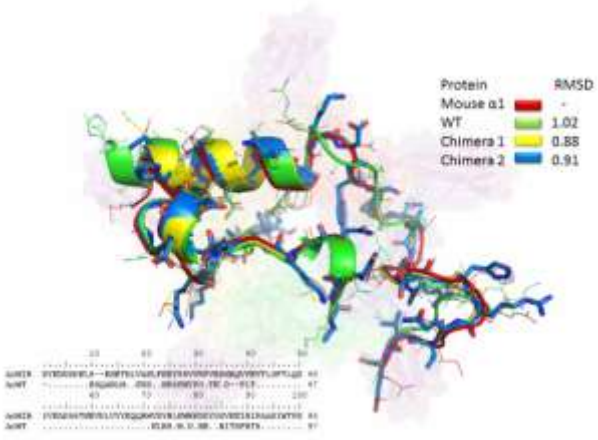
[2]Skaggs Sch. of Pharm. & Pharmaceut. Sciences, Univ. of California, San Diego, La Jolla, CA;
[3]Perelman Sch. of Med., Univ. of Pennsylvania, Philadelphia, PA

Abstract: Since the first report of the acetylcholine binding protein (AChBP) and its structure by Sixma and colleagues in 2001, crystal structures of many complexes have been analyzed in relation to ligand selectivity. However, AChBPs define a sub-species of a nicotinic acetylcholine receptor (nAChR) ectodomain, whose sequence and ligand selectivity are distinct from nAChR subtypes. While providing an atomic resolution view of how nicotinic ligands interact with their binding sites and similar general patterns of “nicotinic” activity, AChBP selectivity differs from specific subtypes of nAChRs and might be treated as one member of a wide family of extracellular domains. To enhance correlation between native nAChRs and the AChBPs, we have generated several chimeric constructs wherein specific regions of the AChBP primary

sequence are replaced with amino acids that correspond with specific nAChR subtypes. In addition to replacing loop C of the AChBP with the sequence of each of the human α and β subtypes we have modified other regions of the subunit interfacial binding site to mimic more closely the binding affinities observed for human nAChRs. We have also modified surface regions that correspond with the area of the muscle α1 subunit implicated in the autoimmune myasthenia gravis (MG). Distinct sequences in the main immunogenic region (MIR) of the α1 subunit have been substituted into the corresponding position of the AChBP sequence. The expressed protein demonstrates an antibody profile directly comparable to that observed in MG patients. To further understanding of the antibody/receptor interactions, we have generated several X-ray structures of our MIR/AChBP chimera. Both C loop and MIR substitutions have enhanced crystallization of several ligand-AChBP complexes yielding valuable information on binding determinants and poses. While we continue to strive for structures of the antibody in complex with this construct the current structures provide a structural template for the development of small molecule or peptidomimetic disrupters of the antibody interaction.



Disclosures: **T.T. Talley:** None. **J. Bobango:** None. **M. Wilson:** None. **K. Gallegos:** None. **J. Lindstrom:** None. **P. Taylor:** None. **J. Luo:** None.

Poster

500. Structure and Function of Nicotinic Receptors and Asics

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 500.10/C37

Topic: B.02. Ligand-Gated Ion Channels

Support: Wings for Life Spinal Cord Research Grant

Title: Active when closed? Coupling to heterotrimeric G proteins enable α7 nAChR mediated calcium store release

Authors: *J. KING, J. NORDMAN, S. P. BRIDGES, M. LIN, N. KABBANI
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Abstract: α7-nicotinic acetylcholine receptors (α7 nAChRs) are pentameric ligand gated channels that enable calcium entry into cells. Although the α7 nAChR is known to rapidly desensitize in response to ligands, its function is critical for long-lasting changes in neuronal activity and structure. To date little is know about the mechanism by which α7 nAChRs mediate persistent changes in cells. Recent studies suggest a role for α7 nAChRs in the regulation of intracellular calcium stores in hippocampal neurons. We have examined the ability of α7 nAChRs to couple heterotrimeric G proteins in developing neural cells. We find that specific residues in the intracellular M3-M4 loop are critical for G protein binding and modulation of IP3 receptor calcium store release. In particular, ligand activation of the α7 nAChR appears to promote an increase in intracellular calcium well beyond the established time frame of channel activation, a process blocked by xestospongin C and attenuated by G protein blockers. The findings support the involvement of G protein signaling in nAChR function and suggest a new framework for considering α7 nAChR driven neuronal modulation.

Disclosures: J. King: None. **J. Nordman:** None. **S.P. Bridges:** None. **M. Lin:** None. **N. Kabbani:** None.

Poster

500. Structure and Function of Nicotinic Receptors and Asics

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 500.11/C38

Topic: B.02. Ligand-Gated Ion Channels

Title: AChBP/insect chimeras as tools for the analysis and development of safer insecticides

Authors: *J. BOBANGO[1], M. WILSON[1], K. GALLEGOS[1], P. TAYLOR[2], T. TALLEY[1]
[1]Dept. of Biomed. and Pharmaceut. Sci., Idaho State Univ., Meridian, ID; [2]Skaggs Sch. of Pharm. and Pharmaceut. Sci., Univ. of California, San Diego, CA

Abstract: The nicotinic acetylcholine receptors (nAChRs), members of the Cys-Loop ligand gated ion channel superfamily, are the primary target of insecticides. Selectivity and resistance

issues of current insecticides dictate a need to research new compounds. Due to the inability to heterologously express insect nAChRs, current assay techniques involve utilizing either truly hybrid receptors or a heterogeneous mix of the binding targets from crude tissue preparations. To circumvent these issues, the soluble acetylcholine binding protein (AChBP) from the mollusk *Aplysia* californica, a good structural surrogate for insect nAChR, is recombinantly expressed and utilized for high throughput assays of binding affinity. Radioligand competition binding assays is utilized to determine the binding affinity differences of wild-type, insect like constructs, and AChBP constructs designed to mimic human nAChR subtypes. This combined with *in situ* Click Chemistry can be used to develop and evaluate analogs of neonicotinoid pesticides. Further, the ability to crystallize the AChBP constructs in the presence of novel ligands allows for structure-guided compound design. In the current study we have generated AChBP/insect chimeras by site-directed mutagenesis in the loop C region of the AChBPs for subtype and species selectivity to mimic target and off-target insects. This was completed for the following insects: *Drosophila* melanogaster (fruit fly), Myzus persicae (green peach aphid), Heliothis virescens (moth), Anopheles gambiae (mosquito), and the off-target honey bee Apis mellifera. We have assayed the affinity of the AChBP/insect chimeras for the nicotinoids nicotine and epibatidine, the neonicotinoids imidacloprid and thiocloprid, and the pyrazinoylimino and trifluoroacetylimino extended neonicotinoid analogs. We have contrasted the affinities relative to wild-type and humanized AChBP. This information will aid the development of new insecticides with greater potency and specificity including a novel series of analogs developed through Click Chemistry.

Disclosures: J. Bobango: None. **M. Wilson:** None. **K. Gallegos:** None. **P. Taylor:** None. **T. Talley:** None.

Poster

500. Structure and Function of Nicotinic Receptors and Asics

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 500.12/C39

Topic: B.02. Ligand-Gated Ion Channels

Support: FDA NicScreen Project

Title: Development and validation of a population patch clamp-based assay for subtype-selective profiling of nicotinic acetylcholine receptors

Authors: G. KIRSCH, Y. KURYSHEV, Z. LIU, L. ARMSTRONG, *C. MATHES, A. M. BROWN
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Abstract: Neuronal nicotinic acetylcholine receptors (nAChRs) belong to a family of pentameric, membrane-bound receptors which function as ionic channels and mediate cholinergic modulation of neurotransmission. Subtypes within the family of neuronal nAChRs are defined by their α- and β-subunit composition, and functional properties such as ligand-sensitivity, pharmacologic profile, Ca^{2+} permeability, and desensitization kinetics. Homopentameric α7 and heteropentameric α4β2 subtypes, in particular, are prominent in brain and have been investigated as therapeutic targets for various neurologic indications including depression, tobacco addiction, neuroinflammation, cognition impairment related to schizophrenia and Alzheimer's disease, and neurodegeneration related to Parkinson's disease. Here we describe development, optimization and validation of medium throughput population patch clamp-based assays for human nicotinic subtypes, $\alpha_3\beta_4$, α_7, $\alpha_4\beta_2$, and $\alpha_3\beta_4\alpha_5$, stably expressed in Chinese hamster ovary (CHO) cells. Cell line development and characterization were performed in both a fluorescent imaging plate reader (FLIPR Tetra) and the IonWorks Barracuda (IWB) automated patch clamp system; only the patch clamp assays are presented here. The cell lines were pharmacologically characterized by both subtype-selective and non-selective reference compounds, including agonists, pore blockers, competitive antagonists and positive allosteric modulators. An IWB platform in 384-well population patch clamp format supported development and validation of automated patch clamp assays in the four different nAChR subtypes. The validation results showed that biophysical characteristics of the receptors (agonist sensitivity and acute desensitization) measured in the IWB system were comparable to those obtained by conventional electrophysiological assays. The potency of reference antagonists for inhibition of the receptor-channels and selectivity of positive allosteric modulators also were very similar between IWB and published conventional voltage clamp results. All four assays were found to be very robust and reproducible, suitable for subtype profiling and medium-throughput screening of compound libraries in agonist, antagonist or positive allosteric modulator modes. The discovery of selective modulators may facilitate development of new therapeutics or adjuvant therapies for treatment of disease conditions such as cognition impairment, depression, addiction, and inflammation.

Disclosures: G. Kirsch: A. Employment/Salary (full or part-time):; ChanTest. 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.; FDA. **Y. Kuryshev:** A. Employment/Salary (full or part-time):; ChanTest Corporation. **C. Mathes:** A. Employment/Salary (full or part-time):; ChanTest Corporation. **Z. Liu:** A. Employment/Salary (full or part-time):; ChanTest Corporation. **L. Armstrong:** A. Employment/Salary (full or part-time):; ChanTest Corporation. **A.M. Brown:** A. Employment/Salary (full or part-time):; ChanTest Corporation. 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.; FDA. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); ChanTest Corporation.

Poster

500. Structure and Function of Nicotinic Receptors and Asics

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 500.13/C40

Topic: B.02. Ligand-Gated Ion Channels

Support: NIHDAO27990

Title: [125I]-iodo-ASEM: A New radioligand for measuring α7 nicotinic receptors

Authors: *T. T. OLSON[1], C. ONONGAYA[1], Y. XIAO[1], Y. GAO[2], R. MEASE[2], M. POMPER[2], A. G. HORTI[2], K. J. KELLAR[1]
[1]Dept. of Pharmacol. & Physiol., Georgetown Univ. Sch. of Med., Washington, DC; [2]Dept. of Radiology, John Hopkins Univ. Sch. of Med., Baltimore, MD

Abstract: Neuronal nicotinic acetylcholine receptors (nAChRs) are pentameric ligand-gated, nonselective cation channels composed of varying α and β subunits. Combinations of these subunits define the nAChR subtypes. Heteromeric nAChRs are composed of α subunits (α2 – α10) in combination with β subunits (β2 – β4), while the α7 subunit forms a homomeric receptor. The homomeric α7 nAChR is found throughout the brain and is believed to play an important role in memory, cognition, schizophrenia, and Alzheimer's disease. Studying the α7 receptor has been difficult due to the limited number of selective ligands available. Currently and historically the most important and selective ligands and tools for studying α7 nAChRs are α-bungarotoxin, which is a protein harvested from Taiwanese krait snake venom, and certain α-conotoxin peptides (Johnson et al. *Mol. Pharmacol*. 1995; Pereira et al., *J Pharmacol Exp Ther*, 1995), which are based on the venom of marine predatory cone snails. These proteins do not readily enter the brain, and therefore are not usually very useful for *in vivo* studies. Moreover, they are sometimes difficult to obtain and/or available only in relatively small quantities. More recently, methyllycaconitine, a norditerpenoid isolated from delphinium seeds has proven to be another excellent high affinity probe for α7 nAChRs (Davies et al., *Neuropharmacolgy*, 1999). Here we investigated iodo-ASEM, a novel selective high affinity α7 ligand (Gao et al., *J Med Chem*,

2013; Horti et al., *J Nucl Med*, 2014) that has the potential to be an excellent ligand for studying α7 nAChRs *in vitro* and *in vivo*. In radioligand binding assays, [^{125}I]-iodo-ASEM demonstrated high binding affinity (<300 pM) and selectivity for α7 nAChRs stably expressed in HEK cells, as well as in solubilized receptors from rat hippocampus (K_d ~3 nM), which expresses a high density of α7 receptors. In radioligand binding competition assays using [^{3}H]-epibatidine to label nAChR binding sites in membranes from cells stably transfected with different nAChR subunit combinations, we further demonstrate the selectivity of iodo-ASEM for the α7 nicotinic receptor compared to other rat and human nicotinic receptor subtypes. [^{18}F]-ASEM has recently been shown to be useful for PET imaging of α7 nAChRs in baboon brain (Horti et al. 2014), indicating its potential as a PET probe for α7 nAChRs in human brain. Further investigation of this new α7-specific ligand should facilitate studies that target the α7 nicotinic receptor and its involvement in neural functions and diseases.

Disclosures: T.T. Olson: None. **C. Onongaya:** None. **Y. Xiao:** None. **Y. Gao:** None. **R. Mease:** None. **M. Pomper:** None. **A.G. Horti:** None. **K.J. Kellar:** None.

Poster

500. Structure and Function of Nicotinic Receptors and Asics

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 500.14/C41

Topic: B.02. Ligand-Gated Ion Channels

Support: NIHDA012976

NIHDA027990

Title: Comparative pharmacology of human α4β2β4 nicotinic acetylcholine receptor subtypes expressed in HEK293 cells using sazetidine-A and AT-1001

Authors: *K. DEDOMINICIS, H. HWANG, M. UDDIN, S. LEE, T. T. OLSON, N. SAHIBZADA, Y. XIAO, B. B. WOLFE, K. J. KELLAR, R. P. YASUDA
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Abstract: Neuronal nicotinic acetylcholine receptors (nAChRs) are present in the central and peripheral nervous system where they mediate and modulate neurotransmission. nAChRs are pentameric ligand gated cation channels that assemble from combinations of different subunits: α2-α10 and β2-β4. These channels can be heteromeric, as in the α4β2 or α3β4 subtypes, or

homomeric, as in the α7 subtype. In this work, we examine the pharmacology of two different ligands, sazetidine-A and AT-1001, at heteromeric nAChRs composed of α4, β2 and β4 subunits. Radioligand binding experiments were performed on membrane homogenates prepared from HEK293 cells expressing α4β2 or α4β4 nAChRs. Both sazetidine-A and AT-1001 compete for [3H]-Epibatidine binding sites on α4β2 and α4β4 homogenates; however, they do so with different affinities. Sazetidine-A competes for α4β2 receptors with a higher affinity than for α4β4 receptors. In contrast, AT-1001 competes for α4β4 receptors with higher affinity than for α4β2 receptors. This is consistent with published data, which has demonstrated that sazetidine-A is selective for β2 containing nAChRs and AT-1001 is selective for β4 containing nAChRs. To further elucidate the binding pharmacology of α4β2 and α4β4 nAChRs with sazetidine-A and AT-1001, we constructed pentameric concatamers using alanine-glycine-serine repeat linkers. The use of concatenated constructs allows us to examine binding parameters of sazetidine-A and AT-1001 at complex nAChR stoichiometries composed of α4, β2 and β4 subunits. Competition binding with sazetidine-A against [3H]-Epibatidine at membrane homogenates from α4β2β4 concatamers fit best to a two-site non-linear regression model, consistent with the selectivity of sazetidine-A for β2 over β4 containing binding sites. The same two-site model was found for competition binding using AT-1001 at these concatamers, with AT-1001 being more selective for β4 over β2 containing binding sites. Further binding studies in conjunction with electrophysiology recordings from these concatameric pentamers expressed in HEK293 cells may provide insight into binding pharmacology for unique α4β2β4 containing nAChRs. The use of these concatameric constructs may facilitate development of ligands with specificity for these complex heteromeric receptors that may be important in some brain regions and/or in certain disease states.

Disclosures: **K. Dedominicis:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Georgetown University holds the patent for sazetidine-A. **H. Hwang:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Georgetown University holds the patent for sazetidine-A. **M. Uddin:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Georgetown University holds the patent for sazetidine-A. **S. Lee:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Georgetown University holds the patent for sazetidine-A. **T.T. Olson:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Georgetown University holds the patent for sazetidine-A. **N. Sahibzada:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Georgetown University holds the patent for sazetidine-A. **Y. Xiao:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Georgetown University holds the patent for sazetidine-A. **B.B. Wolfe:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent

holder, excluding diversified mutual funds); Georgetown University holds the patent for sazetidine-A. **K.J. Kellar:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Georgetown University holds the patent for sazetidine-A. **R.P. Yasuda:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Georgetown University holds the patent for sazetidine-A..

Poster

500. Structure and Function of Nicotinic Receptors and Asics

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 500.15/C42

Topic: B.02. Ligand-Gated Ion Channels

Support: NIH Grant 1R21AG038774

NIH Grant 1S10RR027027

NIH Grant 1T32GM077995

NSF Grant EPS-1004057

Title: Mass spectrometric investigation of human α7-nicotinic acetylcholine receptor interacting proteins in SH-SY5Y cells

Authors: M. J. MULCAHY, *E. HAWROT
Mol Pharmacol, Physiol & Biotechnol, Brown Univ., PROVIDENCE, RI

Abstract: The α7-nicotinic acetylcholine receptor (α7-nAChR) is a ligand-gated ion channel widely expressed in the mammalian CNS and is associated with numerous biological functions and diseases such as Alzheimer's. The α7-nAChR is also the principal high-affinity α-bungarotoxin (bgtx) binding protein in the mammalian CNS. The high-selectivity of bgtx for α7-nAChRs is a powerful tool which has been utilized to specifically isolate the receptor for investigation. SH-SY5Y is a human clonal cell line used to model neurons which endogenously expresses α7-nAChRs. Receptor interacting proteins are associated with the regulation and function of ligand-gated ion channels. To identify α7-nAChR interacting proteins, bgtx-sensitive protein complexes were isolated from SH-SY5Y using bgtx-conjugated affinity resin. Protein was specifically eluted with nicotinic receptor agonist, reduced and alkylated prior to digestion with trypsin in-solution. Proteins isolated and treated by the same method but with affinity resin

lacking bgtx served as controls. Tryptic peptides were separated using high-performance liquid chromatography and analyzed with a Thermo Scientific™ Q Exactive™ hybrid quadrupole-orbitrap mass spectrometer. Spectra were matched to proteins using the Mascot algorithm and resulting data analyzed using ProteoIQ™. A total of 78 proteins, including the α7-nAChR subunit, were identified (1% FDR, >90% probability of correct assignment, identified in multiple replicates, 0% probability in controls). This is the first study in which the α7-nAChR was detected along with its interacting proteins in an endogenous human neuronal model using mass spectrometry. Identified human α7-nAChR interacting proteins are associated with a diverse array of functions which may contribute to the expression, localization, function, or modulation of α7-nAChRs. Identification of endogenous human α7-nAChR-protein interactions provides a foundation to investigate how these interactions may contribute to human disease.

Disclosures: M.J. Mulcahy: None. **E. Hawrot:** None.

Poster

500. Structure and Function of Nicotinic Receptors and Asics

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 500.16/C43

Topic: B.02. Ligand-Gated Ion Channels

Support: USciences Startup Fund

Title: Arylguanidines as potential alpha7 negative allosteric modulators

Authors: *S. N. KHATRI[1], O. ALWASSIL[2], Z. BAZARSKY[3], M. DUKAT[2], M. SCHULTE[3]
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Abstract: Nicotinic acetylcholine receptors (nAChRs) are pentameric, transmembrane, ligand gated ion channels and members of the Cys-loop superfamily of receptors. Varied combinations of α (α2-α10) and β (β2-β4) subunits create different subtypes of neuronal nAChRs. The homomeric alpha7 subtype is expressed in important brain regions mediating learning, memory, and cognition, making them potential drug targets for the treatment of schizophrenia, Alzheimer's and other neurological disorders. They are also implicated in immune system function, angiogenesis, and nociception. meta-Chlorophenylguanidine (MD-354), synthesized by Dr Dukat's laboratory, has been shown to be a partial agonist of the serotonin type 3A (5HT3A)

receptor (EC50 = 3.1 μM) and a negative allosteric modulator (NAM) of the α7 nAChR (IC50 = 41.51 μM). Using a tail flick assay in a mouse model, MD-354 was found to antagonize the antinociceptive effects of nicotine mediated by α7 receptors. The goal of this study was to identify the structural features of MD-354 that are responsible for its action as a negative allosteric modulator at α7 nACh receptors. A series of MD-354 analogs with varying electronic and lipophilic character was synthesized and evaluated for its ability to inhibit ACh-induced responses of Xenopus levies oocytes expressing human α7 nACh receptors in a two electrode voltage clamp assay. Halogen substitution at the meta position of MD-354 was found to increase IC50 values relative to their increasing polarizability (from 21.8 μM to 53.6 μM). A meta-methyl substituent decreased potency (IC50 = 118.4 μM) significantly compared to the halogen-substituted analogs. Methoxy and trifluromethyl substitution produced no significant change as compared to MD-354. Also, the analog with no meta substituent decreased minimal potency (IC50 = 59.8 μM). meta-Substitution seems to have a minimal, yet complex, effect on alpha7 NAM action.

Disclosures: S.N. Khatri: None. **O. Alwassil:** None. **Z. Bazarsky:** None. **M. Dukat:** None. **M. Schulte:** None.

Poster

500. Structure and Function of Nicotinic Receptors and Asics

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 500.17/C44

Topic: B.02. Ligand-Gated Ion Channels

Title: Monitoring pentameric ligand-gated ion channel functionality along the affinity purification process

Authors: *A. V. PANDHARE[1], N. MNATSAKANYAN[1], S. RIELA[2], M. P. BLANTON[3], M. JANSEN[1]
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Abstract: *Xenopus laevis* oocytes are widely used as a heterologous expression system for the study of membrane transport proteins. Most commonly, expression is initiated by the microinjection of poly(A)-cRNA. A unique variant of the oocyte microinjection/expression system, however, utilizes the micro-transplantation of preassembled membrane proteins in vesicles that then integrate into the oocytes plasma-membrane. Our goal is to utilize the latter

system to assess the functionality of affinity-purified and lipid-reconstituted pentameric ligand-gated ion channels (pLGIC), including α4β2 and α3β4 nicotinic acetylcholine receptors (nAChRs), and the *Gloeobacter violaceus* ligand-gated ion channel (GLIC). First, we micro-transplanted HEK-293 cell membranes containing α4β2 or α3β4 nAChRs into oocytes and monitored incorporation into the oocyte plasma-membrane, receptor functionality and pharmacology using two-electrode voltage-clamp (TEVC) recordings. For α4β2 and α3β4 nAChRs, the acetylcholine (ACh) dose-response curves yielded EC_{50} values of 2.28 μM and 147.7 μM, respectively. The EC_{50} values determined for these micro-transplanted neuronal receptors are consistent with those from previously published data. Next, using protein transplantation into oocytes, we also have established an optimal set of conditions for detergent solubilization, affinity purification, and membrane reconstitution that preserves the functionality of a number of pLGICs.

Disclosures: A.V. Pandhare: None. **N. Mnatsakanyan:** None. **S. Riela:** None. **M.P. Blanton:** None. **M. Jansen:** None.

Poster

500. Structure and Function of Nicotinic Receptors and Asics

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 500.18/C45

Topic: B.02. Ligand-Gated Ion Channels

Support: NIH Grant DA026627

NIH Grant DA012242

NIH Grant MH085193

NIH Grant DA019377

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NIH Grant DA030929

NIH Grant GM085237

Title: Differential functional contributions of α4(+)/(-)β2 agonist binding sites in α4β2-nicotinic receptor isoforms

Authors: *P. WHITEAKER[1], J. EATON[1], Y. CHANG[1], J. F. COOPER[2], J. M. LINDSTROM[2], R. J. LUKAS[1], L. M. LUCERO[1]
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Abstract: Selected nicotinic agonists were used to activate and desensitize high-sensitivity (HS; (α4)2(β2)3) or low-sensitivity (LS; (α4)3(β2)2) isoforms of human α4β2-nicotinic acetylcholine receptors (nAChR). Function was assessed using two-electrode voltage-clamp electrophysiology in Xenopus oocytes expressing concatenated pentameric HS or LS α4β2-nAChR constructs (HSP and LSP). Unlike for previously-studied agonists, desensitization induced by the highly-selective agonists A-85380 and sazetidine-A (Saz-A) preferentially reduced α4β2-nAChR HS-phase vs. LS-phase responses. The concatenated-nAChR experiments confirmed that ≈20% of LS-isoform ACh-induced function occurs in an HS-like phase, which is abolished by Saz-A preincubation. Six mutant LSP were generated, each removing a conserved agonist-binding residue within the LS-isoform-only α4(+)/(-)α4 interface agonist binding site. Every mutation reduced the percentage of LS-phase function, demonstrating that this agonist site underpins LS-phase function. Oocyte-surface expression of the HSP and each of the LSP constructs was statistically indistinguishable, as measured using β2-subunit-specific [125I]mAb295 labeling (the presence of two vs. three β2 subunits in HSP vs. LSP nAChR was accounted for). However, maximum function was approximately 5x greater on a "per receptor" basis for unmodified-LSP vs. HSP α4β2-nAChR. Thus, recruitment of the α4(+)/(-)α4 site at higher agonist concentrations appears to augment otherwise-similar function mediated by the pair of α4(+)/(-)β2 sites shared by both isoforms. Further studies modified selected residues implicated in agonist binding at specific α4(+)/(-)α4 or α4(+)/(-)β2 subunit interfaces within HSP and LSP constructs. Significantly, the same mutations applied to one or the other of the nominally-identical α4(+)/(-)β2 agonist binding sites within an HSP construct produced different functional outcomes. Thus, the two α4(+)/(-)β2 agonist binding sites contribute asymmetrically to the function of α4β2 nAChR. These studies elucidate subunit interface bases for differences in pharmacology of α4β2-nAChR isoforms and illustrate new opportunities to selectively manipulate HS vs. LS α4β2-nAChR activity. In turn, since α4β2 nAChR are the predominant neuronal subtype, these discoveries may provide important insights for drug discovery and development.

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

Poster

500. Structure and Function of Nicotinic Receptors and Asics

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 500.19/C46

Topic: B.02. Ligand-Gated Ion Channels

Support: NIHgrant NS-064969

Title: Single-channel kinetic analyses of the mouse neuronal nicotinic acetylcholine receptor channels containing α3 and β4 subunits in hek293 cells

Authors: *P. G. PUROHIT
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Abstract: Background: Neuronal nicotinic acetylcholine receptors (nAChRs), contribute in excitatory transmission, are increasingly receiving attention as therapeutic targets; however, their physiological roles remain elusive. Seventeen homologous subunits organize in unique stoichiometries to form the native pentamers. The agonist concentration-response relationship of these receptors depends on the subunit composition that determines subtype-specific receptor pharmacology. The issue is rarely addressed at single-molecule level. Therefore, here I present kinetic modeling approach on nAChRs containing putative (α-3β-4)2α-3 and (α-3β-4)2β-4 stoichiometries, at single-channel level. Methods: HEK293 cells were transiently co-transfected (15 h) with cDNAs for α-3:β-4 subunits (1:6 ratio) and GFP using calcium phosphate precipitation method. Electrophysiological data were recorded for the next 2 days (23∘C, cell-attached, PBS). Single-channel data were low-pass filtered at 20- and digitized at 50-kHz. QuB suit was used to acquire and analyze the data. Results: Single-channel recordings were generated for acetylcholine, epibatidine, anabasine, choline and TC2559 at multiple concentrations. At sub-micromolar concentrations all agonists, except choline, showed isolated openings/burst with two distinct current amplitudes (L, low and H, high). Open-channel life-times of L-type events were approximately longer compared to the H-type openings. Analyses of only acetylcholine data are presented here. A partially conducting state was observed at lower concentrations; the lifetime and frequency of which decreases at increasing concentrations, from 0.1 to 30 μM. The Closed-Open events (at >100 μM) were clustered and separated by sojourns in long C periods. Based on the intra-cluster open probability (Po) difference, the L- and H-type datasets were segregated using SKM. Single-channel current amplitudes (pA±S.D.) at 100, 300 and 500 μM acetylcholine were 3.2±0.1, 3.1±0.1 and 2.3±0.15 (L) and 5.3±0.3, 5.2±0.1 and 4.9±0.0.4 (H). The intra-clusters dwell times (for L and H) were fitted with one open and three closed exponentials; a predominant C component (95%) likely corresponds to the main gating event. The Po was reduced at increasing concentrations in both populations. Conclusions: 1. The low- and high-Po clusters may represent activity from (α-3β-4)2α-3 and (α-3β-4)2β-4 combinations, respectively. 2. Determination of gating rate and equilibrium constants is experimentally feasible as single C/O components are apparent. 3. The inverse correlation between the agonist concentrations and Po may be due to agonist-induced open channel block.

Disclosures: P.G. Purohit: None.

Poster

500. Structure and Function of Nicotinic Receptors and Asics

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 500.20/C47

Topic: B.02. Ligand-Gated Ion Channels

Title: Identification of bupropion binding sites in GLIC with the photoaffinity probe [^{125}I]-SADU-3-72

Authors: *N. MNATSAKANYAN[1], A. PANDHARE[1], J. R. LEVER[4], D. J. LAPINSKY[5], H. WILMS[2], M. P. BLANTON[3], M. JANSEN[1]
[1]Cell Physiol. and Mol. Biophysics, [2]Neurol., [3]Pharmacol. and Neurosci., Texas Tech. Univ. Hlth. Sci. Ctr., Lubbock, TX; [4]Radiology, and Med. Pharmacol. and Physiol., Univ. of Missouri, Columbia, MO; [5]Pharmaceut. Sci., Duquesne Univ., Pittsburgh, PA

Abstract: Bupropion is clinically used as an antidepressant, as well as smoking-cessation aid. It is known to act as a non-competitive antagonist of nicotinic acetylcholine receptors (nAChRs). Previously, we used the photoreactive bupropion analogue (±)-2-(N-tert-butylamino)-3'-[^{125}I]-iodo-4'-azidopropiophenone ([^{125}I]-SADU-3-72) to identify binding sites in the *Torpedo* (muscle-type) nAChR. To extend our study we investigated bupropion's effect on proton-induced currents in *Gloeobacter violaceus* ligand-gated ion channel (GLIC), a prokaryotic homologue of pentameric ligand-gated ion channels, after expression in *Xenopus laevis* oocytes with two-electrode voltage-clamp recordings. Co-application of bupropion resulted in reduction of pH-induced currents with an IC_{50} value of 25 μM. Affinity-purified and detergent solubilized GLIC expressed in *E. coli* was photolabeled with [^{125}I]-SADU-3-72. In preliminary experiments, we have established bupropion-specific photo-incorporation in the GLIC subunits. Further studies are in progress to identify site(s) of labeling in GLIC, which can be conserved across the eukaryotic members of pLGICs.

Disclosures: N. Mnatsakanyan: None. **A. Pandhare:** None. **J.R. Lever:** None. **D.J. Lapinsky:** None. **H. Wilms:** None. **M.P. Blanton:** None. **M. Jansen:** None.

Poster

500. Structure and Function of Nicotinic Receptors and Asics

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 500.21/C48

Topic: B.02. Ligand-Gated Ion Channels

Support: NIH R01GM56257

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NIH R37GM049202

Title: Atomistic views of positive allosteric modulators acting on the α7 nAChR

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[1]Univ. of Pittsburgh Sch. of Med., Pittsburgh, PA; [2]Anesthesiol., Univ. of Pittsburgh, Pittsburgh, PA

Abstract: α7-nAChR plays an important role in memory, learning, and cognition. It is also a therapeutic target for neurological disorders, such as schizophrenia and Alzheimer's disease. Positive allosteric modulators (PAMs) of α7-nAChR hold therapeutic promise. 1-(5-chloro-2,4-dimethoxyphenyl)-3-(5-methylisoxazol-3-yl)urea (PNU-120596) and 3a,4,5,9b-Tetrahydro-4-(1-naphthalenyl)-3H-cyclopentan[c]quinoline-8-sulfonamide (TQS) are potent and selective PAMs for α7-nAChR. They were thought to act through binding to the transmembrane domain (TMD) of α7-nAChR, but the precise binding sites were uncertain. Here, we applied high-resolution nuclear magnetic resonance spectroscopy (NMR) and surface plasmon resonance (SPR) to determine the binding sites and affinities of PNU-120596 and TQS. Ivermectin, a ligand potentiating α7-nAChR and activating the α7-nAChR TMD channel, was also included in the studies. Three nAChR constructs, including the full-length human α7-nAChR, the TMD alone, and the TMD with the intracellular domain (TMD+ICD), were expressed in E. coli and purified using NiNTA affinity chromatography. The steady-state SPR responses of ligand binding to immobilized α7-nAChR constructs as a function of ligand concentration were measured at 25°C using a Biacore 3000 with the NTA sensor chip. Dissociation constants were derived by non-linear regression analysis using a Langmuir isotherm equation. In agreement with previous functional data, the SPR results show that, among the three tested ligands, PNU-120596 has the highest affinity to α7-nAChR, followed by TQS and ivermectin. Moreover, each ligand has a similar binding affinity to the full-length α7-nAChR, the TMD and the TMD+ICD, confirming that the ligand binding sites are within the TMD. NMR experiments were performed on the α7-nAChR TMD, which forms functional channels that can be activated by ivermectin and potentiated by PNU-120596 and TQS. NOESY and 2D saturation transfer NMR spectra, as well as ligand-induced chemical shifts, revealed intra-subunit binding sites for TQS and PNU-120596

that are located towards the extra- and intra-cellular ends of the TMD, respectively. Although the observed site for PNU-120596 was similar to that suggested previously by mutagenesis data, the TQS site is novel. Ivermectin was also found in a novel site formed between TM3 and TM4 of a single subunit and the surrounding lipids. The atomistic views of PNU-120596, TQS, and ivermectin acting on α7-nAChR are invaluable for the development of new PAMs of therapeutic potentials. This work was supported by NIH (R01GM56257 and R01GM66358 to P.T. and R37GM049202 to Y.X.).

Disclosures: V. Bondarenko: None. **P. Tang:** None. **D.D. Mowrey:** None. **T.S. Tillman:** None. **E. Seyoum:** None. **Y. Xu:** None.

Poster

500. Structure and Function of Nicotinic Receptors and Asics

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 500.22/C49

Topic: B.02. Ligand-Gated Ion Channels

Support: NOAA Sea Grant

Title: Effects of α-conotoxins from the venom of Conus purpurascens on the *Drosophila* α7 nicotinic acetylcholine receptor

Authors: *A. M. RODRIGUEZ, M. HEGHINIAN, T. A. GODENSCHWEGE, F. MARÍ
Florida Atlantic Univ., Boca Raton, FL

Abstract: The venom of *Conus purpurascens* contains several novel α-conotoxins, which are a conserved family of conopeptides that inhibit nicotinic acetylcholine receptors (nAChRs). The decreased functionality of nAChRs is associated with several diseases, including Alzheimer's disease, Parkinson's disease, and nicotine addiction. α7 nAChRs are expressed in the giant fiber system (GFS) of *Drosophila melanogaster*, which is a well-characterized neuromuscular circuit that is responsible for the escape response of the fly. We have developed a *Drosophila* based electrophysiological assay that allows us to characterize α-conotoxins. In this study, conopeptides from *C. purpurascens* were extracted from live specimens using a milking procedure and separated by reverse-phase high performance liquid chromatography (RP-HPLC). A panel of known α-conotoxins, PIA, PIB, and PIC, as well as novel conopeptides, were identified using bioanalytical techniques (Edman degradation, MS, and NMR). Paired nanoinjection of purified conotoxins into *D. melanogaster* with simultaneous

electrophysiological recording of the two muscles used in the escape response was used to screen the compounds. Our findings show that some novel conotoxins from the venom of *C. purpurascens* alter the synaptic transmission of the α7 nAChR-dependent D. *melanogaster* GFS pathway. The continued effort to characterize α-conotoxins is of great importance, as they can be used as probes to study disease pathways and neurodegeneration.

Disclosures: A.M. Rodriguez: None. **M. Heghinian:** None. **T.A. Godenschwege:** None. **F. Marí:** None.

Poster

500. Structure and Function of Nicotinic Receptors and Asics

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 500.23/C50

Topic: G.01. Molecular, Biochemical, and Genetic Techniques

Support: NIH Grant GM57481

Title: The modular character of nicotinic agonists: Minimal pharmacophores and transposable motifs for selectivity and silent agonism

Authors: *N. HORENSTEIN[1], K. CHOJNACKA[1], R. PAPKE[2]
[1]Chem., [2]Pharmacol. and Therapeut., Univ. Florida, Gainesville, FL

Abstract: Tetramethylammonium (tetMA) is the minimal structure that will effectively activate the ion channel of neuronal nicotinic acetylcholine receptors (nAChR). Dimethyldiethylammonium (diMdiEA) is the minimal structure which will selectively activate homomeric alpha7 nAChR. The minimal structure which will produce activation of alpha7 nAChR that is manifest only when the conformational landscape of the receptor has been altered by the binding of a type II positive allosteric modulator (silent agonism) is tetraethylammonium (tetEA). We have tested a series of simple amines and five additional sets of compounds with progressively larger core structures and determined that, for the essential cationic center, single methyl groups are transposable elements that can impart quantal changes in pharmacology. We identify a critical size for the core ammonium group to be approximately 150 Å3, such that analogs with solvent-excluded volume larger than this have profoundly reduced efficacy under normal conditions but may still function as silent agonists. We have previously shown that the selectivity motif of diMdiEA is one of three such motifs which can be placed in a naturally non-selective agonist in order to generate an analog that selectively activates alpha7 nAChR. We now

report that the properties of the core cationic center in a nonselective agonist within much larger global structure, such as 1,1-dimethyl-4-phenylpiperazine (diMPP) , can be modified in the same manner as the simpler amines to establish alpha7 selectivity by extending the N-methyl group to an ethyl group. Replacing the remaining N-methyl group with an ethyl group creates a tetEA substructure within the original diMPP framework that then becomes an alpha7-selective silent agonist. We have extended this approach to three other pairs of diMPP analogs, and our data support the characterization of a transposable core cationic center which is the key regulator of the detailed pharmacological property of nicotinic agonists with extended structures. These data suggest that while the ligand binding domain is tolerant of large molecules, presumably accommodated in the subunit interface, within the subdomain of the core cation recognition center there are stringent requirements for molecules to activate the ion channel and/or induce the conformational changes associated with the desensitized states that can be converted to conducting states by a type II positive allosteric modulator.

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

Poster

500. Structure and Function of Nicotinic Receptors and Asics

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 500.24/C51

Topic: B.02. Ligand-Gated Ion Channels

Support: UNT Health Science Center Intramural Seed Grant Program

American Heart Association (12BGIA8820001)

Title: Acid-sensing ion channel proton sensitivity is modulated by a guanidine containing dietary supplement

Authors: *A. AGHARKAR[1], R. N. SMITH[1], E. B. GONZALES[1,2,3]
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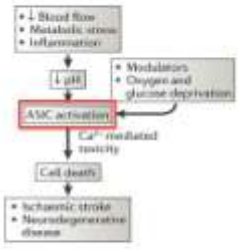
Abstract: Dietary supplements have been the focus of research to identify novel therapeutics for a variety of pathologies, including the prevention of long-term consequences of stroke and reducing pain. Ion channels offer a growing group of molecular targets for treatment, which includes the acid-sensing ion channels (ASICs). The ASICs are sodium selective channels that

are sensitive to changes in extracellular pH, specifically those changes following injury and ischemia. These channels are expressed most prominently in central and peripheral nervous system. The ASIC1a has been implicated centrally in the neurodegeneration following ischemic stroke while ASIC3 is involved in pain sensation. The extracellular domain of ASIC offers several sites for interacting with protons and guanidine compounds. We identified an over the counter dietary supplement (DS) which can modulate the activity of ASICs by altering the channel's proton sensitivity using whole-cell patch-clamp electrophysiology. The dietary supplement increased the ASIC1a pH50 values at physiologically relevant supplement concentrations consistent with suggested dietary supplementation. Our preliminary data show that ASIC3 peak current amplitude is reduced in the presence of the dietary supplement. Future studies will focus on the full characterization of the dietary supplement actions on ASICs. Our findings suggest that dietary supplement may be



neuroprotective.

Disclosures: **A. Agharkar:** None. **E.B. Gonzales:** None. **R.N. Smith:** None.

Poster

500. Structure and Function of Nicotinic Receptors and Asics

Location: Halls A-C

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Program#/Poster#: 500.25/C52

Topic: B.02. Ligand-Gated Ion Channels

Support: National Institute on Aging, Training in the Neurobiology of Aging (T32AG020494)

American Heart Association (12BGIA8820001)

Title: Acid-sensing ion channel modulation by nonproton ligands: the influence of divalent cations

Authors: *R. N. SMITH, E. B. GONZALES
Pharmacol. & Neurosci., UNT Hlth. Sci. Ctr., Fort Worth, TX

Abstract: Acid-sensing ion channels (ASICs) are sodium-selective ion channels activated by extracellular protons. These sodium channels are important components of physiological processes, like action potential propagation, as well as pathophysiological conditions, such as pain and ischemic stroke. Multiple subtypes exist that are widely distributed throughout human physiology and these subtypes respond differently to concentrations of protons in the extracellular space. Crystal structures of ASIC1 revealed that these channels are trimeric and possess a large extracellular domain likely organized for interaction with ligands more complex than protons. In particular, nonproton ligands like 2-guanidine-4-methylquinazoline (GMQ) and divalent cations (Ca2+) can interact with these acid-sensing ion channels to modulate channel activity. Previous electrophysiological studies indicate that reducing extracellular calcium concentrations can activate ASIC3. Calcium appears to lock the channel in a closed state and this calcium block must be removed before the channel can activate with protons or other ligands, like GMQ. The concentration-dependent activation of ASIC3 via GMQ application likewise is dependent on the removal of the calcium block before interacting with the nonproton ligand sensor domain. Here, we probed the role of the calcium block site in GMQ activation of ASIC3. Using full-length ASIC1, ASIC3 and ASIC1/ASIC3 chimeric receptors, we observed that there is a sequential step in the removal of calcium before GMQ activation. To fully resolve these steps, we will substitute other divalent cations for calcium (such as Cd2+, Co2+, Ba2+) and assess the changes in GMQ ASIC activation using patch clamp electrophysiology. We anticipate that higher affinity divalent cations (such as Cd2+) for the ASIC calcium block site will slow GMQ activation. Thus, our work will elucidate the role of divalents and GMQ on ASIC3.

Disclosures: R.N. Smith: None. **E.B. Gonzales:** None.

Poster

500. Structure and Function of Nicotinic Receptors and Asics

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 500.26/C53

Topic: B.02. Ligand-Gated Ion Channels

Title: Tobramycin inhibits acid sensing ion channel (ASIC) homomers 1a, 1b and 2a expressed in CHO cells

Authors: *A. M. ORTEGA[1], K. PÉREZ-TIERRA[1], M. A. GANDINI[2], R. FELIX[2], R. VEGA[1], E. SOTO[1]
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Abstract: Acid-sensing ion channels (ASICs) are ligand-gated cation channels that respond to acidic stimuli, over the years they have taken great relevance because they are involved in a number of neurological diseases such as pain, ischemic stroke, multiple sclerosis. The ASICs are homotrimers or heterotrimers of four ASIC subunits (1...4), each channel conformation displaying distinct properties. The extracellular portion of the pore of the ASIC has negatively charged regions and it is believed that this region facilitates an accumulation of cations near the pore. Tobramycin (Tbr) is a polycationic molecule, fact that makes feasible its binding to the ASIC, modulating the ionic current. In this work we recorded the acid gated current in CHO cells transfected with ASIC1a, 1b and 2a subunits. We found that the Tbr are capable to inhibit the ASIC current depending on the type of ASIC subunit and the state of the channel (open or closed). Tobramycin (100 μm) inhibits the ASIC1b current when it interacts with the channel in closed state (pre-application) (32 ± 5%), and have no significant effect when interaction with the channel is in open state (co-application); in contrast Tbr has an effect on ASIC2a current when co-applied (50 ± 8%). In the case of ASIC1a we noted an inhibition of the peak current in both conditions, pre- and co-application (33 ± 2% and 14 ± 7% respectively). These results suggest that the Tbr is a relevant tool for the study of the ASIC and that analogues of this molecule may constitute be new and valuable drug family.

Disclosures: A.M. Ortega: None. **K. Pérez-Tierra:** None. **E. Soto:** None. **R. Felix:** None. **M.A. Gandini:** None. **R. Vega:** None.

Poster

500. Structure and Function of Nicotinic Receptors and Asics

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 500.27/C54

Topic: C.17. Drugs of Abuse and Addiction

Support: DA034684

Title: ASIC1a in the nucleus accumbens regulates cocaine-associated behaviors

Authors: *A. L. SCHWAGER[1], C. KREPLE[2], C. COSME[1], Y. LU[3], J. WEMMIE[3], R. LALUMIERE[1]
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Abstract: Acid-sensing ion channels (ASICs) are abundantly expressed membrane-bound cation channels that activate upon acidosis of the extracellular fluid. Evidence indicates that ASIC1a, a critical subunit of the ASIC1 channel, is expressed at relatively high levels in the nucleus accumbens (NA), a region known to regulate drug addiction-related behaviors. The present study investigated whether ASIC1a, especially in the NA, regulates two addiction-related behaviors: cocaine conditioned place preference (CPP) and cocaine self-administration. In order to examine the contribution of ASIC1a to cocaine-associated learning and memory, ASIC expression was disrupted globally using Asic1a-/- mice. Mice then underwent CPP, in which they were administered cocaine (10 mg/kg, diluted in 0.9% saline, i.p.) and confined to one side of a two-sided chamber (the cocaine-associated context). During testing, Asic1a-/- mice showed significantly greater cocaine CPP relative to controls. To determine whether the NA was responsible for these effects, ASIC1a expression was restored in the NA of Asic1a-/- mice by injecting 0.5 µl of 70% AAV-Asic1a and 30% AAV-eGFP bilaterally into the NA. Restoring ASIC1a in the NA of Asic1a-/- mice reversed the increased CPP found in the knockout mice. To further confirm the NA as the responsible brain region, site-specific disruption of ASIC1a was achieved by using Asic1aloxP/loxP mice and bilaterally injecting AAV-Cre into the NA. Selective disruption of ASIC1a in the NA also significantly increased preference on the CPP test compared to control mice. These findings suggested that ASIC1a in the NA plays a role in regulating the rewarding/reinforcing properties of cocaine. To determine whether this was the case using a self-administration model, ASIC1a was overexpressed in rats by injecting 0.5 µl of AAV2/1-CMV-Asic1a(mouse) bilaterally into the NA core. Rats were trained to self-administer cocaine in daily two-hour sessions during which active lever presses resulted in an intrajugular cocaine infusion. Rats underwent one day of self-administration with each of five cocaine doses (300, 90, 30, 9, 3 µg/infusion) to create a dose-response curve. Overexpression of ASIC1a in the NA of rats reduced cocaine self-administration relative to AAV2/1-CMV-eGFP controls and produced a rightward shift in the dose-response curve, suggesting that increasing ASIC1a expression and activity in the NA reduced the reinforcing properties of cocaine. Together, these results suggest that ASIC1a in the NA is involved in the rewarding properties of cocaine and, moreover, point to a novel potential therapeutic target for drug addiction.

Disclosures: A.L. Schwager: None. **C. Kreple:** None. **C. Cosme:** None. **Y. Lu:** None. **J. Wemmie:** None. **R. LaLumiere:** None.

Poster

500. Structure and Function of Nicotinic Receptors and Asics

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Program#/Poster#: 500.28/C55

Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Fellowship 5 F31 DC012241

Title: Caenorhabditis elegans: A small, but mighty, tool in understanding addiction

Authors: *S. A. WESCOTT[1,2], M. RAUTHAN[2,3], E. A. RONAN[2], X. Z. S. XU[2]
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Abstract: Motivated behaviors, such as food-gathering, mate-seeking, predator-evasion, and harm-avoidance, are necessary for the propagation and survival of most species, particularly animal species. However, when environmental factors or stimuli subvert the neural mechanisms underlying these adaptive behaviors, maladaptive behaviors and disease states can arise, such as addiction in response to drugs of abuse. For example, in mammals, it is well-documented that most psychoactive drugs of abuse impinge upon the mesolimbic dopamine pathway that plays a vital role in countless motivated behaviors. The intricacy of the mammalian nervous system and the rich behavioral variety of which mammals are capable add layers of complexity to the study of addiction biology. As the hermaphroditic nematode, Caenorhabditis elegans, is capable of self-fertilization, their motivated behaviors are limited largely to foraging and avoidance behaviors. For example, *C. elegans* will move up the gradient of chemicals associated with or predictive of a food source. Meanwhile, these worms will crawl down a gradient of noxious chemicals and will avoid areas of high osmolarity or irradiated with high intensity light. Moreover, myriad studies have demonstrated that *C. elegans* is sensitive to a number of plant alkaloids frequently abused by humans and other mammals, including nicotine and cocaine, among others. In addition to the constrained behaviors *C. elegans* exhibits, the hermaphrodite nervous system consists of only 302 neurons, with a reliably complete and long-established connectome. Furthermore, the genetic amenability and short generation time of these nematodes makes them an attractive model for investigating the manner in which drugs of abuse usurp adaptive neural mechanisms to elicit maladaptive behavioral outcomes. Accordingly, we have developed a behavioral paradigm that allows us to screen mutant worms rapidly for both canonical and novel targets for drugs of abuse, such as acid-sensing ion channels, transient receptor potential channels, and epithelial sodium channels.

Disclosures: **S.A. Wescott:** None. **M. Rauthan:** None. **E.A. Ronan:** None. **X.Z.S. Xu:** None.

Poster

500. Structure and Function of Nicotinic Receptors and Asics

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 500.29/C56

Topic: B.02. Ligand-Gated Ion Channels

Title: The acid-sensing ion channel (ASIC)

Authors: *D. C. BERTRAND[1], S. BERTRAND[1], Y. GAUTSCHI[2], L. SCHILD[2]
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Abstract: Acid-sensing (proton-gated) ion channels belong to the ENaC/degen (Lv et al., 2011)erin superfamily of sodium (Na+) channels that includes the epithelial Na+ channel (ENac). ASIC channels are multimeric channels made of homologous subunits; the subunits ASIC1-4 are encoded by four different genes ACCN1-4 genes.. Evidence support the role of ASICs in pain sensation, expression of fear, neurodegenerarion after ischemia (see for review Wemmie 2013) Spontaneous mutations in ASIC1a have been associated to genetically transmissible diseases such as temporal lobe epilepsies . Activated by low pH, ASIC's display a fast response onset and rapid desensitization. Sharing similarities to other ligand gated such as the neuronal nicotinic acetylcholine receptors, ASIC1 are inactivated (desensitized) by sustained exposure to a condition that otherwise produces activation. Namely, desensitization occurs at pH that are higher (more alkaline) than those causing its activation, which results in activation and desensitization profiles that do not necessarily overlap or yield the so call window currents. Probing ASIC channels function was conducted using heterologous expression in Xenopus oocytes as well as in patch clamp in transfected cells (Vallet et al., 1998;Waldmann et al., 1997;Li et al., 2012). Development of HiClamp, an automated electrophysiological system using expression in Xenopus oocytes offers fast drug application and minimal compound volume allowing multiple measurements in 230 µL of solution and was shown to be optimum for functional investigation of ligand gated channels. In this work we present the latest developments of automated recordings for ASIC1a channels expressed in Xenopus oocytes and revisit physiological and pharmacological properties of this important class of ion channels. Offering new possibilities for functional screening of these rapidly desensitizing channels this work illustrates how to probe effects of libraries of compound in an unattended manner. References Li T, Yang Y, Canessa CM (2012) Impact of recovery from desensitization on acid-sensing ion

channel-1a (ASIC1a) current and response to high frequency stimulation. J Biol Chem 287:40680-40689.Lv RJ, He JS, Fu YH, Zhang YQ, Shao XQ, Wu LW, Lu Q, Jin LR, Liu H (2011) ASIC1a polymorphism is associated with temporal lobe epilepsy. Epilepsy Res 96:74-80. Vallet V, Horisberger JD, Rossier BC (1998) Epithelial sodium channel regulatory proteins identified by functional expression cloning. Kidney Int Suppl 67:S109-S114. Waldmann R, Champigny G, Bassilana F, Heurteaux C, Lazdunski M (1997) A proton-gated cation channel involved in acid-sensing. Nature 386:173-177.

Disclosures: D.C. Bertrand: None. **S. Bertrand:** None. **L. Schild:** None. **Y. Gautschi:** None.

Poster

501. NMDA Receptor Structure and Function

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 501.01/C57

Topic: B.02. Ligand-Gated Ion Channels

Support: Prin 2010–2011

Title: Synaptic availability of GluN2A subunit of NMDA receptors: The role of Rabphilin 3A

Authors: *F. GARDONI[1], J. STANIC[1], M. CARTA[2], A. A. GENAZZANI[3], C. MULLE[2], M. DI LUCA[1]
[1]Univ. Milan, Milano, Italy; [2]Univ. of Bordeaux, Bordeaux, France; [3]Univ. del Piemonte Orientale, Novara, Italy

Abstract: NMDA receptor subunit composition commands receptor function and pharmacological responses. The identity of the GluN2 subunit regulates biophysical and pharmacological properties of the receptor and influences receptor assembly, signaling and localization. Recently, a two-hybrid screening has highlighted Rabphilin 3A (Rph3A) as a new GluN2A partner. Rph3A is a synaptic vesicle-associated protein first identified as a binding partner of Rab3A. Moreover, different studies have indicated that Rph3A can regulate exo- and endocytosis processes at presynaptic sites. Our goal was to characterize Rph3A interaction with GluN2A at postsynaptic sites and to assess its function. Subcellular fractionation assays revealed that Rph3A is present in postsynaptic density (PSD) fractions. Immunofluorescence studies performed in neuronal hippocampal cultures revealed Rph3A colocalization with PSD-95 and GluN2A. With co-immunoprecipitation and GST-pulldown assays, we found Rph3A to be interacting with GluN2A(1349-1389) as well as PSD-95(PDZ3) domains. Therefore, we

designed a cell permeable peptide containing GluN2A(1349-1389) sequence (Tat-2A-40) that disrupts the interaction between Rph3A and GluN2A, reduces the expression of GluN2A in dendritic spines and its surface expression and acutely reduces the amplitude of NMDAR-mediated currents. Moreover, we observed a decrease of hippocampal GluN2B-to-GluN2A developmental perturbation at postsynaptic sites in pups injected with Tat-2A-40 at that crucial development stages. These results demonstrate the presence of Rph3A in the PSD compartment and indicate a function of Rph3A in the modulation of GluN2A localization through the formation of a triple complex with GluN2A and PSD-95.

Disclosures: **F. Gardoni:** None. **J. Stanic:** None. **M. Carta:** None. **A.A. Genazzani:** None. **C. Mulle:** None. **M. Di Luca:** None.

Poster

501. NMDA Receptor Structure and Function

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 501.02/C58

Topic: B.02. Ligand-Gated Ion Channels

Support: NHLBI HL019982

Title: Structural and functional study of N-Methyl-D-Aspartate Receptor-specific antagonistic peptides from Conus species of marine snails: ConPr1, 2, 3, and ConRlB

Authors: ***S. KUNDA**[1,2], T. SNOW[1,2], J. CHERIYAN[2], M. HUR[2], R. BALSARA[1,2], F. J. CASTELLINO[1,2]
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Abstract: Conantokins are naturally occurring, gamma-carboxyglutamate (gla)-containing neuroactive peptides found in the venom of marine snails, which have been studied for their highly selective antagonistic activity towards N-Methyl-D-Aspartate Receptors (NMDAR). Here we report the characterization of peptides from *Conus parius* and *Conus rolani* species of snail identified as Conus parius1, 2, and 3 (ConPr1, 2, 3) and conantokinRlB (ConRlB), which uniquely differs from other conantokins by the presence of a 4-transhydroxyproline (Hyp 'O') residue. These conantokins have been studied for their structural α-helical conformation in the presence or absence of divalent ions, as well as their biological effects downstream of NMDAR activation. ConPr1 and ConPr2 showed an increase in α-helicity on addition of Mg^{2+} ions,

whereas native-apo-ConPr3 is inherently helical. ConRlB displays 62% α-helicity when compared to ConG (100%), in presence of Mg^{2+}. Further elucidation of backbone structure of Mg^{2+}-ConRlB by ^{1}H NMR and TALOSplus software has predicted a disruption in the helix due to the presence of Hyp at position 10. Robust inhibition of intracellular calcium influx [iCa^{2+}] and whole cell recordings was observed in GluN2A$^{-/-}$ mice cortical neurons, but not in GluN2B$^{-/-}$ neurons, revealed GluN2B subunit specific activity of Con Pr peptides and ConRlB. Synthesis of mutant peptides to assess the role of Hyp in 10^{th} position in ConRlB was aimed at elaborating the molecular requisites required by these peptides for their unique structural and functional attributes. ConG[Insert 10 'O'] and ConRl-B[K8N,A9Q,ΔO] behave functionally and structurally similar to their respective parent peptides, ConG and ConRlB, calling to attention the role played by amino acids other than the second inter-gla fragment. In addition, ConPr1, 2, 3 and ConRl-B peptides mildly inhibited NMDA-mediated phosphorylation of CREB at Ser133, a transcription factor required for maintaining long term synaptic activity. Therefore, inhibitory properties displayed by the conantokins antagonize NMDAR-directed current and iCa^{2+} influx, while maintaining the receptor-coupled CREB signaling pathway. Similar experiments were performed with GluN2C$^{-/-}$ and GluN2D$^{-/-}$ neurons. A significant inhibition of iCa^{2+} influx was observed by ConG and ConRlB in DIV9 GluN2D$^{-/-}$ neurons, but not in DIV13-15 GluN2D$^{-/-}$ neurons. In conclusion, biophysical and cellular characterization in conjunction with genetic studies enables correlations of the structure-function relationships of conantokins as allosteric inhibitors of NMDAR.

Disclosures: S. Kunda: None. **T. Snow:** None. **J. Cheriyan:** None. **R. Balsara:** None. **F.J. Castellino:** None. **M. Hur:** None.

Poster

501. NMDA Receptor Structure and Function

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 501.03/C59

Topic: B.02. Ligand-Gated Ion Channels

Support: NSF IOS-1026527

DOD-TS130081

Title: Rapid antidepressant stimulates decoupling of GABAB receptors from GIRK/KIR channels through 14-3-3η

Authors: *E. WORKMAN[1], P. C. G. HADDICK[2], G. DILLY[1], B. ZEMELMAN[1], K. RAAB-GRAHAM[1]
[1]Inst. for Neurosci., The Univ. of Texas At Austin, Austin, TX; [2]Div. of Res., Genentech, South San Francisco, CA

Abstract: A single injection of NMDAR antagonists produces a rapid antidepressant response. Lasting changes in synapse structure and composition underlie the effectiveness of these drugs. However, little is known about the molecular and biochemical mechanisms which initiate these systemic changes. We recently discovered that rapid antidepressants cause a shift in the $GABA_BR$ signaling pathway. With NMDAR antagonists, $GABA_BR$ activation shifts from opening potassium channels to increasing resting dendritic calcium signal and mTOR activity. Herein, we find that $GABA_BR$ signaling to Kir3 (GIRK) channels is reduced with NMDAR blockade. Consistent with these findings, *in vivo* administration of the rapid antidepressant AP5, decreases hippocampal expression levels of Kir3.2 while increasing $GABA_BR2$. Relieving NMDA-dependent repression of 14-3-3η protein expression promotes the decoupling of $GABA_BR$ signaling from Kir3 and is required for the rapid antidepressant efficacy. Taken together, these data suggest that the shift in $GABA_BR$ function requires a loss of $GABA_BR$-Kir3 channel activity mediated by 14-3-3η. Our findings support a central role for 14-3-3η in the efficacy of rapid antidepressants and define a critical molecular mechanism for activity-dependent alterations in $GABA_BR$ signaling.

Disclosures: E. Workman: None. **P.C.G. Haddick:** A. Employment/Salary (full or part-time):; Genentech. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Genentech. **G. Dilly:** None. **B. Zemelman:** None. **K. Raab-Graham:** None.

Poster

501. NMDA Receptor Structure and Function

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Program#/Poster#: 501.04/C60

Topic: B.02. Ligand-Gated Ion Channels

Support: NIH Intramural award to CJM

Title: Investigating the role of NMDA receptor expression in the developmental integration of hippocampal neurogliaform cells

Authors: *E. S. BARKSDALE, R. CHITTAJALLU, X. YUAN, D. COLLINS, K. PELKEY, C. MCBAIN
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Abstract: Appropriate development of interneurons is essential in ensuring normal CNS function. Indeed, the precipitation of a number of neurological disorders can be linked to interneuron dysfunction. Together, the neurogliaform cell (NGFC) and the closely related Ivy cell comprise the most abundant group of interneurons in the CA1 hippocampal region, and their recruitment results in widespread GABA volume transmission. Thus NGFCs/Ivy cells have the potential to impart an inhibitory influence on large numbers of neurons within the hippocampal circuitry. However, in contrast to other interneuron subtypes, relatively little is known about NGFC function and in particular the developmental aspects of their circuit integration. NMDA receptors (NMDARs) have been implicated in a wide variety of cellular phenomena from proliferation/migration through to synaptic refinement/plasticity and recruitment. NGFCs located in the stratum lacunosum moleculare (S-LM) receive excitatory input from the entorhinal cortex via the temporoammonic pathway (TA) and serve as pure feed-forward interneurons, inhibiting distal dendrites of CA1 pyramidal cells. In the current study we show that upon TA stimulation, S-LM NGFCs possess unusually large NMDAR-mediated responses when compared to other CA1 interneuron subtypes. This prompted us to focus on the possible roles of NMDAR expression on varying aspects of NGFC development. The majority of S-LM NGFCs arise from progenitors in the caudal ganglionic eminence (CGE) that specifically express the $5HT_{3A}$ serotonergic receptor. Crossing floxed NR1 knockout mice with mice in which the $5HT_{3A}$ promoter drives expression of both Cre and tdTomato (Ai14 reporter) allowed us to generate deletion of NMDARs in NGFCs. Initial data indicate a decreased density of S-LM NGFCs in $5HT_{3A}Cre:NR1^{-/-}$ when compared to wild-type mice across a number of developmental time points. Furthermore, NGFCs in which functional NMDAR-mediated currents were abolished using our genetic strategy demonstrated a significantly larger AMPA sEPSC frequency but no change in peak amplitude. Importantly, the paired pulse ratio of TA-evoked AMPA EPSCs was unchanged in $5HT_{3A}Cre:NR1^{-/-}$ compared to WT indicating similar release probabilities at TA inputs to WT and NR1 knockout NGFCs. Altogether these data suggest NMDARs play a crucial role in varying aspects of NGFC development including regulating functional synapse formation. We are currently expanding on these data to confirm our initial findings and also to fully investigate the underlying mechanisms involved.

Disclosures: E.S. Barksdale: None. **R. Chittajallu:** None. **X. Yuan:** None. **D. Collins:** None. **K. Pelkey:** None. **C. McBain:** None.

Poster

501. NMDA Receptor Structure and Function

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Program#/Poster#: 501.05/C61

Topic: B.02. Ligand-Gated Ion Channels

Support: Direction Générale de l'Armement

CNRS

Université Aix-Marseille

ANR

Title: Regulation of NMDA receptors functions in the hippocampus: D-serine of glycine?

Authors: *M. LE BAIL[1], S. SACCHI[2], K. AIT OUARES[1], N. A. MOHAMAD NOR HAZALIN[1], L. POLLEGIONI[2], H. WOLOSKER[3], J.-M. BILLARD[4], J.-P. MOTHET[1]
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Abstract: The N-methyl-D-aspartate receptors (NMDARs) are glutamate-gated ionotropic receptors which play a crucial role in synaptic plasticity and cognitive functions. When their function is altered these receptors are also involved in a broad range of neurologic and psychiatric disorders. NMDA receptors activation requires the binding of glutamate and of a coagonist that could be glycine or D-serine. Here we investigated the respective contribution of glycine and D-serine in controlling NMDARs activity in CA1 and dentate gyrus (DG) subregions of the rat hippocampus, a brain area underlying synaptic plasticity and memory formations. To that purpose we recorded NMDAR-mediated field excitatory post-synaptic potentials (fEPSPs) elicited at CA3-CA1 synapses and in DG *stratum moleculare* in response to the stimulation of Schaffer collaterals and to medial Perforant Path fibers respectively in acute hippocampal slices. Selective depletion of endogenous D-serine with applied recombinant D-amino acid oxidase (DAAO) dramatically reduced NMDAR-fEPSPs in the CA1 but not in the DG. Conversely, application of glycine oxidase (GO) to selectively deplete endogenous glycine had no significant effect in CA1 while it significantly impacted NMDA-fEPSPs in the DG. We next showed that pharmacological inhibition of the DAAO potentiated responses in the CA1 but not in the DG, further demonstrating that glycine is the major coagonist for NMDARs in the DG whereas D-serine is the preferred one at CA3-CA1 synapses. This regional compartmentalisation of the coagonist identity is associated to a specific subtype of GluN1/GluN2A or 2B-containing

NMDARs as evidenced by the use of specific antagonists and immunoblot analyses. Strikingly, both amino acids are mandatory for long-term changes in synaptic plasticity. Indeed, enzymatic ablation of their functions occluded the induction and expression of Long Term Potentiation in both DG and CA1. We finally performed experiments in juvenile rats (P9-P14) at the time where GluN1/GluN2B-containing NMDARs over numbered GluN1/GluN2A NMDARs at CA3-CA1 synapses as compared to mature synapses. Here we revealed a developmental switch in the identity of the coagonist since glycine and not D-serine is the preferred coagonist in immature CA3-CA1 synapses. Accordingly, pharmacological inhibition of DAAO at immature CA3-CA1 synapses failed in potentiating NMDA-fEPSPs as compared to mature synapses. Altogether, our observations revealed an unprecedented developmentally and regionally switch in the identity of the coagonist at synaptic NMDARs.

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Poster

501. NMDA Receptor Structure and Function

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 501.06/C62

Topic: B.02. Ligand-Gated Ion Channels

Support: Ekam Imaging

Title: Using fMRI in awake rats to differentiate NMDA receptor antagonists

Authors: *K. MOORE[1], P. KULKARNI[1], M. NEDELMAN[2], C. F. FERRIS[1]
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Abstract: BOLD imaging at 7.0 Tesla was performed on awake rats to examine the dose-dependent changes in brain activity with the NMDA antagonists ketamine and CP101-606. Human and animal studies using 2DG, cFos, PET and MRI have identified several key areas that comprise the ketamine sensitive neural network . With dose escalation the activity of this network shows increasing positive BOLD for both ketamine and CP101-606 in limbic cortex and brain areas associated with emotion and cognition. The pattern of positive BOLD signal change is very similar for both antagonists across a broad range of doses (0.1 - 10mg/kg)(Fig 1). However, at low doses of both drugs (0.1mg/kg) the pattern of negative BOLD signal change is

significantly different. Treatment with CP101-606 results in a robust negative BOLD signal change localized primarily to pontine, cerebellar and brainstem areas. CP101-606 (Traxoprodil) is selective for the NR2B subunit of the NMDA receptor and shown to have neuroprotective effects in Parkinson's models with some evidence of having rapid antidepressant effects similar to ketamine. Given the similarity in positive BOLD between both antagonists it is likely that activation of a common neural circuitry is responsible for antidepression effects while the unique inhibition in brain activity by CP101-606 in the pons, cerebellum and medulla oblongata may provide protection in animals models of neurodegeneration.

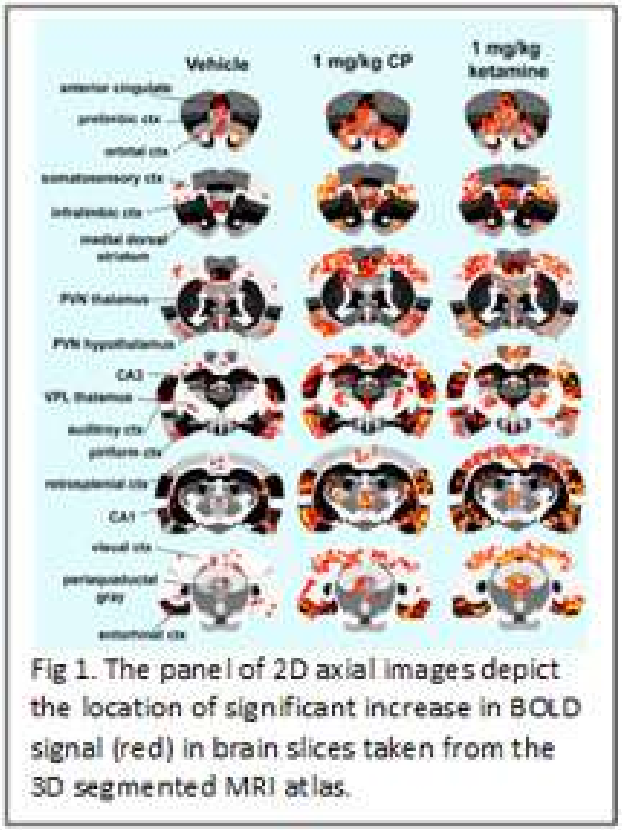

Fig 1. The panel of 2D axial images depict the location of significant increase in BOLD signal (red) in brain slices taken from the 3D segmented MRI atlas.

Disclosures: **K. Moore:** None. **P. Kulkarni:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Ekam Imaging. **M. Nedelman:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Ekam Imaging. **C.F. Ferris:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Animal Imaging Research, Ekam Imaging.

Poster

501. NMDA Receptor Structure and Function

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 501.07/C63

Topic: B.02. Ligand-Gated Ion Channels

Support: Wellcome Trust

Title: A GRIN2A de novo mutation associated with epilepsy and intellectual disability reduces NMDA receptor currents and Mg^{2+} block in cultured primary cortical neurons

Authors: *K. MARWICK, P. A. SKEHEL, G. E. HARDINGHAM, D. J. A. WYLLIE
Univ. of Edinburgh, Edinburgh, United Kingdom

Abstract: *GRIN2A* encodes the GluN2A subunit of the NMDA receptor, a subtype of ionotropic glutamate receptor that displays voltage-dependent block by Mg^{2+} and a high permeability to Ca^{2+}; these receptors play important roles in synaptogenesis and synaptic plasticity. Recently individuals with a range of childhood onset epilepsies and intellectual disability have been found to carry heterozygous missense mutations in this gene, including a *de novo* mutation substituting lysine for an asparagine in the NMDA receptor M2 pore region ($GluN2A^{N615K}$). We hypothesised that this mutation underlies the carrier's early onset epilepsy and severe intellectual disability and sought to explore its functional consequences. As the altered residue comprises part of the ion permeation pathway, we made two-electrode voltage clamp recordings from *Xenopus laevis* oocytes expressing GluN1/$GluN2A^{N615K}$ NMDARs and compared these to wild-type (WT) GluN1/GluN2A NMDARs to assess this mutation's effect on the potency of voltage-dependent Mg^{2+} inhibition. As might be anticipated given the location and nature of the mutation we found that Mg^{2+} (1 mM) block of GluN1/$GluN2A^{N615K}$ mediated currents, at -60 mV, was significantly decreased (5 ± 2% (N615K, n = 13); 89 ± 1% (WT, n = 15)). Furthermore, block by memantine (10 μM) and amantadine (100 μM) was also reduced at GluN1/$GluN2A^{N615K}$ NMDARs compared to WT NMDARs (memantine: 26 ± 1% (N615K, n = 17); 75 ± 2% (WT, n = 17), amantadine: 18 ± 1% (N615K, n = 17); 44 ± 3% (WT, n = 17)). Intriguingly, block by ketamine was unaffected by the mutation, whereas we observed a significant increase in the block produced by dextromethorphan (56 ± 3% (N615K, n = 9); 44 ± 2% (WT, n = 8)). We next used whole-cell patch-clamp recordings to evaluate NMDAR-mediated currents in mouse primary cortical pyramidal neurons that were transfected with either $GluN2A^{WT}$ or $GluN2A^{N651K}$ subunits. We observed a significant decrease in Mg^{2+} (1mM) sensitivity (50 ± 5% (N615K, n = 10); 93 ± 2% (WT, n = 9)) and a significant decrease in current density (36 ± 6% (N615K, n = 10); 61 ± 2% (WT, n = 9)). In summary, the disease associated mutation $GluN2A^{N615K}$ has substantial effects on NMDA receptor inhibition by both endogenous and exogenous channel blockers. **Acknowledgements**: This work was funded by The Wellcome Trust via an Edinburgh Clinical Academic Training PhD Fellowship.

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Poster

501. NMDA Receptor Structure and Function

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Program#/Poster#: 501.08/C64

Topic: B.02. Ligand-Gated Ion Channels

Support: NIH Grant R01 MH045817

NIH Grant T32 NS073548

Title: Voltage dependence of NMDA receptor inhibition by memantine and by ketamine depend on duration of glutamate application and on receptor subtype

Authors: ***N. G. GLASGOW**[1], J. W. JOHNSON[2]
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Abstract: NMDA receptors (NMDARs) are a class of ionotropic glutamate receptor that are essential for neuronal development, synaptic plasticity, learning, memory formation, and cell survival. NMDARs are typically composed of GluN1 and GluN2 subunits. There are 8 GluN1 splice variants arising from a single gene and 4 GluN2 subunits (GluN2A-GluN2D) expressed from 4 separate genes. NMDAR subtypes are defined by the identities of the subunits present in the receptor (e.g. the GluN1/2A NMDAR subtype contains two GluN1 and two GluN2A subunits). NMDARs play critical roles in normal neuronal physiology, and NMDAR dysregulation is implicated in many neurological disorders including Alzheimer's disease, schizophrenia, depression, and neuropathic pain. Inhibition of NMDARs with open channel blockers, such as memantine, which is approved for treatment of Alzheimer's disease, has broad potential for treatment of neurological disorders. Another NMDAR open channel blocker, ketamine, has shown promise as a rapidly-acting antidepressant and as a treatment for neuropathic pain. Although memantine and ketamine share similar voltage dependence, kinetics and IC50s at NMDARs, their clinical effects vary significantly, suggesting that their mechanisms of NMDAR inhibition may differ in ways that are not yet well understood. There has been recent debate over the efficacy of inhibition by memantine and ketamine when NMDARs are activated by synaptic glutamate release as opposed to when NMDARs are activated under steady-state conditions (e.g., Xia et al., 2010; Emnett et al., 2013). Further, whether memantine and ketamine

exhibit similar voltage dependence during synaptic activation and during steady-state activation is unknown. Therefore, we compared inhibition of heterologously expressed GluN1/2A and GluN1/2B receptors activated by synaptic-like glutamate applications (2-4 ms) and by long (>10 s) glutamate applications at 3 membrane voltages (Vms): -65, -45, and -25 mV. We focused on NMDARs containing GluN2A and GluN2B subunits because they are the GluN2 subunits most commonly expressed at synapses. We have previously shown at a holding Vm of -65 mV that memantine and ketamine display NMDAR subtype-dependent inhibition during synaptic-like glutamate activations. At holding Vms of -45 and -25 mV we found for both memantine and for ketamine that inhibition did not depend on NMDAR subtype, or on whether responses were activated by synaptic-like or by long glutamate applications. Thus, voltage dependence of NMDAR inhibition by memantine and by ketamine depends both on duration of glutamate application and on NMDAR subtype.

Disclosures: **N.G. Glasgow:** None. **J.W. Johnson:** None.

Poster

501. NMDA Receptor Structure and Function

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 501.09/C65

Topic: B.02. Ligand-Gated Ion Channels

Support: NIH R01AG032132

Title: Transmembrane conformational signaling at NMDA receptors independent of ion flow during synaptic plasticity

Authors: *K. B. DORE, J. AOW, R. MALINOW
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Abstract: Traditionally, ion-channels achieve signaling through transmembrane passage of ions. Whether ion-channels can transmit transmembrane conformational information in the absence of ion passage is not well established. Here we use Förster resonance energy transfer (FRET) imaging of fluorescently tagged proteins expressed in neurons to monitor conformational changes within the NMDA receptor (R) complex. We show that extracellular ligand binding to the NMDAR drives a rapid ion-flow independent conformational change in its cytoplasmic domain (cd). In conditions that produce LTD, a form of synaptic plasticity, ligand binding to the NMDAR is sufficient to drive a transient FRET reduction between NMDARcd and phosphatase

1 (PP1) as well as a long-lasting FRET reduction between NMDARcd and calcium/calmodulin-dependent protein kinase II (CaMKII) that depends on PP1 activity. These results demonstrate a novel signaling mechanism for ion channels and suggest that ligand binding to the NMDAR drives NMDARcd-complexed PP1 to dephosphorylate and redirect NMDARcd-bound CaMKII during LTD.

Disclosures: K.B. Dore: None. **J. Aow:** None. **R. Malinow:** None.

Poster

501. NMDA Receptor Structure and Function

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 501.10/C66

Topic: B.02. Ligand-Gated Ion Channels

Support: NIGMS Grant GM48677

Title: Classification of neuronal cell types in the mouse brainstem through cell specific constellations

Authors: *S. RAGHURAMAN[1], A. GARCIA[2], K. J. CURTICE[1], R. TEICHERT[1], J.-M. RAMIREZ[2], B. OLIVERA[1]
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Abstract: The mammalian brain consists of an extraordinary number of cell types. A deeper understanding of circuits, networks and behavior requires that we identify and study specific neuronal cell types. Comprehensive classification of these cell types is impeded partly by their molecular complexity and by the inability to identify each cell type by multiple molecular markers. Here we describe an approach to resolve these issues, which allowed us to define different cell types and to refine their molecular characterization by using target-selective conotoxins. We studied a heterogeneous population of cells obtained from a network in mouse brainstem that generates breathing rhythm. To classify cell types in this region, we used an approach that we call constellation pharmacology. The ventral respiratory columns from mice were isolated and dissociated to obtain a heterogeneous cell population. Using calcium imaging, we then monitored the simultaneous responses of ~200 cells to a panel of pharmacological challenges. The responses to such pharmacological perturbations enabled us to categorize these cells into three major cell classes and subclasses based on the constellations of ion channels and

receptors expressed by each cell. We found that a subset of these cells that responded to substance P, putatively inspiratory pre-Bötzinger complex (preBötC) neurons, also responded to histamine and bradykinin. We further examined the existence of these cell classes in an intact slice through electrophysiological studies and confirmed that the preBötC neurons responsive to substance P in an intact slice exhibited similar responsiveness to bradykinin and histamine. Thus, this approach provided a platform to classify cell types with crucial physiological roles in behavior and to generate hypotheses to test the roles of modulatory inputs to these defined cell classes in an intact system. To further refine the definition of these cell classes, we used target-selective conotoxins that differentiated between complex molecular isoforms. For example, NMDA receptors that are found in various combinations of subunits are difficult to study due to the lack of target selective tools. Using conantokin-Rl-B, a conotoxin that selectively targets NR2B subunits of NMDA receptors, we demonstrate its expression only in a specific subset of the defined cell types. Thus, using constellation pharmacology as our approach, we defined various cell types within the ventral respiratory column of the mouse brainstem and studied the expression of complex NMDAR isoforms within these cell types using target selective conotoxins.

Disclosures: S. Raghuraman: None. **A. Garcia:** None. **K.J. Curtice:** None. **R. Teichert:** None. **J. Ramirez:** None. **B. Olivera:** None.

Poster

501. NMDA Receptor Structure and Function

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Program#/Poster#: 501.11/C67

Topic: B.02. Ligand-Gated Ion Channels

Title: PSD-95 differentially regulates trafficking of NMDA receptors based on their subunit composition

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Abstract: The function of PSD-95 has been viewed primarily as restricting NMDA or AMPA receptors to synapses. We report that the splice variants of the NR1 subunit impart unique trafficking and targeting properties on both individual subunits, and assembled full-length NMDA receptors, in the context of PSD-95. NR2B/NR1-3/4 receptors in the presence of PSD-95

are targeted to the trans-Golgi network (TGN) and directly to synapses, reconstituting the effect of AP5 on NMDA receptor targeting. NR2B/NR1-1/2 receptors are clustered on the cell surface in the presence of PSD-95 and found in recycling endosomes at lower expression levels. PSD-95 PDZ-binding to NR1-3 or NR1-4 triggers a differential interaction between PSD-95 and NR1 that blocks clustering and imparts TGN and synaptic targeting. On the other hand, PSD-95 binding to the NR2B PDZ-binding domain and sequences upstream causes well-characterized clustering, and recycling endosomal localization. These results, along with prior work related to SAP97, another PSD-95 family MAGUK, suggest these proteins may generally operate as trafficking switches, employing ligand-specific architectures to mediate differential sorting and targeting of their respective binding partners.

Disclosures: S.H. Standley: None. **M. Avetisyan:** None. **M. Ronilo:** None.

Poster

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Topic: B.02. Ligand-Gated Ion Channels

Support: NINDS R01NS032123

Title: Autocrine boost of NMDAR current in hippocampal CA1 pyramidal neurons by a PMCA-dependent pH shift

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Abstract: Neural activity causes a surface membrane alkalosis due to activation of the plasma membrane calcium ATPase (PMCA), a transporter that extrudes calcium in exchange for two protons from the extracellular space. We previously showed that increasing effective buffering with exogenous carbonic anhydrase (XCAR, which reduces the surface alkaline shift) can significantly diminish the NMDAR-EPSC of a singly-activated, hippocampal, CA1 pyramidal neuron (PN). This suggested that an alkalosis is normally responsible for a large fraction of NMDAR current, and that this surface pH shift originates locally, from the recorded cell. The pH-dependent modulation of the NMDAR would accordingly be regarded as an autocrine effect. Indeed, splice variants of PMCA2 have been localized to dendritic spines and associated with

post-synaptic density proteins (1,2), placing them in close proximity to post-synaptic NMDARs. Here, we have further tested this autocrine hypothesis. Whole cell recordings of pure NMDAR EPSCs (VH = +50 mV) were obtained from CA1 PNs in juvenile mouse hippocampal slices. To assess locality of the modulation, we reduced stimulus intensity to generate responses of just 30 - 55 pA. The addition of XCAR reduced these EPSCs by 25 +/- 3.2 % ($n = 9$, $p < 0.001$), which was no different from its effect on larger EPSCs of ranging 166 - 386 pA ($p = 0.6$). AMPAR EPSCs of similar small size (32 - 49 pA) were unaffected by XCAR (VH = -70 mV). To directly test the autocrine nature of the modulation, we blocked activation of the PMCA in the recorded cell. When patch pipettes contained 20 mM BAPTA, after 12 min. of dialysis the NMDAR EPSCs were reduced by 23 +/- 4.2 % ($n=7$, $p < 0.001$) compared to responses at breakthrough. Moreover, after reduction of the EPSCs by BAPTA, external XCAR had no effect on EPSC amplitude. Similarly, when pipettes contained the PMCA inhibitor carboxyeosin (1 μM), EPSCs were reduced by 19 +/- 4 % after 12 min. of dialysis ($n = 7$, $p < 0.01$), and the effect of XCAR again was occluded. These data link the effects of XCAR to activation of the PMCA, and indicate that the alkaline boost of NMDAR currents is an autocrine effect that likely occurs at the level of single boutons. (1) Burette, A.C., Strehler, E.E. & Weinberg, R.J. *Neurosci.* **169,** 987-993 (2010), (2) DeMarco, S.J. & Strehler, E.E. *J. Biol. Chem*. **276,** 21594-21600 (2001).

Disclosures: H. Chen: None. **M. Chesler:** None.

Poster

501. NMDA Receptor Structure and Function

Location: Halls A-C

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Topic: B.02. Ligand-Gated Ion Channels

Support: NHLBI HL019982

Title: Ischemia-induced excitoxicity is attenuated by GluN2B-specific conantokin-G in a rat model of focal brain ischemia

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Abstract: Conantokin-G (ConG) is a GluN2B-specific potent N-methyl-D-aspartate receptor (NMDAR) antagonist, which displays neuroprotective activity on neurons that have

extrasynaptically activated NMDAR. The neuroprotective effect of ConG was tested in an *in vivo* rat model of Middle Carotid Artery Occlusion (MCAO) with 2 μM ConG administered intrathecally 30 min post occlusion. The animals were evaluated at 4 hr and 26 hr after injury induction. A 30% reduction in edema volume and a 50% reduction in infarct size were observed at 4 hr post-MCAO. Though, reduction in edema and infarct size was not observed at 26 hr post-MCAO, neurological recovery was significant at this time point. ConG treated rats showed significant recovery of the brain cytoarchitecture and neuronal integrity in the penumbra region as demonstrated by hematoxylin & eosin staining and Microtubule Assisted Protein-2 immunostaining. This was accompanied by decreased number of degenerated neurons by con-G at both 4 and 26 hr in the ipsilateral penumbra. MCAO-induced loss of GluN1 and GluN2B localization around the soma and proximal dendrites was reinstituted by ConG administration at 4 and 26 hr, but ConG had no effect on restoring MCAO-induced loss of GluN2A localization, which showed increased perinuclear presence in the brains of ConG treated rats. These data provide evidence that ConG ameliorates the detrimental effects of ischemic stroke via the NMDARs by repairing certain neurological and neuroarchitectural deficits, as well as reconstituting neuronal localization of GluN1 and GluN2B subunits in the penumbra.

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Poster

501. NMDA Receptor Structure and Function

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Title: Activity-induced synaptic delivery of GluN2A-containing NMDA receptors is mediated by endoplasmic reticulum chaperone Bip and participates in fear memory

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Abstract: The N-methyl-D-aspartate receptor (NMDAR) is mainly composed of GluN1 and GluN2A or GluN2B subunits in hippocampus and cortex. Since the GluN2A- and GluN2B-containing NMDARs possess distinct functional properties, their relative amount and dynamic regulation on these synapses play a major role in controlling Ca2+-dependent signaling and synaptic plasticity. Previous studies have suggested that neurons occupy multiple trafficking pathways and intracellular reserve pools for synaptic receptor transport. So far, accumulative evidence reveals that KIF17 selectively mediates GluN2B transport along microtubules and tyrosine phosphorylation regulates surface level of GluN2B-containg NMDARs, although its relevance to physiology has not been established. However, the mechanism of rapid increase of synaptic GluN2A-containing NMDARs remains unknown, and as well does its implication in learning and memory. We found in this study that newly-inserted synaptic GluN2A-containing NMDARs in response to neuronal activity were directly assembled and delivered from dendritic ER. We identified a well-studied ER chaperone protein Bip to retain GluN2A subunits in dendritic ER through the selective interaction between them and mediate the activity-induced synaptic delivery of the GluN2A-containing NMDAR. And interestingly, this regulatory process was effectively abolished by interrupting the interaction between Bip and GluN2A. Furthermore, *in vivo* application (i.p.) of the interfering peptide before training effectively disrupted the fear memory formation in mice. These findings uncover a new mechanism for activity-induced supply of synaptic GluN2A-containing NMDARs that are initially from a reserve pool of GluN2A subunit in a Bip-bond form in dendritic ER, and demonstrate a relevance of this process to memory formation.

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Poster

501. NMDA Receptor Structure and Function

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Topic: B.02. Ligand-Gated Ion Channels

Support: NIH Grant MH078823

NIH Grant MH101874

Title: Novel reagents for cell biological and biochemical assessment of ketamine targets

Authors: *C. M. EMNETT[1], H. LI[2], A. BENZ[1], X. JIANG[1], J. BOGGIANO[1], C. ZORUMSKI[1], D. WOZNIAK[1], D. REICHERT[2], S. MENNERICK[1,3]
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Abstract: Ketamine is of interest in neuropsychiatry as a rapidly acting antidepressant. Although NMDAR antagonism is likely relevant for triggering antidepressant effects of ketamine, other cellular and biochemical targets could also be important. Ketamine is rapidly metabolized into norketamine, which has a longer half-life (~12 h vs ~3 h) and biological activity possibly similar to ketamine. Here we offer new chemical biological tools that may help identify novel targets of ketamine and norketamine. We first explored actions of (R,S)ketamine and (R,S)norketamine in the forced swim test (FST). Ketamine significantly reduced immobility in C57BL/6J mice at 3 ($p=0.021$) and 10 mg/kg ($p=0.002$), during the FST after a 3 h post-injection delay. These doses did not affect locomotor activity. Norketamine also reduced immobility at 3 ($p=0.039$) and 10 mg/kg ($p=0.003$) using the same test parameters. Thus, norketamine had a similar behavioral effect as ketamine in this antidepressant screen. We examined NMDAR actions of ketamine, norketamine and first-generation chemical biology analogs in hippocampal cultures. We found that norketamine was active but ~4-fold less potent than ketamine at native NMDARs. Norketamine exhibited slower kinetics of block and more steady-state voltage dependence. At 10 μM, both compounds suppressed the peak of NMDAR EPSCs by ~20%, but ketamine more strongly accelerated EPSC decay time constants. These results confirm the potential relevance of norketamine and suggest that analogs of ketamine/norketamine would be useful. To that end, we exploited the versatility of bio-orthogonal click chemistry to develop bi-functional analogues of ketamine/norketamine to probe intracellular targets. We introduced an alkyne “click” handle into the ketamine/norketamine structure (alkyne-norketamine) at the key nitrogen atom via alkylation with 5-iodo-1-pentyne. Alkyne-norketamine exhibited potency equivalent to norketamine and voltage dependence similar to ketamine, thereby yielding an NMDAR-active, bi-functional probe. Introducing the alkyne with an amide linkage abolished NMDAR antagonism, presumably by interacting with nitrogen protonation. Imaging neurons following incubation in 10 μM alkyne-norketamine revealed intracellular labeling following Cu2+ catalyzed cycloaddition of azide-AlexaFluor488, demonstrating the utility of the probe for cell biological studies and revealing the potential for relevant intracellular targets. Thus, we advance first-generation chemical biology tools to aid identification and characterization of cellular and molecular targets of ketamine/norketamine relevant to antidepressant effects.

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Poster

501. NMDA Receptor Structure and Function

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Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

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Topic: B.02. Ligand-Gated Ion Channels

Support: NSERC Grant 238569-2011

Title: CaMKII-α is necessary for normal development of NMDA receptors on zebrafish Mauthner cells

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Abstract: Calcium/calmodulin dependent protein kinase 2 (CaMKII) is a multifunctional protein that is highly enriched in the synapse. It plays important roles in neuronal functions such as synaptic plasticity, synaptogenesis and neural development. Gene duplication in zebrafish has resulted in the occurrence of seven CaMKII genes (*camk2a*, *camk2b1*, *camk2b2, camk2d1, camk2d2, camk2g1* and *camk2g2*) that are expressed in a developmentally regulated manner. In this study, we used single cell, real-time quantitative PCR to investigate the expression of CaMKII genes in individual Mauthner cells (M-cells) of 2 days post fertilization (dpf) zebrafish embryos. We found that out of seven different CaMKII genes, only CaMKII-α was expressed in the M-cells at detectable levels, while all other isoforms were undetectable. To investigate a developmental role for CaMKII-α, we used two separate antisense morpholino oligonucleotides to knock down CaMKII-α expression in embryonic zebrafish; one was targeted to block the translation of mRNA and the other was designed to disrupt proper splicing of pre-mRNA. We recorded miniature excitatory postsynaptic currents (mEPSCs) mediated by NMDA receptors, which exhibited a biexponential decay with a τ_{fast} of ~30 ms and τ_{slow} of ~300 ms. Morpholino knockdown of CaMKII-α resulted in a 40% reduction in the amplitude of the slow component of the mEPSCs, with very little effect on either the amplitude of the fast component, the frequency or kinetics of the mEPSCs. These results suggest that CaMKII-α is present in embryonic M-cells and that it plays a role in the normal development of excitatory synapses. Our findings pave the way for determining the function of specific CaMKII isoforms during the early stages of M-cell development.

Disclosures: B. Roy: None. **D.W. Ali:** None.

Poster

501. NMDA Receptor Structure and Function

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Topic: B.02. Ligand-Gated Ion Channels

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Title: Differential localization of NMDA receptors in cerebellar stellate cells

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Abstract: Modification of the strength of excitatory synapses has been long thought to be the neural basis for learning and memory. Nonetheless, it is now accepted that inhibitory synapses can also undergo plasticity and probably contribute to most forms of experience-dependent plasticity by altering the balance between excitatory and inhibitory inputs. Therefore, understanding the crosstalk between these inputs is essential. We and others have shown that activation of extrasynaptic NMDA receptors in cerebellar GABAergic interneurons leads to both short and long term changes in the strength of GABAergic synapses. Here we determined the effects of NMDAR subunit specific inhibitors on somatic and dendritic NMDA receptors in stellate cells. Previous data from our lab using GluN2D KO mice indicated the presence of GluN2B and Glun2D subunits in cerebellar stellate cells. First we applied 0.1 μM PPDA and 0.2 μM CPP to outside-out patches excised from the soma of cerebellar Purkinje and Golgi neurons that express di-heteromeric GluN2D and tri-heteromeric GluN2B/2D NMDA receptors, respectively. Our results showed that PPDA inhibited GluN2D-containing NMDA receptors, whereas CPP selectively blocked tri-heteromeric GluN2B/2D NMDA receptors. Using these antagonists as well as the GluN2B antagonist Ifenprodil (3 μM), we then characterized NMDA receptors located in the soma of stellate cells. We found that PPDA and Ifenprodil inhibited NMDA-evoked currents in outside-out patches excised from the soma of stellate cells, whereas 0.2 μM CPP had no effect. Our data suggests that stellate cells express both GluN2B and GluN2D di-heteromeric NMDA receptors in the soma. Finally, we activated dendritic NMDARs by repetitive stimulation of parallel fibers and recorded dendritic currents recorded at +40 mV in the presence of the AMPA receptor antagonist NBQX. We found that PPDA and Ifenprodil

reduced dendritic currents by ~40% and in contrast CPP only blocked less than 20% of the NMDAR currents. Thus di-heteromeric GluN2B and GluN2D NMDA receptors, but not tri-heteromeric GluN2B/2D NMDA receptors, are located in the soma and the dendrites of stellate cells. Because a low concentration of CPP blocked an NMDAR-dependent increase in GABA release, the tri-heteromeric GluN2B/2D receptors appear to be mostly restricted to the axons of these cells. This differential subcellular distribution of NMDA receptors with distinct pharmacological and electrophysiological properties may function to finely tune the crosstalk between excitation and inhibition in the molecular layer of the cerebellar cortex.

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Poster

501. NMDA Receptor Structure and Function

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NARSAD

Spanish Ministry of Science (SAF2010-20636 and CSD2008-00005)

Title: GluN3A expression restricts the localization of NMDA receptors to synapses via modulation of surface dynamics

Authors: I. M. GONZÁLEZ-GONZÁLEZ[1], J. A. GRAY[2], R. A. NICOLL[2], L. GROC[3], *I. PEREZ-OTANO[1]
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Abstract: NMDA-type glutamate receptors (NMDARs) guide the activity-dependent remodeling of excitatory synapses and associated dendritic spines during critical periods of postnatal brain development. Whereas mature NMDARs composed of GluN1 and GluN2 subunits mediate synapse plasticity and promote spine growth and stabilization, juvenile NMDARs containing

GluN3A subunits are thought to inhibit these processes via yet unknown mechanisms. One key factor controlling the synaptic concentration of NMDARs is their ability to swap in and out of synapses via lateral diffusion. This ability depends on the type of GluN2 subunits comprising the receptor complex; whereas GluN2A subunits confer stability to NMDARs, GluN2B-containing subtypes are more mobile. Because GluN2A levels progressively increase during postnatal brain development, it has been hypothesized that the replacement of GluN2B by GluN2A subunits underlies the stabilization of NMDARs at synapses and the associated consolidation of neural networks. Information regarding the mobility of GluN3A-containing subtypes has been conspicuously lacking, despite biochemical and EM evidence suggesting that overexpression or down-regulation of GluN3A might modify receptor localization and stability. By taking advantage of high resolution live cell imaging, native GluN2 were followed using a single-molecule tracking based on the detection of quantum dots (QDs) in cultured hippocampal neurons. Here we report that variations in GluN3A expression differentially modulate the surface dynamics of NMDARs depending on the type of GluN2 subunit, and thereby drive developmental changes in the synaptic enrichment of GluN2-NMDARs.

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Poster

501. NMDA Receptor Structure and Function

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Topic: B.02. Ligand-Gated Ion Channels

Support: RFBR 14-04-00227

RFBR 14-04-31707

Title: Peculiarities of homocysteine as an agonist of NMDA receptors

Authors: *D. A. SIBAROV[1], P. A. ABUSHIK[1], R. GINIATULLIN[2], S. M. ANTONOV[1]
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Abstract: High level of homocysteine (HCY) in blood or in cerebrospinal fluid, often originating from distorted methionine metabolism, is a significant risk factor for the heart attack, stroke and neurodegenerative disorders. In central and peripheral nervous systems HCY acts as an agonist of NMDA receptors (NMDARs) and metabotropic (mGluR5) glutamate receptors. Previously we showed that the neurotoxic effect of HCY in rat cotrical and trigeminal sensory neurons is determined by intracellular Ca2+ oscillations (doi:10.1111/jnc.12615). Pro-apoptotic action of HCY in neurons results from cumulative effect of Ca2+ entry via NMDA receptors and mGluR5 provoked secondary release of Ca2+ from intracellular stores. Here, using whole cell patch-clamp recording from rat cortical neurons in primary culture we studied the agonist properties of HCY in comparison with NMDA. A fraction of neurons responding to 30 μM NMDA, generated inward currents also to HCY (100 μM) application. In neurons exhibiting comparable peak currents to both NMDA and HCY, the amplitude of late HCY-induced steady-state current was 2-folds magnitude lower than for NMDA. The EC50 value for HCY measured at the steady state currents was as low as 14.4 ± 1.3 μM. In the nominal absence of extracellular Ca2+ NMDA-induced currents did not reveal the receptor desensitization. In contrast, HCY-induced currents demonstrated the same degree of desensitization independently on the presence of extracellular Ca2+. Both Mg2+, as a noncompetitive, and AP5 as the competitive NMDARs antagonist, inhibited HCY induced currents. Interestingly, HCY-induced currents were insensitive to ifenprodil, the selective inhibitor of GluN2B subunit of NMDARs. NMDA-induced currents of neurons that did not respond to HCY were almost completely blocked by ifenprodil. Taking together these observations allow us to suggest, that HCY does not act on GluN2B subunits. Based on known predominant expression of GluN2A and GluN2B subunits in cortical neurons, we hypothesize that HCY selectively interacts with the binding site of the GluN2A subunit containing NMDARs. Thus, HCY acts as an effective agonist of NMDARs, causing Ca2+ independent rapid desensitization with the low amplitude of the steady-state currents. HCY can strongly interfere with the NMDARs-mediated synaptic transmission in concentrations comparable with those which occur *in vivo* during hyperhomocysteinemia.

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Poster

501. NMDA Receptor Structure and Function

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Topic: B.02. Ligand-Gated Ion Channels

Title: D-Cycloserine selectively alters kinetics of evoked NMDA responses in the rat entorhinal cortex

Authors: A. M. LENCH, P. V. MASSEY, *R. S. JONES
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Abstract: The NMDA receptor (NMDAr) coagonist binding site partial agonist D-cycloserine (DCS) has been implicated as a potential therapeutic target in a number of CNS diseases. In particular the anticonvulsant effects of DCS have been identified but are yet to be fully explored and may point to novel approaches for the treatment of epilepsy. We have examined the effects of DCS, applied cumulatively from 1 to 30 μM, on evoked NMDA receptor responses (eNMDAr), recorded under voltage clamp in neurones of the entorhinal cortex in rat brain slices. Interestingly, DCS had no observable effect on the amplitude of these responses (e.g. at 30 μM, mean amplitude was 187.6±54.1pA compared to 174.7±34.2 pA in control) but significantly decreased their decay time (Emax=65.8±5.0% control, IC50 = 2.1μM, n=6). When a saturating concentration of D-serine (1mM) was applied following DCS (10μM) the amplitude of eNMDAr increased to 122.1±4.9% control (n=7). As D-serine is a full agonist, this indicated that the endogenous level of binding was 81.9±3.2% of maximum. This value is very similar to the reported efficacy of DCS at NR2A/NR2B containing receptors. This indicates that the lack of effect of DCS on eNMDAr amplitude is due to a match between this efficacy and the endogenous level of activation. The low efficacy partial agonist, 1-Aminocyclobutane-1-carboxylic acid, was seen to decrease eNMDAr amplitude and decay time, supporting this conclusion. It is plausible that the kinetic effect underlies the anticonvulsant properties of DCS. We show this computationally using a Pinksy-Rinzel network model, demonstrating that a 35% reduction in NMDAR decay time results in large reductions in predicted durations of bursting activity. The allosteric properties of NMDAr coagonist binding site partial agonists have been extensively described though it is remarkable that in an endogenous situation the kinetic effects of DCS occur selectively at synaptic NMDA receptors. These allosteric effects may therefore underlie the anticonvulsant effect of DCS by reducing the ability of neuronal networks to sustain rhythmic activity.

Disclosures: **A.M. Lench:** None. **R.S. Jones:** None. **P.V. Massey:** None.

Poster

501. NMDA Receptor Structure and Function

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Program#/Poster#: 501.21/D5

Topic: B.08. Synaptic Plasticity

Support: CDMRP award W81XWH-04-1-0065/PR030035

NINDS grant NS045964

Title: Chronic GluN2B inhibition reduced axon length and sphere of axonal arborization in dentate/hilar border interneurons, but did not affect GABAergic synapses onto dentate granule cells

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Abstract: We showed previously that chronic treatment with distinct classes of N-methyl-D-aspartate receptor (NMDAR) antagonists exerted differential effects on seizure-like events (SLE) in organotypic hippocampal slice cultures. SLE involving dentate granule cells were dramatically reduced following chronic treatment (17-21 days) with the GluN2B-selective antagonist, Ro25,6981, but were increased in cultures treated with the non-subtype-selective antagonists, memantine or D-APV. Since GABAergic transmission plays a critical role in the regulation of hippocampal activity, we hypothesized that chronic Ro25,6981 would promote expansion of interneuron axonal fields as well as the number of GABAergic synapses onto dentate granule cells, while memantine or D-APV would elicit opposite effects. To investigate potential changes in morphology, interneurons at the dentate/hilar border were filled with neurobiotin and digitally reconstructed. Treatment with Ro25,698, but not the other NMDAR antagonists dramatically reduced total axonal length and sphere of axonal arborization. Axonal branch points and putative boutons mirrored these results, but branch point and bouton density were unchanged. Dendritic length and sphere of dendritic arborization also were reduced following chronic Ro25,6981, but this reduction was not specific to GluN2B inhibition as other NMDAR antagonists caused similar reductions. Double immunofluorescence for GAD 65/67 (GABA synthetic enzymes) and NeuN (for somata) or MAP2 (for dendrites) was then performed to quantify neuron number and numbers of putative GABAergic synaptic boutons apposed to granule cell somata and dendrites, respectively. Quantification showed that the number of neurons and putative GABAergic synapses onto granule cells were lower following D-APV or memantine treatment compared to vehicle. Similar decreases have been described in epilepsy models and may contribute to the increased SLE expression in slice cultures following chronic treatment with D-APV and memantine. In contrast, we found equivalent numbers of putative GABAergic synapses onto granule cells in vehicle- and Ro25,6981-treated cultures, despite reduced axonal fields in individual interneurons and no change in the number of GABAergic neurons. These changes together with electrophysiological results suggest that one possible mechanism for reduced SLE following chronic Ro25,6981 is that individual interneurons may have a reduced ability to synchronize activity across widespread populations of neurons.

Disclosures: **S.B. Bausch:** None. **Y. Wang:** None. **D. Lapides:** None. **K. Quinn:** None.

Poster

501. NMDA Receptor Structure and Function

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Program#/Poster#: 501.22/D6

Topic: B.02. Ligand-Gated Ion Channels

Support: "Capitale Umano" grant by Fondazione Con Il Sud

Title: Combined effect of genetic variants in GRIN2B on prefrontal function during working memory performance

Authors: ***G. PERGOLA**[1], P. DI CARLO[1], L. FAZIO[1], A. RAIO[1], R. MASELLIS[1,2], B. GELAO[1], A. RAMPINO[1,2], G. BLASI[2], A. BERTOLINO[1,2]
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Abstract: N-methyl-D-aspartate receptors (NMDAR) are important modulators of prefrontal activity during working memory processing. Furthermore, they concur in mediating glutamate-dependent neural plasticity and are involved in neurodevelopmental processes. For example, among the subunits composing NMDAR, GluN2B are more abundant during neurodevelopmental stages than they are in adults - in which GluN2A is prevalent. Here, we tested the combined effect of genetic variants in the GluN2B gene (GRIN2B) on GRIN2B prefrontal mRNA expression. Once this effect was detected, we also investigated how these genetic variants together affect prefrontal activity during working memory in healthy human subjects. SNPs were identified using Braincloud (http://braincloud.jhmi.edu/). We found five SNPs significantly associated with GRIN2B expression: rs2160517, rs219931, rs11055792, rs17833967, rs12814951 (all $p < .05$, corrected with Benjamini-Hochberg FDR). Thus, based on signal detection theory, we computed a composite genetic score to account for the combined effect of these SNPs on expression. Machine learning indicated that this score was negatively correlated with gene expression ($N = 148$, $R^2 = .17$, $p < 10^{-6}$). Then, we genotyped the SNPs of interest in 134 healthy individuals (56 females; mean age ± SD was 28 ± 7 years (range 19-53) and determined the associated genetic score. Functional magnetic resonance images were also acquired while these participants performed a working memory task (n-back). We estimated the association of genetic score with dorsolateral prefrontal cortex activation using linear regression models, controlling for age, gender, IQ, socioeconomic status, and handedness. With this aim,

we performed an ROI analysis including bilateral Brodmann Areas 8, 9, and 46. We found a significant cluster of 157 voxels in the left superior frontal gyrus (Brodmann Area 8; $z = 3.9$; $p < .05$, peak-level family-wise error correction; coordinates in MNI space: $x = -32$; $y = 20$; $z = 58$). In particular, results suggested that activation was negatively correlated with GRIN2B genetic score. Adding behavioral performances as nuisance variables in the statistical analysis did not modify the results. This study suggests that genetic variants in GRIN2B have additive effects on gene expression. The combined effect of these genetic variants is also associated with more distal and complex phenotypes related to prefrontal function and cognitive processing. These findings may help to shed light on the biological basis of brain disorders with a complex genetic background.

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Poster

501. NMDA Receptor Structure and Function

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Program#/Poster#: 501.23/D7

Topic: B.02. Ligand-Gated Ion Channels

Support: NIH NINDS NS065371

NIH NINDS NS086368

NIH NINDS NS071802

NIH NINDS NS007480

Title: Synthesis, mechanism of action, and structural determinants for a novel class of GluN2C/D-selective NMDA receptor antagonists

Authors: *S. A. SWANGER[1], S. S. ZIMMERMAN[2], T. M. ACKER[2], K. M. VANCE[1], C. A. MOSLEY[2], D. C. LIOTTA[2], S. F. TRAYNELIS[1]
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Abstract: NMDA receptors are ionotropic glutamate receptors that mediate excitatory synaptic transmission and are involved in many neurological disorders. These tetrameric receptors are

composed of two GluN1 and two GluN2 subunits (GluN2A-2D), and the GluN2 subunits exhibit distinct developmental and regional expression patterns in the brain. Therefore, subunit-selective pharmacological modulation of NMDA receptors is a promising strategy for studying NMDA receptor function in specific neuronal circuits and for developing therapies for neurological disorders. We have discovered a novel class of GluN2C/D-selective antagonists (class 1063) that are >500-fold selective for GluN2C/D-containing receptors over GluN2A/B-containing receptors. The 1063 compounds had low micromolar potencies at GluN2C- and GluN2D-containing receptors, when tested on recombinant NMDA receptors in Xenopus oocytes. Furthermore, inhibition of GluN2C/D-containing receptors by 1063 is voltage-independent and could not be surmounted by increasing concentrations of agonists; this is consistent with a noncompetitive mechanism of action. Using GluN2A/D chimeric receptors, we identified a critical region for 1063 inhibition near the first transmembrane domain of the GluN2 subunit. Mutation of a single residue (C590) that is not conserved between GluN2C/D and GluN2A/B blocks inhibition, suggesting this region may be a molecular determinant of action. In addition, 1063 compounds inhibited triheteromeric receptors containing GluN1/GluN2A/GluN2C subunits, which may enhance their utility for studying neural circuits. Importantly, the 1063 series of NMDA receptor antagonists inhibited GluN2C/D-containing NMDA receptors in transiently transfected cultured rat neurons. These data suggest that this new class of GluN2C/D-selective antagonists may be a valuable tool for studying NMDA receptor function, and could be further developed for potential use in treating neurological disease. This research was supported by funding from NIH-NINDS [NS065371 (SFT), NS086368 (SAS), NS071802 (TMA), T32-NS007480 (KMV)].

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Poster

501. NMDA Receptor Structure and Function

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Bantly Foundation

Title: Distributed kinetic effects of oxysterol positive modulators on NMDA channels

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Abstract: N-methyl-D-aspartate receptors (NMDARs) are essential for learning and memory and are important in neuropsychiatric disorders including Alzheimer's disease and schizophrenia. NMDAR gating can be manipulated by allosteric modulators, resulting in the alteration of channel opening probability (Po). Recently, we reported on a novel endogenous oxysterol NMDAR positive allosteric modulator, 24(S)-hydroxycholesterol (24(S)-HC), and we showed that synthetic oxysterol analogs potentiate NMDARs with high specificity and potency. Oxysterol potentiation increases agonist efficacy and channel Po. Here we explored biophysical mechanisms of potentiation by analyzing the activity of individual GluN1a/GluN2A channels in transfected HEK293 cells. At saturating agonist concentration (300 μM NMDA), single channels in cell-attached patch recordings exhibited three clearly resolved closed states and two open states, consistent with observations from previous studies. Application of the oxysterol analog SGE-301 (2 μM) decreased the mean duration of long channel closures and increased the mean duration of both short and long channel openings. There was no change in the relative contributions of various closed-time and open-time components. We observed minimal modal channel behavior, examined by time-binned Po analysis. We interpreted oxysterol-induced changes by applying several five-state kinetic models from previous work to our data. Fits to a linear gating scheme produced a paradoxical slowing of the rate of forward transition between the two open states and slowing of several backward rate constants. Together, these changes produced the expected potentiation of simulated macroscopic steady-state currents. Two five-state branched gating models were indistinguishable from the linear model by log likelihood values. Like the linear model, both predicted distributed changes to multiple rate constants. The linear model better mimicked synaptic-like pulses of agonist and modulation by SGE-301 than the branched models. Taken together, we show that oxysterols have distributed effects on several aspects of channel behavior. Our results elucidate the mechanism by which oxysterols augment NMDAR function and contribute to novel therapeutic interventions.

Disclosures: **A.J. Linsenbardt:** A. Employment/Salary (full or part-time):; Washington University. **S.M. Paul:** A. Employment/Salary (full or part-time):; Sage Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Sage Therapeutics. **J.J. Doherty:** A. Employment/Salary (full or part-time):; Sage Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual

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Poster

502. Purine and Other G-Protein Coupled Receptors

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Topic: B.03. G-Protein Linked Receptors

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CAPES

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NARSAD

Title: Adenosine A2A receptor blockade therapeutically reverts synaptic dysfunction and altered mood and memory performance in mice subjected to chronic unpredictable stress

Authors: *R. A. CUNHA[1,2], M. P. KASTER[1,3], N. J. MACHADO[1], H. B. SILVA[1], A. NUNES[1], A. ARDAIS[1,4], M. M. SANTANA[1], Y. BAQI[5,6], C. E. MULLER[5], A. S. RODRIGUES[3], L. O. PORCIÚNCULA[4], J.-F. CHEN[7], A. R. TOMÉ[1,8], P. AGOSTINHO[1,2], P. M. CANAS[1]
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Abstract: Repeated stress elicits neurochemical and neuroanatomical changes that negatively impact on brain functioning and constitute a trigger or risk factor for psychiatric disorders such as depression and neurodegenerative disorders involving cognitive impairment in both humans and animal models. Since caffeine consumption (adenosine receptor antagonist) correlates inversely with depression and adenosine A2A receptor (A2AR) antagonists normalize aberrant plasticity and afford neuroprotection, we tested the role of A2AR in the control of the behavioural, electrophysiological and neurochemical modifications caused by chronic unpredictable stress (CUS, composed by uncontrollable daily stressors such as tilted cage, food/water deprivation, foot shock, paired caging, continuous light, wet bedding) for 3 weeks in adult mice. CUS led to an up-regulation of A2AR selectively in glutamatergic terminals in the hippocampus, increased the morning plasma levels of corticosterone, induced anxiogenic (open field, elevated plus maze) and depressive-like behaviour (forced swimming, tail suspension, sucrose preference) and decreased memory performance (object displacement, object recognition, modified Y maze, Morris water maze). These behavioural changes were accompanied by a decrease of synaptic plasticity (long term potentiation) and a reduced density of synaptic proteins (SNAP-25, syntaxin and vesicular glutamate transporters type 1, vGluT1) in the hippocampus without overt neuronal damage (Cresyl violet and FluoroJade C) or microgliosis (CD11b immunoreactivity). All these CUS-induced changes were prevented by each of the following treatments: 1) caffeine (1 g/L, p.o., starting 3 weeks before and throughout CUS); 2) KW6002 (3 mg/kg, p.o.; selective A2AR antagonist, starting 4 days before and throughout CUS); 3) global genetic deletion of A2AR; 4) selective A2AR deletion in forebrain neurons. Notably, A2AR blockade was not only prophylactic but also therapeutically relevant since, after the end of the CUS protocol, a 3-weeks treatment with the A2AR antagonist SCH58261 (0.1 mg/kg, i.p.) reversed the mood and memory impairments as well as the synaptic dysfunction caused by CUS. These results herald a key role of neuronal A2AR in the control of chronic stress-induced modifications and prompt the hypothesis that A2AR may be an attractive and effective target for the development of novel corrective strategies for brain disorders that involve a synaptic dysfunction, as now observed for the maladaptive responses to chronic stress.

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Poster

502. Purine and Other G-Protein Coupled Receptors

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Topic: B.03. G-Protein Linked Receptors

Support: MH083767

DK064862

NS058525

AA019357

DK59802

Title: Hypoxia/reoxygenation increase caspase-1 activity via the A2A adenosine receptor

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Abstract: Anxiety is one of the most prevalent comorbidities associated with acute lung injury (ALI) and acute respiratory distress syndrome (ARDS). Activation of the innate immune system is a known inducer of anxiety-like behaviors. Adult male BALB/cJ mice were exposed to 6% oxygen for 2 hours (hypoxia). We found that hypoxia increased circulating adenosine concentration by more than two-fold. Moreover, we see that hypoxia induced a four-fold increase in staining for active caspase-1 in the amygdala when compared to normoxic controls in wild type but not A2A adenosine receptor (AR) KO. Finally, hypoxia increased anxiety-like behaviors in wild-type mice by 45% in the open field test and 86% in the elevated zero-maze, while A2A AR KO mice were resistant to hypoxia-induced anxiety-like behaviors. Our results indicate that hypoxia induces anxiety-like behaviors, at least in part, by increasing adenosine signaling via A2A AR to increase caspase-1 activity.

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Poster

502. Purine and Other G-Protein Coupled Receptors

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Topic: B.03. G-Protein Linked Receptors

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NIH grant NS073947

Title: Intracellular control of A2A receptor signaling and behavior as revealed by optoA2AR approach

Authors: X. HOU[1,2], M. P. SURPRIS[1], P. LI[1], J. ZHENG[2], *J.-F. CHEN[1]
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Abstract: Adenosine A2A receptors (A2ARs) in the nucleus accumbens (Acb) play an important role in control of motor, sleep and addiction. A2AR can exert its biological responses through G-protein-dependent as well as G-protein independent signaling pathways. Recently, at least six G-protein accessory proteins have been identified to bind to the C-terminus of A2ARs to affect A2AR signaling. This raises a possibility that the interaction between the intracellular domain of A2AR and G proteins and G-protein accessary proteins can directly affect down-stream cellular signaling and function. To specifically define the role of the intracellular domain of the A2AR in dictating intracellular signaling and behavior in Acb, we have developed a chimeric rhodopsin-A2AR (optoA2AR) protein which confers light responsiveness with the extracellular and transmembrane domains of rhodopsin and A2AR signaling with the intracellular domains of the A2AR. In this study, we selectively expressed the optoA2AR in the striatopallidal neurons by injecting the Cre-dependent AAV-optoA2AR-mCherry into the Acb of Adora2a-Cre mice. The striatopallidal neuron-specific expression pattern was confirmed by the localization of optoA2AR in enkephalin-positive, but not substance P-positive cells in the striatum. Light activation of optoA2AR in the striatopallidal neurons triggered MAPK phosphorylation in the striatum and suppressed motor activity, but had no effect on recognition memory when light was delivered during the training or immediately after the training. These findings illustrate the utility of the optoA2AR approach to investigate the intracellular control of A2AR signaling in behaving animals, and suggest that the intracellular domain of the A2AR may be important in dictating A2AR signaling and behavioral responses.

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Poster

502. Purine and Other G-Protein Coupled Receptors

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FCT (PTDC/SAU-NSC/122254/2010)

QREN (CENTRO-07-ST24-FEDER-002006)

NARSAD

CNPq (Ciência sem Fronteiras)

Title: Adenosine A2A receptors control impulsivity and synaptic plasticity in the prelimbic medial prefrontal cortex - interaction with dopamine D2-like receptors

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Abstract: Caffeine and adenosine A2A receptors (A2AR) control stress-induced emotional and memory alterations that involve information processing in the prelimbic medial prefrontal cortex (PLmPFC). A2AR control synaptic plasticity in different brain regions and can form heteromers and antagonize dopamine D2-like receptors (D2R). However, the role of A2AR in the PFC is essentially unknown and may underlie the ability of caffeine to restore cognitive flexibility in an animal model of attention deficit and hyperactivity disorder (ADHD). Thus, we now tested if A2AR blockade with its selective antagonist (SCH58261) controlled synaptic transmission and plasticity in the PFC and if the effects of A2AR depend on D2R function, a critical modulator of PFC function. We recorded population spikes in layer V of slices from male Wistar rats (6 weeks) upon stimulation of layer II/III and long-term potentiation (LTP) was induced by 1 priming 100Hz (0.5s duration) train, followed 15 min later by 4 trains of 100Hz (0.5s duration, every 10s). SCH58261 (50nM) was devoid of effects on basal synaptic transmission but decreased LTP amplitude (23.5±3.8% over basal with SCH58261 versus 40.3±5.3% in control slices, n=6, $p<0.05$). Silencing A2AR selectively in the mPFC (with a lentivirus expressing a shRNA downregulating A2AR, shA2AR) triggered an impulsive phenotype, evaluated in a delayed reward paradigm (34.5±3.3% of larger reward choices in shA2AR versus 64.0±6.7% in control rats, n=8, $p<0.01$). Since impulsive phenotype has also been linked to loss of D2R function and A2AR and D2R can heteromerize, we tested if D2R impacted PLmPFC LTP and interacted with A2AR. D2R blockade with supiride (10μM) decreased LTP amplitude

(26.8±6.7% with sulpiride versus 40.7±6.5% in control, n=6, P<0.05), as did A2AR blockade. Furthermore, with the simultaneous blockade of A2AR and D2R, LTP amplitude (20.7±5.1%, n=5) was not significantly different from SCH58261 or sulpiride (p>0.05), revealing mutually occlusive effects. These results document a novel role of A2AR in the control of adaptive plastic changes in the PFC in tight interaction with D2R, with relevance for the control impulsivity in conditions such as ADHD in view of the clear impulsive phenotype in rats with silenced A2AR.

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Poster

502. Purine and Other G-Protein Coupled Receptors

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Topic: B.03. G-Protein Linked Receptors

Support: APQ1/FAPERJ

INCT

PROAP/CAPES

Proppi/UFF

Title: Caffeine potentiates D-aspartate-mediated GABA release in the developing chick retina

Authors: *D. D. FERREIRA[1], B. STUTZ[2], R. A. M. REIS[2], F. G. DE MELLO[2], R. C. C. KUBRUSLY[1]
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Abstract: Aim: We are investigating the effects of caffeine in GABAergic system, analyzing the purinergic receptors influence in regulating NMDA receptor (NMDAR) activity in retinal explants of chick embryo. Methods and Results: Retinas from 13-day-old chick embryo (Gallus gallus domesticus) were used for [3H]-GABA uptake and release, high performance liquid chromatography and cAMP assay. All experiments were approved by the Ethics Committee of CCS-UFRJ. D-aspartate (500µM) (D-asp) increased GABA-release in 4.5 fold when compared to basal levels (B) (B=2.21±0.05, n=6; D-Asp=9.95±0.24, n=6). Caffeine (500µM) (Caf+D-Asp)

elevated D-asp-induced GABA release in 60% (Caf+D-Asp=16.10±2.29, n=7). The release was inhibited in the presence of NNC-711 (NNC), GABA transporter-1 (GAT-1) blocker (NNC=2.42±0.33, n=3), and MK-801 (MK), NMDAR antagonist (MK=3.18±0.58, n=3). Caffeine did not modify [3H]-GABA uptake in 5, 30 and 60min (5min:Ctrl=96.14±1.73; Caf=101.4±11.84; 30min:Ctrl=290.2±58.58; Caf= 247.4±29.65; 60min:Ctrl=312.1±6.76; Caf=313.3±36.13; n=2) and did not increase the release of D-asp (Ctrl=9.64±1.45, Caf= 8.78±1.22) or glutamate at basal (Ctrl=14.29±2.98, Caf=12.94±2.61; n=6) or stimulated conditions (Ctrl=15.40±3.23, Caf=11.66±3.31; n=6). Caffeine's effect was mimicked by the adenosine A1 receptor DPCPX (DPCPX=21.95±1.42, n=4) and by the adenylyl cyclase activator forskolin (FK) (FK=18.76±1.82, n=4); and was blocked by the PKA inhibitor, H-89 (H-89=11.58±0.98, n=4), tyrosine kinase inhibitor genistein (Gen=9.19±0.31, n=3) and src family kinase (SFK) inhibitor PP1 (PP1=9.76±0.42, n=3). Forskolin-stimulated cAMP levels (F) were reduced in the presence of the A1 receptor agonist CHA (F=2317±447, n=3; CHA=1094±420, n=2). The GluN2B subunit-containing NMDAR antagonist ifenprodil (IF) inhibited caffeine's effect at 10uM (IF=8.78±0.35; n=6). Conclusion: Our data suggest that caffeine potentiates D-asp-induced GABA release, which is mediated through reversion of GAT-1 and is dependent of NMDAR. Caffeine seems to promote this effect by antagonizing the adenosine A1 receptor and cAMP levels and PKA may be involved in it. Additionally, phosphorylation of the GluN2B containing-NMDAR by SFK may participate in the potentiation of D-asp-induced GABA release by caffeine.

Disclosures: D.D. Ferreira: None. **B. Stutz:** None. **R.A.M. Reis:** None. **F.G. de Mello:** None. **R.C.C. Kubrusly:** None.

Poster

502. Purine and Other G-Protein Coupled Receptors

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

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Topic: B.03. G-Protein Linked Receptors

Support: CAPES

CNPq

FAPERJ

PRONEX-MCT

Title: Modulation of ERK phosphorylation by A1 adenosine receptor in cultures of avian retinal glial cells: Involvement of PKC and Src kinase

Authors: *A. DOS SANTOS-RODRIGUES[1], M. R. PEREIRA[1], I. L. A. DA SILVA[1], S. A. RODRIGUES[1], L. R. LEÃO-FERREIRA[2], R. PAES-DE-CARVALHO[1]
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Abstract: Adenosine is an important modulator of neuronal survival and differentiation and also participates in neuroprotection mechanisms. Besides its classical role in the regulation of adenylyl cyclase activity, increasing evidence indicates that adenosine receptors regulate different signaling pathways including MAP kinase cascade which appear to have important roles in several neural and glial functions. Glial cells play fundamental roles in the CNS such as regulation of synaptic transmission as well as neurotransmitter uptake and metabolism. In the present work expression of adenosine A1 receptors and regulation of extracellular-regulated kinase (ERK) activity of cultured retinal glial cells by adenosine was evaluated. Expression of A1 receptor in purified cultures of glial cells obtained from 11 day-old chick embryo retinas was detected by (3H) DPCPX binding and western blot. Purified cultures were incubated with selective kinase inhibitors and adenosine receptor selective agonists or antagonists for determination of phosphorylated ERK (pERK) level by western blotting or immunocytochemistry. Incubation of cultured glial cells with adenosine deaminase (0.5 U/ml) or DPCPX (10 μM), an A1 receptor-selective antagonist, reduced basal pERK level by approximately 50% indicating that endogenous adenosine regulates ERK phosphorylation through activation of A1 receptor. Incubation with CHA (1 μM), an A1 receptor-selective agonist, induced an increase of 120% in pERK levels, an effect completely blocked by DPCPX. Basal pERK level was also reduced 30-50% by PD98059, a MEK inhibitor, PP1, a Src inhibitor, or Chelerythrine chloride (100 nM), a PKC inhibitor. Furthermore, these selective inhibitors completely blocked CHA-induced ERK phosphorylation. Immunocytochemistry data revealed that A1 receptor-induced increase in pERK level is mainly localized in the cytosol. These results demonstrate that A1 adenosine receptor is expressed in retinal glial cells and modulate ERK signaling through a pathway involving PKC and Src kinase.

Disclosures: **A. dos Santos-Rodrigues:** None. **M.R. Pereira:** None. **I.L.A. da Silva:** None. **S.A. Rodrigues:** None. **L.R. Leão-Ferreira:** None. **R. Paes-de-Carvalho:** None.

Poster

502. Purine and Other G-Protein Coupled Receptors

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NSF Grant IOS-0843585

JSPS KAKENHI 23790303

JSPS KAKENHI 25860193

Title: Adenosine-based anticonvulsant mechanisms underlying ketogenic diet

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Abstract: A ketogenic diet (a low-carbohydrate high-fat protocol) was designed in the 1920s to mimic fasting to treat epilepsy. In recent decades, ketogenic diet has increasingly been noted as a useful therapy for medically-refractory epilepsy using antiepileptic drugs. Despite decades of clinical use, the neural mechanisms underlying the anticonvulsant effects of a ketogenic diet are poorly understood, and to date there has been no established *in vitro* preparation recapitulating its neural effects *in vivo*. To elucidate this, we used a complementary approach which included *in vivo* dietary treatment followed by characterizing acute brain slices using electrophysiology. We fed rats and mice a ketogenic or control diet for 2-3 weeks, prepared acute hippocampal slices, and performed electrophysiology and pharmacology in the seizure-prone CA3 region of hippocampus. Slices from ketogenic diet-fed animals showed reduced excitability, and seizure propensity depended on maintaining a reduced extracellular glucose level. This reduced excitability was not observed from control diet-fed rats and mice. The effects of the ketogenic diet could be reversed with blockers of adenosine A_1 receptors and were absent in slices obtained from ketogenic diet-fed mice lacking adenosine A_1 receptors, thus identifying specific mechanisms mobilized by the diet that influenced neuronal activity. These results suggest that the reduction of neuronal activity through activation of adenosine A_1 receptor is one of the key mechanisms underlying anticonvulsant effects of ketogenic diet.

Disclosures: M. Kawamura: None. **D.N. Ruskin:** None. **S.A. Masino:** None.

Poster

502. Purine and Other G-Protein Coupled Receptors

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Topic: B.03. G-Protein Linked Receptors

Support: NIH Grant T32 HD041697

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Title: A novel role of adenosine A2B receptors in mediating calcium-activated small conductance potassium (SK) channel function at the synapse

Authors: *A. K. GARSKE[1], L.-R. B. WEITZEL[1], R. J. TRAYSTMAN[2], P. S. HERSON[1]
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Abstract: After ischemic injury adenosine levels are highly elevated in the brain. The role of adenosine receptors in ischemic injury has been studied, however very little is known about the A2B adenosine in the brain. In peripheral organ ischemia-reperfusion causes the upregulation of A2B receptors and organ protection. In contrast, we made the remarkable observation that inhibition of A2B receptors provides robust protection against stroke injury in mice. Therefore, we used electrophysiology methods to explore potential mechanisms to explain the injurious role of A2B receptors in brain ischemia. Patch clamp recordings from adult mouse hippocampus demonstrate that pharmacologic activation of the A2B receptor leads to a reduction in SK channel mediated current. Synaptic recordings implicate this A2B regulation of SK channels is present at the synapse. Additionally, our findings suggest that this potential interaction between A2B receptor and SK channel activity may be mediated by a mechanism involving protein kinase A (PKA) dependent signaling. These findings propose a novel signaling interaction between A2B receptor signaling and SK channel functional activity, which may provide an improved understanding of SK channel regulation at the synapse. Further, these observations may have implications in neuronal excitability and efficacy of synaptic transmission, particularly after ischemic injury.

Disclosures: A.K. Garske: None. **L.B. Weitzel:** None. **R.J. Traystman:** None. **P.S. Herson:** None.

Poster

502. Purine and Other G-Protein Coupled Receptors

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Program#/Poster#: 502.10/D18

Topic: B.03. G-Protein Linked Receptors

Title: The GAIN domain of the adhesion GPCR BAI1 regulates the constitutive activity of the receptor

Authors: *R. H. PURCELL, R. A. HALL
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Abstract: Adhesion GPCRs are a family of more than 30 receptors that undergo autoproteolytic cleavage to separate their long, extracellular N termini (NT) from their 7-transmembrane regions. These receptors share a conserved G protein Autoproteolysis-Inducing (GAIN) domain, which mediates this proteolytic activity (Arac et al. 2013). Previous studies have shown that loss of the NT augments receptor signaling activity in several adhesion GPCR family members including brain-specific angiogenesis inhibitor 1 (BAI1), a Galpha12/13-coupled receptor that has been shown to regulate dendritic spine morphology in hippocampal neurons (Duman et al. 2013) and signal to the Rho, Rac, and ERK pathways (Duman et al., 2013; Stephenson et al. 2013, Park et al. 2007). BAI1 exhibits significant constitutive activity in cultured cells, but the mechanisms regulating receptor activity are currently unknown. We hypothesized that the GAIN domain of BAI1 might be a key regulator of receptor signaling activity. We prepared a Flag-tagged BAI1 GAIN domain construct and found that the GAIN domain binds robustly to both the BAI1-NT and full-length BAI1. Moreover, we found that co-transfection of the BAI1 GAIN domain modulates the constitutive activity of the full-length receptor, as assessed in multiple distinct assays. These data suggest that the constitutive activity of BAI1 is regulated by GAIN domain binding, which has implications for understanding how this receptor is activated in native cell types in the brain.

Disclosures: R.H. Purcell: None. **R.A. Hall:** None.

Poster

502. Purine and Other G-Protein Coupled Receptors

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Topic: B.03. G-Protein Linked Receptors

Support: the Ministry of Education, Science, Sports and Culture; grant (C) 24591264 from Grant-in-Aid for Scientific Research

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Title: The subcellular localization and local function of Gs-linked receptor GPR3 in neuronal cells

Authors: *T. MIYAGI[1], S. TANAKA[2], I. HIDE[2], T. SHIRAFUJI[2], N. SAKAI[2]
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Abstract: G-protein coupled receptor (GPR) 3 is a member of GPRs that constitutively activates adenylate cyclase. We have reported that the expression of GPR3 in cerebellar granular neurons (CGNs) enhances neurite outgrowth and modulates proliferation of CGNs. However, the subcellular localization and local function of GPR3 have not been fully elucidated. In the present study, we investigated the distribution of GPR3 in the CNS utilizing the GPR3 knockout LacZ knock-in mouse. X-gal staining for these mice revealed that GPR3 was distributed in the cortex, striatum, hippocampus, medial habenular nucleus, and cerebellum. We further determined the subcellular localization of GPR3 in cerebellar granular neurons (CGNs) using fluorescent-tagged GPR3. In CGNs, GPR3 was distributed along the plasma membrane and in endoplasmic reticulum and goldi body. To precisely analyze time-dependent distribution of GPR3 *in vivo*, we examined the distribution of GPR3 in CGNs using time-lapse imaging. Surprisingly, fluorescent punctae of GPR3 were translocated along the neurite in both directions. Moreover, translocation of GFP-GPR3 punctae was completely abrogated by administration of blebbistatin, which is a potential myosinIIinhibitor. We further asked if local distribution of GPR3 affects local activation of cAMP-PKA signaling pathway. PKA FRET indictor (AKAR3EV) analyses revealed that up-regulation of GPR3 resulted in activation of PKA in cell body and neurites, whereas local activation of PKA in the tips of neurite was inhibited by administration of blebbistatin or transfection of GPR3 siRNA in SH-SY5Y cells. There results thus indicated that GPR3 may be related with local PKA activation, thereby affect neurite outgrowth and neuronal polarization.

Disclosures: **T. Miyagi:** None. **S. Tanaka:** None. **I. Hide:** None. **T. Shirafuji:** None. **N. Sakai:** None.

Poster

502. Purine and Other G-Protein Coupled Receptors

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Topic: B.03. G-Protein Linked Receptors

Support: NIH Grant 3R01HL059949-15S1

Title: Pharmacological implications of A2AR-D2R heteromerization; significance for Parkinson's disease

Authors: *C. N. HATCHER-SOLIS[1], D. E. LOGOTHETIS[2]
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Abstract: Appreciation of the existence of oligomeric G protein-coupled receptor (GPCR) complexes with distinct signaling properties from their homomeric counterparts is growing. Yet, the effect of heteromerization on the pharmacology of many GPCR homomers remains unknown. We have undertaken the task to examine the effect of heteromerization on G_s signaling through the adenosine 2A receptor ($A_{2A}R$) and G_i signaling through the dopamine receptor type 2 (D_2R). Signaling through the $A_{2A}R$-D_2R heteromeric complex is of great interest as this heteromer is an emerging therapeutic target for Parkinson's disease. Previous work suggests the $A_{2A}R$ and D_2R display reciprocal antagonism, with $A_{2A}R$-mediated negative allosteric modulation of the D_2R and D_2R-mediated negative allosteric modulation of the $A_{2A}R$. We sought to develop an electrophysiological assay for $A_{2A}R$-D_2R heteromer and homomer signaling. Our objective is to determine using this assay whether combining D_2R full agonists and $A_{2A}R$ inverse agonists targeting the $A_{2A}R$-D_2R heteromer will potentiate dopaminergic, G_i signaling more than sole stimulation of the D_2R. We heterologously expressed GIRK channels that can serve as reporters for GPCR signaling through G_i and G_s. *Xenopus laevis* oocytes injected with cRNA for GIRK1, GIRK2, D_2R, $A_{2A}R$, and $G_{\alpha s}$ were used for our heterologous expression system and currents were measured using two-electrode voltage-clamp. GIRK channels were used as reporters for GPCR signaling because GPCR activation leads to direct $G\beta\gamma$-mediated stimulation of the GIRK current. Preliminary data have demonstrated that heteromer formation decreases dopamine-elicited G_i signaling through the D_2R and CGS-21680-elicited G_s signaling through the $A_{2A}R$. Furthermore, this reciprocal antagonism seemed to occur through a wide cRNA GPCR injection ratio. Currently, we are examining crosstalk by assessing whether addition of agonists or inverse agonists to the $A_{2A}R$ is able to decrease or increase respectively the D_2R-mediated G_i

signaling through the $A_{2A}R$-D_2R heteromer. Modulation of G_s signaling through the $A_{2A}R$ by D_2R ligands is also being examined. Once we have characterized G_s and G_i signaling through the $A_{2A}R$-D_2R heteromer in our heterologous expression system, we will validate the results in native cells to ensure the physiological relevance of such signaling. Characterization of the signaling pathway through the $A_{2A}R$-D_2R heteromer will provide insight into what ligands optimize dopaminergic signaling, which may advance Parkinson's disease pharmacotherapy.

Disclosures: C.N. Hatcher-Solis: None. **D.E. Logothetis:** None.

Poster

502. Purine and Other G-Protein Coupled Receptors

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Topic: B.03. G-Protein Linked Receptors

Support: NS 061068 to MLD

UNAM PASPA Program to EBNR

Title: Design and synthesis of N-substituted indoles as selective hMT2 melatonin receptor ligands

Authors: *M. L. DUBOCOVICH[1], E. B. NARANJO-RODRIGUEZ[2], A. LIRA-ROCHA[2], O. ESPEJO-GONZALEZ[2], R. V. RAJNARAYANAN[1]
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Abstract: The goal of this study was to design, synthesize and iteratively optimize a mini-library of N-substituted indoles with high selectivity for the MT_2 melatonin receptors. Theoretical models of human melatonin receptors were generated using the x-ray crystal structures of rhodopsin [PDB ID: 1L9H] and beta-2 adrenergic G-protein-coupled receptor [2RH1; 3NYA; 3SN6]. To enhance the predictability of these models, a curated list of ligands with affinity for melatonin receptors were used to generate pseudo-receptor spaces (PRS) and as shape/volume filters of 3D melatonin receptor models. An ensemble of functionally relevant receptor conformations of melatonin receptors were generated by high-throughput hierarchical helical search by translation and rotation of transmembrane helices followed by combinatorial docking

of PRS of active melatonin ligands. A total of ~15 million ligand-melatonin receptor complexes were analyzed to gauge the conformational dynamics and ligand induced changes in receptor architecture. MT_1 receptor rotamers with TM3 and TM5 at 330 and 240 degrees positioned H195, S110 and S114 to provide space within the binding site to accommodate melatonin. A further translation in the x-direction was required to enhance melatonin binding through H-bonding interactions with S110 and S114. Using these predictive MT_1 and MT_2 melatonin receptor models we designed, synthesized and iteratively optimized a mini-library of N-substituted indoles. In silico analysis of the top MT_2 ligand, with Ki values of 17.0 $\pm$ 1.4 nM (n=6) and 1848$\pm$1.4 nM (n=6) for MT_2 and MT_1 receptor, respectively [MT_1/MT_2 affinity ratio = 115] revealed several residue clashes [VAL111, ILE112, ILE115 and PHE116] in the MT_1 receptor binding pocket and none in the MT_2 receptor pocket, indicative of MT_2 selectivity. The high number of ligands with MT_2 selectivity in this library can be attributed to the ring stacking interactions with W264, F209, H208 and hydrophobic interactions with M120, V124, I125, F129, L172, Y183, F192, V205 and L267. Furthermore, the MT_2 melatonin receptor binding constants of these compounds was proportional to the hydrophobicity of the N-aryl substituent. Optimization of this lead molecule could result in highly selective MT_2 melatonin receptor ligands.

Disclosures: M.L. Dubocovich: None. **E.B. Naranjo-Rodriguez:** None. **A. Lira-Rocha:** None. **O. Espejo-Gonzalez:** None. **R.V. Rajnarayanan:** None.

Poster

502. Purine and Other G-Protein Coupled Receptors

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Topic: B.03. G-Protein Linked Receptors

Support: Boehringer Ingelheim Ulm University BioCenter (BIU) N2

International Graduate School in Molecular Medicine Ulm (IGradU)

Title: Differential expression of cannabinoid receptors, monoacylglycerol lipase and fatty acid amide hydrolase in neurons and glia cells

Authors: M. ENGELSKIRCHEN[1], N. PASQUARELLI[1,2], H. BAYER[1], J. HANSELMANN[1], *P. WEYDT[1], B. FERGER[2], A. WITTING[1]

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Abstract: The endocannabinoid system (ECS) represents a potential therapeutic target for neurodegenerative diseases. ECS consists of the cannabinoid receptors CB_1 and CB_2, the endocannabinoids (eCBs) and the enzymes involved in synthesis and degradation of the eCBs. In our study we investigated the expression of CB_1 and CB_2 and of the endocannabinoid hydrolyzing enzymes monoacylglycerol lipase (MAGL) and fatty acid amide hydrolase (FAAH) in different cell types of the brain. The expression levels of CB_1, CB_2, FAAH and MAGL were quantified in primary mouse neurons, astrocytes, oligodendrocytes and microglia on mRNA level by qPCR and on protein level by Western blot. The cellular localization of CB_1, CB_2, FAAH and MAGL was investigated by immunocytochemistry. We found that CB_1 receptors were highly expressed in neurons and CB_2 receptors were highly expressed in microglia. MAGL and FAAH were mostly expressed in astrocytes, whereas microglia expressed relatively low levels of MAGL and FAAH. Our results confirm that CB_2 ligands will mainly induce an effect on microglia, whereas CB_1 ligands will mainly induce effects in neurons. Inhibitors of MAGL and FAAH will target mainly astrocytes that might play a key role in mediating neuroprotective and anti-inflammatory effects related to MAGL and/or FAAH inhibition.

Disclosures: M. Engelskirchen: None. **P. Weydt:** None. **N. Pasquarelli:** None. **H. Bayer:** None. **J. Hanselmann:** None. **B. Ferger:** A. Employment/Salary (full or part-time):; Boehringer Ingelheim Pharma GmbH & Co. KG. **A. Witting:** None.

Poster

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SLS project grant SLS-331631

Title: Activating the histamine H1 receptor induces gamma oscillations in the rat hippocampus

Authors: *R. H. ANDERSSON[1,2], D. PAPADIA[2], D. GALTER[1], A. FISAHN[2]
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Abstract: From a neurophysiological perspective wakefulness is characterized by mild depolarization of principal neurons of cortical structures and parts of the thalamus and the cessation of slow-wave activity. Histamine is a biogenic amine, which is a key transmitter in the arousal and wakefulness system of the mammalian brain. It has been shown to have largely excitatory actions in the hippocampus where it promotes burst firing in pyramidal cells. Because the hippocampal area CA3 has a strong tendency to generate gamma oscillations (30-80Hz) when exposed to an excitatory neurotransmitter we hypothesized that histamine may promote such rhythmic network activity. Indeed we found that histamine dose-dependently elicits gamma oscillations and this histamine-induced rhythmogenesis depends on the H1 receptor but not H2 or H3. We also showed that rhythmogenesis was independent of indirect release of acetylcholine. Using *in-situ* hybridization with probes for the H1, H2 and H3 receptors respectively, we observed that they were all expressed in *stratum pyramidale* with little expression outside this layer. The H2 and H3 receptors were also expressed in CA1 whereas the H1 receptors were almost exclusively expressed in CA3, suggesting that they might have a specialized role there. Both pyramidal cells and fast-spiking interneurons exhibited a depolarization as well as a decrease in membrane resistance in response to histamine. We did not observe any effect on synaptic charge transfer for excitatory or inhibitory postsynaptic currents recorded in pyramidal cells. Rather, it was an increased synchronization of inhibitory postsynaptic currents that seems to underlie the rhythmogenic effect of histamine.

Disclosures: **R.H. Andersson:** None. **D. Galter:** None. **A. Fisahn:** None. **D. Papadia:** None.

Poster

502. Purine and Other G-Protein Coupled Receptors

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Topic: B.03. G-Protein Linked Receptors

Support: NIDCD DC005782

Title: Molecular recognition of ketamine by discrete olfactory G-protein coupled receptors

Authors: *J. HO[1], L. GAO[3], J. M. PEREZ-AGUILAR[4], J. G. SAVEN[3], R. ECKENHOFF[5], H. MATSUNAMI[2]
[2]Mol. Genomics & Microbiology, [1]Duke Univ. Med. Ctr., Durham, NC; [3]Dept. of Chem., Univ. of Pennsylvania, Philadelphia, PA; [4]Dept. of Physiol. and Biophysics, Weill Cornell Med. Col., New York, NY; [5]Dept. of Anesthesiol. and Critical Care, Perelman Sch. of Medicine, Univ. of Pennsylvania, Pennsylvania, PA

Abstract: Ketamine is a well-known N-Methyl-D-aspartate (NMDA) receptor antagonist with a wide variety of pharmacological effects, including sedation, analgesia, hallucinations, and general anesthesia. However, the underlying molecular mechanisms that mediate these effects, particularly the anesthetic effects, remain unknown. Specifically, ketamine's interactions with G-protein coupled receptors (GPCRs) have been little studied. We aim to use olfactory GPCRs (ORs) as a model to study the ketamine-GPCR interaction by taking advantage of their ligand-binding diversity and sequence and structural similarity for insights into the ketamine binding pocket. Using an in-vitro screen for OR ligand activation, we identified 4 ORs (MOR136-1, MOR136-3, MOR136-5 and MOR139-1) that respond to ketamine in a dose-dependent manner. We then applied a combination of molecular homology modeling and site-directed mutagenesis to examine key ketamine binding residues in the MOR136-1, and were able to increase (D109), reduce (S105) and abolish (S112) its ketamine response via single site mutations at the indicated residues. Finally we were able to introduce ketamine response in a non-responder, MOR136-4, again by a single site mutation at Y104. All four residues are located in the third transmembrane domain (TM3), suggesting its importance in ketamine binding and response. Our results suggest that GPCRs could, at the minimum, serve as functional targets for ketamine, and our ability to abolish and introduce responsiveness in specific receptors suggest a signature binding pocket that can be used to further explore other protein structures for more candidate ketamine receptors.

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Poster

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Topic: B.03. G-Protein Linked Receptors

Support: DA011322

Title: When entourage gets in the way: The curious case of 2-og and 2-lg

Authors: *N. MURATAEVA, K. MACKIE, A. STRAIKER
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Abstract: 2-Arachidonoylglycerol (2-AG) is the most abundant endogenous cannabinoid in the brain and is a high efficacy agonist at both cannabinoid receptors (CB1 and CB2). Over the past years the synthesis, degradation and signaling of 2-AG have been investigated in some detail. However, several other endogenous monoacylglycerols have been isolated from various tissues, but their pharmacology has not been fully explored. Two of these are 2-linoleoylglycerol (2-LG) and 2-oleoylglycerol (2-OG), also a GPR119 agonist. The current data suggest that these compounds do not bind to the cannabinoid receptors. Nor do they affect intracellular free Ca2+ levels or adenylyl cyclase activity in a CB1-dependent manner. However, the presence of these compounds has been reported to potentiate the activity of 2-AG and slow its degradation, possibly through competitive inhibition of 2-AG degradation. This phenomenon has been dubbed the 'entourage effect' and may be a means to regulate synaptic activity. To clarify the activity of these lipids at the CB1 receptor we employed patch-clamp and cell-based assays. For the former we used cultured autaptic hippocampal neurons, i.e. self-synapsing neurons that have the necessary cellular machinery for several forms of endocannabinoid-mediated synaptic plasticity. This includes the 2-AG-, CB1-, and MAGL-dependent retrograde form of neuronal signaling known as depolarization-induced suppression of excitation (DSE), making it a useful model to test for a potential entourage effect. Our electrophysiological data show that 2-OG and 2-LG do not inhibit neurotransmission via CB1 when applied to autaptic neurons. However, these compounds fail to potentiate the 2-AG-dependent DSE. Instead 2-OG and 2-LG behave as antagonists at the CB1 receptor, attenuating DSE. This result is inconsistent with an 'entourage effect'. Interestingly 2-OG and 2-LG do internalize CB1 receptors in CB1-HEK cells, as shown by an on-cell western assay, indicating that these compounds do activate CB1 receptors under some conditions. Our results suggest 1) that these compounds may serve as functional antagonists under certain conditions and, interestingly, 2) that these compounds may exhibit functional selectivity in their signaling. Our results suggest that the relationship between 2-AG and its congeners may be more nuanced than previously appreciated.

Disclosures: **N. Murataeva:** None. **K. Mackie:** None. **A. Straiker:** None.

Poster

502. Purine and Other G-Protein Coupled Receptors

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 502.18/D26

Topic: B.03. G-Protein Linked Receptors

Support: NIH Grant NS25296

Title: Functional G-protein coupled receptor 35 (GPR35) in rat hippocampal CA1 stratum radiatum interneurons

Authors: *M. ALKONDON, E. F. R. PEREIRA, S. W. TODD, M. LANE, E. X. ALBUQUERQUE
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Abstract: G-protein coupled receptor 35 (GPR35) was de-orphanized after the discovery that kynurenic acid (KYNA) acts as an agonist of this receptor. Abundant evidence supports that GPR35 exists primarily in peripheral tissues. Here, we tested the hypothesis that GPR35 exists in the hippocampus and remains a molecular target for the actions of KYNA in the CNS. Fluorescence immunohistochemical staining using an antibody anti-NeuN (a neuronal marker), an antibody anti-GFAP (a glial marker), and an antibody anti-GPR35 revealed that neurons in the stratum oriens, stratum pyramidale and stratum radiatum of the CA1 field of the hippocampus express GPR35. To determine whether these receptors are functional, we tested the effects of various GPR35 agonists on the frequency of spontaneous action potentials recorded as fast current transients (CTs) from stratum radiatum interneurons (SRIs) under cell-attached configuration in rat hippocampal slices. Bath application of the GPR35 agonists zaprinast (1-10 μM), dicumarol (50-200 μM), pamoic acid (500-1000 μM), and amlexanox (3 μM) produced a concentration- and time-dependent reduction in the frequency of CTs. Superfusion of the hippocampal slices with the GPR35 antagonist ML145 (1 μM) increased the frequency of CTs and reduced the inhibitory effect of zaprinast and dicumarol. At concentrations ranging from 200 nM to 1000 nM, KYNA also decreased the frequency of CTs. The present results demonstrate that functional GPR35s are expressed by CA1 SRIs and suggest that these receptors can be molecular targets for KYNA's actions in the hippocampus.

Disclosures: M. Alkondon: None. **E.F.R. Pereira:** None. **S.W. Todd:** None. **M. Lane:** None. **E.X. Albuquerque:** None.

Poster

502. Purine and Other G-Protein Coupled Receptors

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Program#/Poster#: 502.19/D27

Topic: B.03. G-Protein Linked Receptors

Support: NIH Grant R01AG036794

Title: The role of CB2 cannabinoid receptors in regulating synaptic transmission

Authors: *J. KIM
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Abstract: Major endocannabinoids (e.g., 2-arachidonoylglycerol, anandamide) and cannabis components (e.g., Δ9-tetrahydrocannabinol) can activate two types of cannabinoid receptors--CB1 and CB2 receptors (CB1R and CB2R, respectively). In the brain, the effects of cannabinoids have been studied mostly in relation to CB1R in part because early evidence indicated that CB2R was present in the immune system but not in the brain. However, CB2Rs have been recently discovered in the brain and implicated in various neuropsychiatric phenotypes, suggesting their involvement in a variety of brain functions such as anxiety, addiction, depression and schizophrenic behaviors. Despite recent information about the presence and behavioral effects of CB2Rs, it is largely unknown how CB2Rs regulate neuronal functions at the cellular level. In the hippocampus, CB2Rs are located in the soma and dendrites of neurons, especially near synaptic contacts, as well as in microglia, but the role of CB2Rs in modulating synaptic function remains elusive. The objective of this study is to determine the role of CB2Rs in regulating synaptic transmission. Postsynaptic currents were recorded from CA1 pyramidal neurons in organotypic cultures of the rat hippocampus. CB2R agonists were found to change the properties of glutamatergic synapses via extracellular signal-regulated kinases. This study reveals a novel function of CB2 receptors in the hippocampus. It will be interesting to further identify the signaling cascade and CB2R location that are involved in the synaptic regulation.

Disclosures: J. Kim: None.

Poster

502. Purine and Other G-Protein Coupled Receptors

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Program#/Poster#: 502.20/D28

Topic: B.03. G-Protein Linked Receptors

Support: CIHR Grant MOP-102713

DFG GRK1459

Title: Subcellular mobility of the trans-Golgi-associated PDZ protein PIST in endocrine cells

Authors: W. ALSHAFIE[1], Y. PAN[1], H. J. KREIENKAMP[2], *T. STROH[1]
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Abstract: The Protein Interacting Specifically with Tc10 (PIST) is a Golgi resident PDZ protein implicated in sorting at the trans-Golgi-network (TGN). For instance, PIST protects Somatostatin (SOM) Receptor subtype 5 (sst5) from lysosomal degradation by trapping it in the Golgi before its engagement in the recycling pathway (Bauch et al, 2014). Here we used immuno labelling and confocal microscopy followed by fluorescence intensity profile analysis to examine the effect of somatostatinergic agonists on PIST subcellular distribution and its association with trafficking adaptors in the endocrine AtT20 cell line that endogenously expresses several SOM receptor subtypes (sst1, 2, 4, and 5). As expected, we observed a high peak of PIST immunofluorescence at the TGN under control non-stimulated conditions. Interestingly, following stimulation with the sst2-selective agonist L779,976 (0 to 60 minutes), while the main peak of PIST was still associated with the Golgi, over time smaller fluorescence peaks appeared at the cell periphery associated with PIST-positive vesicular structures. They appeared to be located just beneath the plasma membrane as visualized by immuno labelling for the sst2A receptor. This apparent mobilization of PIST positive structures from TGN to the cell periphery appeared to rely on sst2 activation since L779,976 is highly selective for sst2 over other SOM receptor subtypes. We also observed an increased co-localization of PIST and a recycling adaptor, Rab11a, following sst2 stimulation suggesting a possible role for PIST in the recycling pathway. Based on these observations, we propose an important role for PIST in the regulation of trafficking from the Golgi compartment including the regulation of recycling.

Disclosures: W. Alshafie: None. **Y. Pan:** None. **H.J. Kreienkamp:** None. **T. Stroh:** None.

Poster

502. Purine and Other G-Protein Coupled Receptors

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Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 502.21/D29

Topic: B.03. G-Protein Linked Receptors

Support: HSFC

CFI

SHRF

Title: Contribution of adenosine A1 and A2A receptors to hypoxia-reperfusion synaptic potentiation in rat hippocampus

Authors: *J. STOCKWELL, Z. MING, Z. CHEN, F. S. CAYABYAB
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Abstract: Adenosine is a principal regulator of synaptic depression in various neuronal insults such as hypoxia/ischemia. Hypoxic insult to the vulnerable hippocampus induces a substantial increase in extracellular adenosine and subsequent synaptic depression, which is mediated by activation of the adenosine A1 receptor. The inhibitory adenosine A1 receptors are widely believed to be neuroprotective in these insults, whereas the excitatory A2A receptors promote neurotoxicity. We previously showed that a 20-minute hypoxic insult followed by a 45-minute normoxic reperfusion period induced rapid synaptic depression in hypoxia followed by significant post-hypoxic potentiation during reintroduction of oxygen. Accordingly, we showed that A1 receptors mediate synaptic depression through internalization of AMPA glutamate receptors, namely GluA1 and GluA2 AMPA receptor subunits. We hypothesized that the significant post-hypoxic potentiation is caused by A2A receptor activation, which we have shown causes increased GluA1-AMPA receptor surface expression. We tested hippocampal slices incubated in SCH 442416, an A2A receptor antagonist, and DPCPX, an A1 receptor antagonist followed by a 20-minute hypoxic insult and a 45-minute normoxic washout. Electrophysiological fEPSP recordings show that SCH 442416 prevented post-hypoxic potentiation, allowing fEPSPs to recover to baseline levels, and was accompanied by less GluA1 upregulation compared to control, as shown by biochemical analysis. Paired-pulse ratio (PPR) analysis showed significant paired-pulse facilitation in hypoxia in presence of SCH 442416 (decreased probability of neurotransmitter release), similar to control. However, during reperfusion of normoxic solutions, SCH 442416 caused recovery of PPR to baseline levels, while control slices showed paired-pulse depression (increased probability of transmitter release). Interestingly, DPCPX not only reduced hypoxia-induced synaptic depression, but also prevented post-hypoxic potentiation and GluA1 surface expression. These results suggest that a cross-talk between the adenosine A1 and A2A receptors primes A2A receptors for adenosine-mediated excitotoxic potential in cerebral ischemia/reperfusion injury.

Disclosures: **J. Stockwell:** None. **Z. Ming:** None. **Z. Chen:** None. **F.S. Cayabyab:** None.

Poster

502. Purine and Other G-Protein Coupled Receptors

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Program#/Poster#: 502.22/D30

Topic: B.03. G-Protein Linked Receptors

Support: NIH R01 NS083410

NIH 5T32NS041218

Title: Astrocyte-derived MMP-1 as an effector of PAR1-dependent neuronal excitability

Authors: *M. ALLEN[1], A. SMART[2], X. CHEN[3], G. P. AHERN[2], R. DZAKPASU[3], K. MAGUIRE-ZEISS[1], K. CONANT[1]
[1]Neurosci. and Interdisciplinary Program in Neurosci., Georgetown Univ., Washington, DC; [2]Pharmacol. and Physiol., [3]Physics and Pharmacol. and Physiol., Georgetown Univ. Med. Ctr., Washington, DC

Abstract: Reports from the last decade overwhelmingly implicate synaptic dysfunction in most, if not all, Autism Spectrum Disorders (ASD). Synaptic structure and function are tightly coordinated by signal exchange between both glia and neurons. Understanding the mechanism(s) through which glia and neurons regulate synaptic transmission is critical to understanding ASD-associated synaptic deficits. Several groups report finding astrocyte activation and neuronal hyperexcitability in ASD mouse models. Interestingly, activated astrocytes release matrix metalloproteinases (MMPs), a family of extracellular matrix protein proteases. MMPs may potentiate neuronal excitability through activation of glial-neuronal signaling, which may contribute to the hyperexcitability phenotype found in ASD. MMP-1 is known to activate protease-activated receptor 1 (PAR1), a G-protein-coupled receptor (GPCR) that is highly expressed in both astrocytes and neurons. Previous studies have shown activation of astrocytic PAR1 canonical G protein signaling triggers a Ca^{2+}-dependent release of glutamate that potentiates neighboring neurons. Therefore, we questioned whether astrocyte-derived factors play a role in astrocyte to neuron signaling *in vitro* and *in vivo*. More specifically, we investigated the astrocytic modulation of neuronal responses via MMP-1 mediated activation of PAR1. In the present study, we utilized novel transgenic mice that overexpress the potent PAR1 activating protein, human MMP-1 (hMMP1), under the direction of an astrocyte specific promoter to assess astrocyte to neuron signaling. First, we use a combination of complementary mass spectrometric methods to measure MMP-1 specific activation of PAR1. Next, we examine the role mobilization of intracellular astrocyte calcium plays in the release of glial glutamate with

several calcium imaging experiments. Lastly, we employ the use of multielectrode arrays (MEAs) to study the effect of astrocyte-derived factors on neuronal action potential frequency. MMP1-mediated activation of PAR1 in astrocytes has not yet been reported in the context of neurodevelopmental disorders, thus this proposal offers an innovative approach to assess the therapeutic potential of PAR1 inhibitors.

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Poster

502. Purine and Other G-Protein Coupled Receptors

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Topic: B.03. G-Protein Linked Receptors

Support: NIH Intramural Grant Z01 DK031116-27

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Title: Design, synthesis and characterization of high affinity fluorescent agonist and antagonist ligands of G protein-coupled P2Y receptors

Authors: *K. A. JACOBSON[1], E. KISELEV[1], P. JAYASEKARA[1], M. O. BARRETT[2], V. KATRITCH[3], S. PAOLETTA[1], C. WEITZER[2], E. HAMMES[1], R. BALASUBRAMANIAN[1], Z.-G. GAO[1], Q. ZHAO[4], R. C. STEVENS[3], T. K. HARDEN[2]
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Abstract: Extracellular nucleotides acting at Gq- and Gi-coupled P2Y receptors (P2YRs) modulate biological processes in many organs and tissues. We explore novel P2YR agonists and antagonists, to identify selective agents as pharmacological probes and potential therapeutic agents. We designed fluorescent conjugates of functionalized congeners that display high P2YR

affinity, for characterization of these GPCRs in living cells by flow cytometry and in cell membranes. Fluorescent agonists are mostly internalized consistent with agonist-induced receptor internalization, and this labeling is attenuated by specific P2YR ligands. Examples are MRS4129 and MRS4162, which are fluorescent pyrimidine nucleotides, respectively, selective for the P2Y6R (EC50 9 nM, phospholipase C activation) and high affinity pan-agonist at P2Y2R,P2Y4R and P2Y6R (expressed in astrocytoma cells). We synthesized P2Y14R fluorescent antagonists based on potent and highly selective 2-naphthoic acid derivative PPTN. We modeled the hP2Y14R based on recent hP2Y12R X-ray structures and simulated docking, suggesting that a piperidine of PPTN is accessible for tethering fluorophores. Click chemistry was used to conjugate functionalized PPTN alkyne derivatives and azide-bearing fluorophores. Flow cytometry showed high specific P2Y14R binding of AlexaFluor488 derivative MRS4174 (Ki 80 pM, cAMP inhibition in P2Y14R-expressing CHO cells). Known P2Y ligands inhibited cell labeling consistently with affinity order. Thus, the 3D knowledge of ligand recognition in GPCRs promises to enable drug discovery through design of fluorescent molecular probes for P2YRs. This approach demonstrates the predictive power of GPCR homology modeling and the value of applying newly determined X-ray structures to the medicinal chemistry of GPCRs.

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Poster

502. Purine and Other G-Protein Coupled Receptors

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Topic: B.02. Ligand-Gated Ion Channels

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SQM 2014

Title: Novel insights on the allosteric mechanism of alfaxolone interaction with rP2X4 receptor opening, lessons from structural biology analysis

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Abstract: Danio rerio P2X4 receptor crystals (zfP2X4R) with and without ATP in the orthosteric site offered the possibility of understanding at the structural/atomic level the function of this receptor channel. Alfaxolone, a prototype neurosteroid, elicited a positive allosteric modulation of the rP2X4R through an interaction occurring likely in the transmembrane (TM) rP2X4R domain; larger steroid concentrations elicited per se an ionic current, suggesting pore opening in the absence of ATP (Codocedo et al., 2009). We aimed at characterizing the steroid binding site and understand how the structure of the pore changes in response to alfaxolone binding to the TM domain of rP2X4R. Based on the crystallized zfP2X4R in the apo and holo (ATP-bound) states, corresponding rP2X4R models of the extracellular and TM domains for both states were built, including the modelling of the N and C-terminus cytoplasmic tails which are absent in the crystallized zfP2X4R. The rP2X4R model was embedded in an artificial lipid membrane environment; all atom molecular dynamics (MD) simulations with three docked alfaxolone molecules were developed during 100 nanoseconds. MD calculations not only allowed identifying the steroid binding site, but also the eventual conformational alternations elicited by steroid binding. Results reveal that in the apo state, 3 alfaxolone molecules interact with the TM domain throughout the simulation; however, only a single steroid forms hydrogen bonds across the subunit interface of the TM, while the other two steroid molecules only bind to the TM domain of the binding subunit. Channel activation involved the separation between the TM helices of neighboring subunits (Hattori & Gouaux, 2012). Single alfaxolone binding may partially open the rP2X4R pore, as evidenced by the increases in the solvent accessible surface area for Val-47 (subunit A) and Asn-338 (subunit C) when comparing the apo state with alfaxolone and apo state without alfaxolone, a feature also observed between the apo and holo structures of rP2X4R and zfP2X4R. These data provides insights of the direct alfaxolone effect, helping to understand the motion of the pore during rP2X4R channel gating. The interaction of neurosteroids eliciting a negative allosteric modulation will be promptly examined on the rP2X4R.

Disclosures: J.T. García-Huidobro: None. **N. Alveal:** None. **C.H. Navarrete:** None. **N.P. Barrera:** None.

Poster

502. Purine and Other G-Protein Coupled Receptors

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Topic: B.03. G-Protein Linked Receptors

Support: NIH grant NS026880

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Title: When BigLEN met GPR171: A tale of a recently deorphanized neuropeptide/receptor system

Authors: *E. N. BOBECK, J. WARDMAN, I. GOMES, L. DEVI
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Abstract: Receptor systems of the hypothalamus are considered attractive targets for the development of therapeutics regulating food intake, satiety and body weight regulation. Recent neuropeptidomics studies have demonstrated that ProSAAS-derived peptides are among the most abundant peptides present in the hypothalamus; these peptides are greatly enriched in the agouti related peptide neurons of the arcuate nucleus. Additional studies have implicated Big LEN (b-LEN), the peptide derived from ProSAAS in acute feeding. Recently we deorphanized GPR171, as a hypothalamic G protein-coupled receptor for b-LEN. GPR171 is a Gαi/o coupled receptor activated by the C-terminal region of b-LEN. Overexpression of GPR171 leads to an increase and knock-down leads to a decrease in b-LEN-binding and signaling. In the hypothalamus GPR171 expression complements the expression of b-LEN; the levels and activity of GPR171 are elevated in mice lacking b-LEN. Lentiviral shRNA-mediated knock-down of GPR171 in the hypothalamus leads to alterations in food intake and metabolism. To further investigate the role of GPR171 in the orexigenic properties within the arcuate nucleus, a designer receptor exclusively activated by designer drugs (DREADD) approach was used targeting b-LEN containing, agouti related peptide neurons. Activation of these neurons with clozapine-N-oxide induced food intake; the role of b-LEN and other neuropeptides released from these neurons in food intake is currently being investigated. To explore the physiological involvement of GPR171 in modulating additional behaviors, we examined its distribution in mouse brain and find expression in dentate gyrus, pedunculopontine nucleus, and preriaqueductal gray suggesting potential role for GPR171 on a number of behavioral outcomes. Further studies are underway to investigate the role of GPR171 in addiction, Parkinson’s disease, and pain processing. This work

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Poster

502. Purine and Other G-Protein Coupled Receptors

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Program#/Poster#: 502.26/D34

Topic: B.03. G-Protein Linked Receptors

Title: Potential association between a mutation in the brain-expressed receptor GPR37L1 and a novel inherited neurological disorder

Authors: ***M. M. GIDDENS**[1], E. G. FARROW[2], S. E. SODEN[2], R. A. HALL[1]
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Abstract: GPR37-like-1 (GPR37L1) is a G protein-coupled receptor that is expressed almost exclusively in the central nervous system. This receptor has been previously shown by our lab to be activated by prosaposin, a secreted factor with neuroprotective and glioprotective actions (Meyer et al., Proc. Natl. Acad. Sci. USA, 2013). Stimulation of GPR37L1 by prosaposin mediates cytoprotective signaling in astrocytes and other cell types in the brain. Recently, exome mapping in a large, consanguineous family of Middle Eastern descent has identified a point mutation in the GPR37L1 gene as the single candidate mutation for inheritance of a previously-undescribed recessive monogenic disorder. Affected children develop severe migraines and seizures around the onset of puberty, which is followed by cortical atrophy and ultimately death by the late teens. The mutation in the GPR37L1 gene results in a lysine to asparagine substitution (K349N) in the middle of the receptor's third cytoplasmic loop. To better understand the functional consequences of the GPR37L1 K349N mutation and assess whether this mutation underlies the pathology observed in the affected family, we have created a GPR37L1 expression construct with the K349N mutation. Ongoing studies are assessing the expression, trafficking, signaling and function of the K349N mutant receptor in transfected cells. These studies will shed new light on the fundamental properties of GPR37L1 and potentially provide insights regarding potential treatment options for the affected family.

Disclosures: **M.M. Giddens:** None. **E.G. Farrow:** None. **S.E. Soden:** None. **R.A. Hall:** None.

Poster

502. Purine and Other G-Protein Coupled Receptors

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Program#/Poster#: 502.27/D35

Topic: B.03. G-Protein Linked Receptors

Title: GPR37 promotes oligodendroglial survival

Authors: *B. COLEMAN, R. HALL
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Abstract: GPR37 is an orphan G protein-coupled receptor that is highly expressed in the brain. Previous work from our lab has shown that GPR37 is a receptor for the secreted neuroprotective and glioprotective factor prosaposin and that prosaposin acts through GPR37 to protect cultured astrocytes from oxidative stress-induced cell death (Meyer et al., Proc. Natl. Acad. USA, 2013). Interestingly, several reports indicate that GPR37 is expressed at particularly high levels in oligodendrocytes (Imai et al., Cell, 2001; Cahoy et al., J. Neurosci., 2008). Furthermore, prosaposin has also been shown to enhance the survival of oligodendroglia *in vitro* and also promote myelination *in vivo* (Hiraiwa et al., Proc. Natl. Acad. Sci. USA, 1997; Hiraiwa et al., Glia, 1999). However, despite the aforementioned evidence for GPR37 expression in oligodendroglia, the role(s) that GPR37 and its ligand prosaposin may play in oligodendroglial biology remain uncharacterized. Using enriched oligodendrocyte cultures and mixed glial cultures generated from either wild type (WT) or GPR37 knockout (GPR37KO) C57Bl/6 neonatal mice, we investigated the role of GPR37 in oligodendroglial survival. We found that oligodendrocytes generated from GPR37KO mice are less viable in culture than oligodendrocytes generated from WT mice. Furthermore, we also observed that GPR37KO oligodendrocytes are more susceptible than their WT counterparts to death induced by oxidative stress via hydrogen peroxide treatment. Together, these data suggest that GPR37 is important for oligodendroglial survival and protection against insults. Ongoing studies in this area seek to elucidate the signaling pathways downstream of GPR37 that promote the survival of oligodendroglia.

Disclosures: B. Coleman: None. **R. Hall:** None.

Poster

502. Purine and Other G-Protein Coupled Receptors

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Topic: B.03. G-Protein Linked Receptors

Title: Guanyl nucleotide modulation of the dopamine D3 receptor in rat brain cerebellum lobule 9&10

Authors: *N. C. STRATMAN, C. J. SCHMIDT
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Abstract: The functional nature of the dopamine D3 receptor (D3R) has typically been investigated in recombinant cell lines, and many studies have identified weak or nonexistent G-protein dependent coupling in these systems. It has not been clear if recombinant cells lines have the appropriate G-proteins or secondary messenger systems necessary for adequate responses of D3R activation. As such, investigation of the D3R in its native environment may provide insights into to the mechanisms needed to elicit intracellular responses. Since the D3R is only moderately expressed in the same brain regions of high levels of dopamine D2 receptors (D2R) such as in the ventral striatum, discrimination between the two receptors has proved difficult with non-selective ligands. However, the D3Rs are moderately expressed in cerebellum lobules 9 and 10 without the presence of the D2R. The current investigation profiled the binding of radiolabeled D3R agonists in rat cerebellum lobules 9 and 10 in the absence or presence of guanyl nucleotides to identify modulation of the D3R as an indication of activation of the receptor through coupling to native G-proteins. Autoradiography has been utilized to provide the most sensitive method to quantify changes in radioligand binding.

Disclosures: N.C. Stratman: A. Employment/Salary (full or part-time):; Pfizer Inc. **C.J. Schmidt:** A. Employment/Salary (full or part-time):; Pfizer Inc..

Poster

502. Purine and Other G-Protein Coupled Receptors

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Topic: B.03. G-Protein Linked Receptors

Support: 1ZIAMH002386

1ZIAMH002592

Title: GPCR-Gs-NCS/Rapgef2 coupling: A novel pathway to ERK activation in neuroendocrine cells

Authors: M. V. EIDEN[1], A. C. EMERY[2], *L. E. EIDEN[2]
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Abstract: It is generally accepted that G-protein coupled receptors (GPCRs) that engage Gs and activate adenylate cyclase exert effects on neuronal function through the activation of multiple cAMP sensors, including protein kinase A, the Rap guanine nucleotide exchange factors Epac1 and 2, cyclic nucleotide-gated calcium channels, and cyclic nucleotide-gated potassium channels. We have characterized a cAMP-dependent factor mediating neuritogenesis through ERK phosphorylation, initiated by PACAP occupancy of the family B GPCR PAC1, and termed this the neuritogenic cAMP sensor (NCS). We have recently identified the NCS as the protein product of the Rapgef2 gene. We now report on some new features of NCS/Rapgef2 function in neuroendocrine cells, based on the creation of a 293 cell line expressing hRapgef2, and preliminary results on NCS/Rapgef2 coupling to GPCRs in addition to the PAC1 receptor. We have reported that functional full-length Rapgef2 is expressed in cell lines of neuroendocrine lineage, but not in several non-neuroendocrine cell lines commonly used for high-throughput GPCR ligand and antagonist screening (Emery et al., *Science Signaling* **6**: ra51, 2013). To study the role of this protein in GPCR-initiated cAMP signaling, *in cellula,* we have therefore created a series of indicator cell lines based on the 293_CREB-luciferase reporter gene line (Promega), and a 293_Elk-luciferase reporter gene line constructed via sequential retroviral transduction/limiting dilution cell cloning, in which we have transduced various human GPCRs, and/or hRapgef2, each under the control of the CMV promoter, via introduction as retroviral vector integrants. We have also introduced GPCRs via retroviral gene delivery into rat neuroendocrine NS-1 cells, in which neurite extension caused by elevation of cAMP is mediated exclusively through the NCS/Rapgef2->Rap->Raf->MEK->ERK signaling pathway. We find that a diverse array of family B (PAC1, VPAC1, VPAC2) GPCRs, and a family A (D1) Gs-coupled GPCR, cause either ERK-dependent neuritogenesis in NS-1 cells, or ERK activation sensitive to adenylate cyclase inhibition in 293 cells only when expressing both hRapgef2 and the appropriate GPCR, when cells are stimulated by the cognate receptor ligand PACAP, VIP or SKF 81297. The availability of cell lines expressing GPCR and hRapgef2 from single-copy genes, to avoid both receptor reserve, and signaling pathway misactivation, and amenable to high-throughput screening for GPCR-initiated activation of ERK, should be useful tools in GPCR-based CNS drug discovery.

Disclosures: **M.V. Eiden:** None. **A.C. Emery:** None. **L.E. Eiden:** None.

Poster

502. Purine and Other G-Protein Coupled Receptors

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 502.30/D38

Topic: B.03. G-Protein Linked Receptors

Support: Fondazione Banco di Sardegna

Title: Tricyclic and tetracyclic antidepressants activate LPA_1 lysophosphatidic acid receptor signaling in glial cells

Authors: *P. ONALI, S. DEDONI, M. C. OLIANAS
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Abstract: Although inhibition of presynaptic monoamine reuptake or metabolism is generally considered the main mechanism of action of antidepressants (AD), there is a large body of evidence indicating that the pharmacological actions of these drugs can also derive from their direct interaction with additional molecular targets. We have previously reported that different classes of AD can activate LPA_1 lysophosphatidic acid (LPA) receptor in Chinese hamster ovary cells to induce growth factor receptor transactivation. In the present study we extended this observation by examining the ability of different AD to induce LPA_1 signaling in glial cells, which are considered a relevant cellular target of AD therapeutic action. We found that in C6 glioma cells the tricyclic AD amitriptyline, desipramine, imipramine and nortriptyline, the tetracyclic AD mianserin and mirtazapine increased the phosphorylation state of extracellular signal-regulated kinases 1 and 2 (ERK1/2) in a pertussis toxin (PTX)-sensitive manner. The $LPA_{1/3}$ antagonist Ki16425 and the selective LPA_1 antagonist AM966 reduced the stimulatory effect on ERK1/2 phosphorylation elicited by either amitriptyline, mianserin or LPA. The LPA receptor antagonists also counteracted the increased phosphorylation of the transcription factor CREB and the prosurvival protein kinase Akt induced by either amitriptyline or mianserin. Similar results were obtained in rat cortical astrocytes, where amitriptyline and mianserin activated ERK1/2 in a manner sensitive to PTX, Ki16425 and AM966. Moreover, treatment of C6 glioma cells with LPA_1 siRNA inhibited ERK1/2 phosphorylation by amitriptyline and mianserin. The data provide evidence that in glial cells tricyclic and tetracyclic AD activate LPA_1 receptor coupled to signaling pathways regulating cell survival and differentiation.

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

Poster

503. Serotonin and GABA Transporters

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 503.01/D39

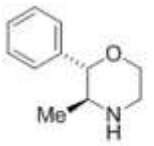
Topic: B.05. Transporters

Support: NIH Grant DA12970

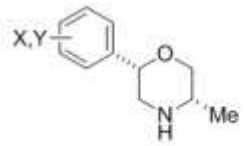
Title: Dopamine and serotonin transporter release activity of phenmetrazine analogs

Authors: *A. M. DECKER[1], A. LANDAVAZO[1], J. S. PARTILLA[2], B. E. BLOUGH[1], M. H. BAUMANN[2], R. B. ROTHMAN[2]
[1]RTI Intl., RTP, NC; [2]Natl. Inst. on Drug Abuse, Baltimore, MD

Abstract: Monoamine releasers represent one class of compounds currently being evaluated as potential agonist medications to treat psychostimulant addictions. However, due to activation of dopamine neurons, these agonist medications are often abused. Previous evidence suggests that serotonin elevations can counteract the stimulant and reinforcing effects of dopamine. Additional research indicates that withdrawal from drugs of abuse is associated with deficits in both dopamine and serotonin. One possible advantage of using dual dopamine/serotonin releasers as agonist medications is their combined ability to provide the necessary stimulant-like properties (dopamine release) while reducing abuse liability (serotonin release). Recently, the monoamine releaser (+)-phenmetrazine produced favorable results in cocaine discrimination and self-administration studies in rhesus monkeys. Phenmetrazine was once approved as an appetite suppressant but was discontinued due to its high abuse liability. Our study set out to evaluate the dopamine and serotonin transporter release activity of a series of phenmetrazine analogs with the goal of identifying compounds that have dual dopamine/serotonin release activity. The analogs were synthesized using either an epoxide opening-cyclization method or a Grignard addition-cyclization method. Dopamine and serotonin transporter release activity was measured in freshly prepared rat brain synaptosomes. Our results show that several analogs are more potent dual dopamine/serotonin releasers than phenmetrazine, especially in the 5-methyl-2-phenylmorpholine series. We further demonstrate that compounds with the (2*S*,5*S*) configuration in this series produced the most effective dopamine and serotonin release activity. In conclusion, our results indicate that the (2*S*,5*S*)-5-methyl-2-phenylmorpholine series represents a good lead in our efforts to identify dual dopamine/serotonin releasers that could potentially be used as agonist medications for psychostimulant abuse.



(+)-phenmetrazine



(2*S*,5*S*)-5-methyl-2-phenylmorpholines

Disclosures: **A.M. Decker:** None. **A. Landavazo:** None. **J.S. Partilla:** None. **B.E. Blough:** None. **M.H. Baumann:** None. **R.B. Rothman:** None.

Poster

503. Serotonin and GABA Transporters

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 503.02/D40

Topic: B.05. Transporters

Title: Gat1 expression in human brain: alternate transcript validation and expression analysis of novel RNAs in patients with schizophrenia

Authors: ***H. MITCHELL**, M. I. MIGHDOLL, G. URSINI, J. SHIN, A. JAFFE, R. TAO, D. R. WEINBERGER, J. E. KLEINMAN, T. M. HYDE
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Abstract: Abnormal GABA-mediated neurotransmission is a consistent postmortem finding in schizophrenia. In the dorsolateral prefrontal cortex (DLPFC) of subjects with schizophrenia, studies have revealed alterations in GABA signaling, and lower expression of GABA transporter 1 (GAT1) mRNA compared to healthy controls. GAT1 is a widely expressed GABA reuptake transporter in neuronal and glial membranes of the neocortex. Here we examined GAT1 mRNA expression and alternative splicing in the DLPFC. PolyA enriched RNA was extracted from post-mortem human DLPFC grey matter (n=107 controls, n=107 patients with schizophrenia) and then purified and enriched with PCR to create a final cDNA library for high throughput sequencing using the Illumina HiSeq2000. The Illumina Real Time Analysis module was used to perform image analysis and base calling, followed by the BCL Converter to generate FASTQ files containing sequence reads. Pair-end reads of cDNA sequences obtained by the HiSeq2000 were aligned to the human genome reference (UCSC hg19) by splice-read mapper (TopHat v2.0.4), providing known transcripts from Ensembl Build GRCh37.67. Multiple novel RNA species were identified in the GAT1 locus. Specifically, we detected 50 novel splicing events. Although no differences were found in the global expression of the GAT1 gene between controls

and patients with schizophrenia, the expression of a novel exon located between exons 7 and 8 was greater in patients (p=0.04). Two novel exons, that partially overlap known exons, were also over expressed in patients, based on a junction analysis (chr3:11034616-11058805; p=.02468, chr3:11072966-11075082; p=.00096). Finally, we detected novel non- coding RNAs spanning the GAT3 and GAT1 loci. Our RNA sequencing analysis identified multiple novel RNAs in the GAT1 locus, demonstrating the complexity of the transcriptional regulation of this gene. Preliminary analyses also suggest that those novel transcripts predicted to alter the protein coding sequence are over expressed in patients with schizophrenia.

Disclosures: **H. Mitchell:** A. Employment/Salary (full or part-time):; Lieber Institute for Brain Development. **M.I. Mighdoll:** A. Employment/Salary (full or part-time):; Lieber Institute for Brain Development. **G. Ursini:** A. Employment/Salary (full or part-time):; Lieber Institute for Brain Development. **J. Shin:** A. Employment/Salary (full or part-time):; Lieber Institute for Brain Development. **A. Jaffe:** A. Employment/Salary (full or part-time):; Lieber Institute for Brain Development. **R. Tao:** A. Employment/Salary (full or part-time):; Lieber Institute for Brain Development. **D.R. Weinberger:** Other; Lieber Institute for Brain Development. **J.E. Kleinman:** A. Employment/Salary (full or part-time):; Lieber Institute for Brain Development. **T.M. Hyde:** A. Employment/Salary (full or part-time):; Lieber Institute for Brain Development.

Poster

503. Serotonin and GABA Transporters

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 503.03/D41

Topic: B.05. Transporters

Support: NIH Grant SC1GM086344

Title: Revised ion/substrate coupling stoichiometry of GABA transporters

Authors: S. L. WILLFORD, C. M. ANDERSON, S. R. SPENCER, *S. ESKANDARI
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Abstract: Plasma membrane γ-aminobutyric acid (GABA) transporters (GAT) are electrogenic transport proteins that couple the cotranslocation of Na^+, Cl^-, and GABA across the plasma membrane of neurons and glia. GATs regulate the synaptic and extrasynaptic concentrations of GABA in the brain and, thus, they modulate inhibitory GABAergic signaling in the nervous system. A fundamental property of the transporter that determines its ability to concentrate

GABA in neurons and glia and, hence, reduce synaptic and extrasynaptic GABA concentrations, is the ion/substrate coupling stoichiometry. An accurate knowledge of the stoichiometry is essential to understanding the contribution of GATs to GABAergic signaling in health and disease. The currently accepted GAT stoichiometry is 2 Na^+ : 1 Cl^- : 1 GABA, however, this model is inconsistent with several experimental results. In the present study, we expressed GAT1 and GAT3 in *Xenopus laevis* oocytes and utilized a thermodynamic approach, as well as uptake under voltage clamp experiments, in order to determine a definitive stoichiometry for the GABA transporters. Voltage-clamped GAT1-expressing oocytes were preloaded with GABA and then superfused with solutions containing different external concentrations of Na^+, Cl^-, or GABA (while the concentrations of the other two co-substrates remained the same). For any given substrate concentration, the reversal potential (V_{rev}) of transporter-mediated current was recorded. The shifts in V_{rev} for a 10-fold change in the external Na^+, Cl^-, and GABA concentration were 84 ± 4 mV, 30 ± 1 mV, and 29 ± 1 mV, respectively. We then measured Na^+, Cl^-, and GABA fluxes under voltage clamp in GAT3-expressing cells in order to determine the ratio of charge flux to substrate flux. We found that for every Na^+ ion translocated across the plasma membrane by GAT3, 0.7 ± 0.1 elementary charge enters the cell. For every Cl^- ion translocated across the plasma membrane, 2.0 ± 0.2 elementary charges enter the cell. For every GABA molecule translocated across the plasma membrane, 2.1 ± 0.1 elementary charges enter the cell. Altogether, the thermodynamic and flux measurements are inconsistent with the currently-accepted 2 Na^+ : 1 Cl^- : 1 GABA stoichiometry model, but rather strongly suggest a 3 Na^+ : 1 Cl^- : 1 GABA coupling stoichiometry for the GABA transporters. The revised stoichiometry has important implications for the role of the GABA transporters in establishing the resting concentration of GABA in the brain, as well as for the contribution of these proteins to regulating GABAergic synaptic activity.

Disclosures: **S.L. Willford:** None. **C.M. Anderson:** None. **S. Eskandari:** None. **S.R. Spencer:** None.

Poster

503. Serotonin and GABA Transporters

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 503.04/D42

Topic: B.05. Transporters

Support: ANR grant 08-JCJC-0087

Aquitaine-Euskadi grant / convention Neuroscience n°9

Ministère de l'Education Nationale, de la Recherche et de la Technologie

Title: Reduction of GAT-3 expression is responsible of tonic inhibition in globus pallidus neurons in experimental parkinsonism

Authors: *M. CHAZALON[1], C. MIGUELEZ[2], S. MORIN[1], S. CRISTÓVÃO-FERREIRA[3], S. H. VAZ[3], A. M. SEBASTIAO[3], J. BAUFRETON[1]
[1]neuroscience, Inst. Des Maladies Neurodegeneratives, Bordeaux, France; [2]Univ. of the Basque Country, Fac. of Pharmacy, Dept. of Pharmacol., Vitoria-Gasteiz, Spain; [3]Fac. of Medicine, and Unit of Neurosci., Inst. of Pharmacol. and Neurosciences, Inst. of Mol. Med., Lisbon, Portugal

Abstract: The external globus pallidus (GPe, equivalent of GP in rodents) is the first relay of the indirect pathway of the basal ganglia network and receives its main source of inhibition from the striatum. In Parkinson's disease, the striato-pallidal pathway is believe to become hyperactive, leading to an increase of extracellular GABA levels ([GABA]e) in the GP which contribute to the hypoactivity of pallidal neurons observed in animal-models of the disease. The molecular mechanisms involved in the hypoactivity of GP neurons remain however poorly understood, we thus undertaken series of experiments to better characterize the impact of dopamine-deprivation on GABAergic inhibition properties in GP. Using patch-clamp recordings in acute brain slices, we found the presence of a permanent form of inhibition, commonly called tonic inhibition (TI) but only in GP neurons of 6-hydroxydopamine-treated (6-OHDA) rats or mice. Interestingly, TI persisted in dopamine-depleted slices in presence of tetrodotoxin (TTX, 1μM) suggesting that it is independent of neuronal activity and not the consequence of striatal neuron's hyperactivity. Using pharmacology and δ-knock-out mice, we demonstrated that TI is mediated by δ-containing GABAA receptors in GP neurons, but that these receptors are not overexpressed in 6-OHDA animals. We thus investigated the contribution of neuronal and glial GABA transporters, called GAT-1 and GAT-3, respectively. GAT-1 blockade lead to the same change in the magnitude of TI in control and dopamine-depleted conditions suggesting that this transporter is important in the regulation of [GABA]e but is not implicated in the TI observed in 6-OHDA rodents. On the other hand, whereas GAT-3 blockade produces a substantial increase in TI in control rats, it didn't significantly increase TI in dopamine-depleted animals suggesting that glial uptake of GABA is impaired in experimental Parkinsonism. Immunohistochemistry experiments showed a reduction of GAT-3 staining in GP astrocytes in 6-OHDA rats suggesting a down-expression of the transporter. We are currently testing that GAT-3 expression is reduced in astrocytes in 6-OHDA-treated rats using western blot analysis. In addition, we are investigating the impact of tonic inhibition on the excitability and pattern of GP neurons in acute brain slices using extracellular multi-electrode recordings. Taken together these results suggest that the increase of [GABA]e observed in experimental Parkinsonism in GP is caused, at least in part, by a dysfunction of GAT-3 transporters leading to a persistent form of inhibition in pallidal neurons.

Disclosures: **M. Chazalon:** None. **C. Miguelez:** None. **S. Morin:** None. **S. Cristóvão-Ferreira:** None. **S.H. Vaz:** None. **A.M. Sebastiao:** None. **J. Baufreton:** None.

Poster

503. Serotonin and GABA Transporters

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 503.05/D43

Topic: B.05. Transporters

Support: CONACYT México 152326

CONACYT México 169023

Title: CB1 and GPR55 receptors regulate GABA uptake in gliosomes and synaptosomes respectively in the rat globus pallidus

Authors: ***M. MUNOZ ARENAS**[1], R. SANCHEZ-ZAVALETA[2], A. BAEZ-CORDERO[3], F. PAZ-BERMUDEZ[2], D. LIMON[1], B. FLORAN[2]
[1]Lab. De Neurofarmacologia, Puebla, Mexico; [2]Physiology, Biophysics and Neurosciences, CINVESTAV-IPN, México. D.F., Mexico; [3]Lab. de Neurofarmacología, BUAP, Puebla, Mexico

Abstract: Globus pallidus express GABA transporters: GAT-1 and GAT-3 in neural terminals and glial cells respectively. GABA concentration in the synaptic cleft is regulated by these transporters and regulates motor behavior. In addition, it has been shown that GABA uptake is modulated by the different compounds acting on cannabinoid receptors. Therefore, the aim of this work was to study the role of CB1 and GPR55 receptors on the activity of GAT-1 and GAT-3 in the globus pallidus of the rat. [^{3}H]GABA uptake was measured after activating the CB1 and GPR55 receptors in synaptosomes (nerve terminals) and gliosomes (enriched astrocytes membrane somas) from globus pallidus of normal rats. We found that CB1 receptor activation inhibits [^{3}H]GABA uptake mediated by GAT-3 in gliosomes, while compounds with selectivity of GPR55 inhibit [^{3}H]GABA uptake mediated by GAT-1 in synaptosomes. We propose that cannabinoid compounds blocks GABA uptake at GAT-1 and GAT-3 in the GP by means of the different location of transporters in cellular elements. This study also shows the importance of the endocannabinoid system in the control of the GABAergic transmission of the globus pallidus.

Disclosures: **M. Munoz Arenas:** None. **R. Sanchez-Zavaleta:** None. **F. Paz-Bermudez:** None. **D. Limon:** None. **B. Floran:** None. **A. Baez-Cordero:** None.

Poster

503. Serotonin and GABA Transporters

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 503.06/D44

Topic: B.05. Transporters

Support: National Institute on Aging Intramural Research Program

NIH Grant NS050275

Dr. Miriam and Sheldon G. Adelson Medical Research Foundation

Michael S. and Karen G. Ansari ALS Center for Cell Therapy and Regeneration Research

Title: The astrocytic transporter Slc7a10 (Asc-1) is required for glycinergic inhibitory function

Authors: *J. T. EHMSEN[1], Y. LIU[2], Y. WANG[2], J. D. ROTHSTEIN[1], S. H. SNYDER[1], M. P. MATTSON[1], A. HOKE[1]
[1]Johns Hopkins, BALTIMORE, MD; [2]Natl. Inst. of Aging, Baltimore, MD

Abstract: Slc7a10 (Asc-1) is a sodium-independent neutral amino acid transporter known to be the primary mediator of D-serine transport in the brain. Slc7a10 transports a number of additional amino acids including glycine, L-alanine, L-serine, and L-cysteine, as well as their D-enantiomers. We find that Slc7a10 is enriched in cerebellar Bergmann glia and within a subset of astrocytes of the caudal brain and spinal cord, in a distribution corresponding to high densities of glycinergic inhibitory synapses. Accordingly, we find that spinal cord glycine levels are significantly reduced in Slc7a10-null mice and that spontaneous glycinergic postsynaptic currents in motor neurons of mice lacking Slc7a10 show substantially diminished amplitude, identifying the likely etiology of sustained myoclonus and early postnatal lethality previously described for these animals. These observations establish a critical role for astrocytic Slc7a10 in glycinergic inhibitory function in the central nervous system, and implicate SLC7A10 as a candidate gene in human hyperekplexia and stiff person syndrome.

Disclosures: J.T. Ehmsen: None. **Y. Liu:** None. **Y. Wang:** None. **J.D. Rothstein:** None. **S.H. Snyder:** None. **M.P. Mattson:** None. **A. Hoke:** None.

Poster

503. Serotonin and GABA Transporters

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 503.07/D45

Topic: B.05. Transporters

Support: NIH Grant DA022378

NDEPSCoR AURA Award

Title: Outer-gate residues determine species-specific differences in recognition of MDMA as a substrate by the serotonin transporter

Authors: *B. FELTS[1], E. L. BARKER[2], L. K. HENRY[1]
[1]Univ. of North Dakota Sch. of Med. & He, Grand Forks, ND; [2]Dept. of Medicinal Chem. and Mol. Pharmacol., Purdue Univ., Lafayette, IN

Abstract: The Na^+/Cl^-–dependent serotonin transporter (SERT) functions to reuptake serotonin (5-HT) into pre-synaptic terminals following neurotransmission. In addition, SERT is also the target of the drug of abuse 3,4-methylenedioxymethamphetamine (MDMA, ecstasy). MDMA is recognized as a substrate by human SERT (hSERT) and transported into presynaptic neurons, where it mediates robust reverse transport (efflux) of 5-HT through SERT and into the synapse. Previously published work has demonstrated that, unlike hSERT, MDMA is not recognized as a substrate by *Drosophila* SERT (dSERT), as MDMA is unable to elicit substrate-induced currents or produce measurable substrate efflux. However, MDMA is able to bind to dSERT and antagonize 5-HT uptake, as revealed through competitive uptake analysis, albeit with an eight-fold drop in potency when compared to hSERT. Importantly, hSERT and dSERT display similar K_M values for 5-HT transport, suggesting that differences in substrate recognition are not universal for all SERT substrates. As substrate recognition occurs at the molecular level, we focused on amino acid differences between hSERT and dSERT that reside in the proposed permeation pathway. Interestingly, there is divergence in the residues that form the outer gate structure, which in hSERT is composed of R104 and E493 and likely form a salt-bridge. In dSERT, the Arg is conserved (R99) but the charged Glu is replaced by a polar Asn (N484). We found that a reciprocal switch of this single differential amino acid in hSERT and dSERT resulted in both a gain of function for dSERT, where dSERT N484E was able to recognize and transport MDMA, and a loss of function for hSERT, where hSERT E493N can no longer transport MDMA. These reciprocal mutants retained 5-HT transport competency. Our findings suggest a negative charge opposite of the Arg at the outer gate may be necessary for recognition

of MDMA as a substrate. To further characterize the role of the outer-gate residues in MDMA recognition we investigated SERT from *C. elegans* (ceSERT), as its outer gate is composed of the universally conserved Arg (R125) and an Asp (D517). Like in hSERT, this forms a true salt bridge at the extracellular gate, however the variation in acidic residues between hSERT and ceSERT has not been examined for its effect on MDMA recognition. Our aim is to utilize the functional similarities and differences between hSERT, dSERT and ceSERT to unravel the role of the salt bridge in MDMA recognition as a substrate.

Disclosures: B. Felts: None. **E.L. Barker:** None. **L.K. Henry:** None.

Poster

503. Serotonin and GABA Transporters

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 503.08/D46

Topic: B.05. Transporters

Support: NIH Grant MH090256

Title: Dissecting the role of integrins in the modulation of the serotonin synapse

Authors: *M. MAZALOUSKAS, A. M. CARNEIRO
Pharmacol., Vanderbilt Univ., Nashville, TN

Abstract: Recent evidence from our laboratory has indicated that obligate integrin heterodimers containing the β3-subunit (ITGB3) influence the serotonin system in platelets. Whereas fibrinogen, an activator of αIIbβ3 (also known as glycoprotein GPIIb/IIIa), enhances the activity of the antidepressant-sensitive serotonin (5-hydroxytryptamine, 5-HT) transporters (SERTs, SLC6A4) in platelets, the genetic disruption of integrin β3 in mice diminishes platelet SERT activity. Thus the SERT•β3 complex appears to serve as an important regulatory element in 5-HT signaling. Recent studies from our group and others have begun to elucidate a functional interaction between SERT and β3 integrin in the central nervous system, where integrin signaling contributes to neuronal migration and synaptic differentiation during development, as well as aids in the synaptic plasticity of mature neurons. Whereas chronic administration of the selective serotonin reuptake inhibitor (SSRI) paroxetine has been shown to enhance the levels of integrin β3 mRNA in cultured cells, the SSRI fluoxetine has been shown to induce increases in synaptogenesis in rat hippocampal CA1 and CA3 subfields. Given that SSRIs may indirectly influence 5-HT1A receptors to increase integrin expression and the formation of new synapses,

we examined the role of SERT•β3 complexes in serotonergic synapses in the raphe nuclei of the midbrain, the major source of 5-HT neurons in the brain. Our immunohistochemical analyses of the raphe nuclei indicate that integrin β3 is expressed in approximately 1/3 of serotonergic synapses. We find that the absence of integrin β3 in the midbrain of β3 null mice (Itgb3-/-) results in a decrease in the number of serotonergic synapses without affecting the total number of neurons, thus implicating integrin β3 in serotonergic synaptogenesis. Utilizing mouse lines expressing a gain-of-function mutant form of integrin β3, we will examine whether newly formed synapses express integrin β3 and assess the activation state and downstream signaling of integrin β3 using biochemical approaches. Proteomic approaches will be utilized to further identify key determinants of serotonergic synaptogenesis on fluorescence-activated cell sorted serotonergic synaptosomes containing integrin β3. Elucidating the role of integrins in governing serotonergic synaptogenesis may provide insight into the molecular mechanisms that underlie neuropsychiatric disorders and reveal novel, druggable targets for the treatment of several psychiatric illnesses.

Disclosures: M. Mazalouskas: None. **A.M. Carneiro:** None.

Poster

503. Serotonin and GABA Transporters

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 503.09/D47

Topic: B.05. Transporters

Support: NIMH Grant 090256-01A1

Title: Regulation of serotonin transporters by focal adhesion proteins

Authors: *M. DOHN, A. CARNEIRO
Pharmacol., Vanderbilt Univ., Nashville, TN

Abstract: The monoamine serotonin is a critical mediator of a broad range of physiological and behavioral functions. Serotonin plays roles in platelet aggregation, cardiac function, and digestion, whereas dysfunction of serotonin neurotransmission has been implicated in depression, anxiety, and autism spectrum disorders. While serotonin signaling is transduced by a large family of receptors, extracellular clearance, and thus inhibition, of serotonin occurs primarily via one protein, the serotonin transporter (SERT). SERT has been shown to interact both genetically and physically with components of focal adhesion (FA) complexes. FAs are

large multiprotein complexes that connect the cytoskeleton to the extracellular matrix via transmembrane integrin proteins. Interactions between synaptosomal and FA proteins are thought to modulate synaptogenesis and synaptic function. In platelets, FA formation modulates SERT function via the association of adaptor proteins with the carboxy-terminus of SERT, followed by inactivation of the transporter and translocation to endocytosis-ready membranes. The FA adaptor protein Hic-5 is a key modulatory protein, and Hic-5/SERT associations depend on the activation of intracellular signaling pathways. While functional interactions of SERTs with FAs have been established in platelets, little is known on the role of these proteins in neurons. Here we dissect the effect of these interactions on SERT function. Using serotonin uptake assays in HEK293 cells transduced to express SERT and Hic-5, we demonstrate that Hic-5 suppresses SERT transporter activity. Deletion and point mutants are also utilized to uncouple protein-protein interactions and map potential interaction domains. We will then extend those studies to primary neuronal cultures to decipher how the association of SERT with FA complexes modulates SERT trafficking to axons, plasma membrane insertion, and uptake capacity. From these studies we aim to provide a greater understanding of SERT regulation by FA proteins.

Disclosures: M. Dohn: None. **A. Carneiro:** None.

Poster

503. Serotonin and GABA Transporters

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 503.10/D48

Topic: B.05. Transporters

Title: Cellular and ultrastructural localization of organic cation transporter 3 suggest a dendritic and glial monoamine clearance mechanism

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Abstract: Transporter-mediated uptake determines the duration and extent of diffusion of released monoamines. Most studies of monoamine clearance have focused on the presynaptic reuptake transporters SERT, NET and DAT. We have recently demonstrated the expression of an additional, largely uncharacterized, high-capacity monoamine clearance mechanism, OCT3 (organic cation transporter 3), throughout the rat brain. In contrast to DAT, NET and SERT,

OCT3 has higher capacity and lower affinity for substrates, is sodium-independent and multi-specific, with the capacity to transport dopamine, norepinephrine, and serotonin. OCT3 is insensitive to inhibition by cocaine and antidepressants, but is inhibited by corticosterone. Thus, OCT3 represents a novel, glucocorticoid-sensitive monoamine clearance mechanism. However, little is known about the subcellular localization of the transporter and its proximity to sites of monoamine release. This information is critical for the integration of OCT3 into models of the regulation of monoaminergic neurotransmission. In this study, we used immunochemical techniques at both the light and electron microscopic level to examine the distribution and phenotype of OCT3-expressing cells, and the subcellular localization of OCT3 in the rodent amygdala and retrosplenial cortex (RSC). OCT3-immunoreactive perikarya and punctae were observed throughout the BLA and RSC, with particularly high densities in the intercalated cell groups of the amygdala and the granular RSC. In both areas, dense perinuclear staining was observed. Dual immunofluorescence revealed OCT3 expression in close proximity to, but not on fibers expressing either tyrosine-hydroxylase or dopamine-beta-hydroxylase. In the BLA, OCT3 immunoreactive punctae were observed in close proximity to D1 receptor + punctae. OCT3-was observed in neurons and glia in both areas. Immuno-electron microscopy revealed similar patterns of OCT3-like immunoreactivity in BLA and RSC. OCT3 immunostaining was associated with plasma membranes of dendritic spines adjacent to putative monoamine release sites. In several dendrites, dense OCT3 immunostaining was associated with mitochondria adjacent to the plasma membrane. In addition to dendritic staining, OCT3 immunostaining was observed in glial processes, and in a small number of presynaptic terminals. In both RSC and BLA, dense OCT3-immunostaining was associated with the nuclear envelope. These data suggest that represents a post- or peri-synaptic clearance mechanism, and raise interesting questions regarding potential roles of this transporter in the intracellular disposition and metabolism of substrates.

Disclosures: **P.J. Gasser:** None. **J. Chan:** None. **M. Hurley:** None. **V.M. Pickel:** None. **J.E. Hill:** None.

Poster

503. Serotonin and GABA Transporters

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Topic: B.05. Transporters

Support: NIH Grant R01-MH064489-S1 (LCD)

NIH Grant R01-MH093320 (LCD, WK)

NARSAD Independent Investigator Award (LCD)

Title: Mechanisms contributing to lack of antidepressant efficacy in juveniles and adolescents

Authors: *N. MITCHELL[1], R. FRASER[2], W. OWENS[1], R. HORTON[1], M. VITELA[1], G. GOULD[1], W. KOEK[3], L. DAWS[1]
[1]Physiol., [2]Pharmacol., [3]Psychiatry, 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 (5-HT) 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; however, there was no difference in expression of hippocampal 5-HT transporter (SERT), the target protein of SSRIs, to account for the reduced SSRI efficacy. The increase in extracellular 5-HT following SSRI administration is thought to trigger downstream events required for therapeutic effects. Thus, our data raise the possibility that transporters capable of 5-HT uptake other than SERT may be present in disproportionately higher levels during juvenile and adolescent periods thereby preventing extracellular 5-HT from climbing to therapeutically relevant levels following SSRI treatment. Decynium-22 (D22) is a blocker of organic cation transporters (OCTs) and the plasma membrane monoamine transporter (PMAT), low affinity, but high capacity transporters for 5-HT. We found that in juvenile and adolescent mice, the density of [3H]D22 binding sites in hippocampus are greater than in adults. Western blot analysis using specific antibodies revealed that increased [3H] D22 binding was most likely driven by increased PMAT expression in young mice relative to adults. These data suggest that D22 may have antidepressant activity in juvenile and adolescent mice. In our preliminary studies we found that D22 (0.01mg/kg) produced antidepressant-like effects in juvenile but not adult mice. Using *in vivo* chronoamperometry, an electrochemical technique which allows for sub-second measurements of region specific 5-HT clearance in brain, studies are underway to determine whether the antidepressant-like effects of D22 are related to its ability to inhibit 5-HT clearance. Our results suggest that significant uptake of 5-HT by PMAT and/or OCTs may limit the therapeutic efficacy of SSRIs, providing a mechanistic basis for poor treatment response to SSRIs particularly in juveniles and adolescents.

Disclosures: **N. Mitchell:** None. **R. Fraser:** None. **W. Owens:** None. **R. Horton:** None. **M. Vitela:** None. **G. Gould:** None. **W. Koek:** None. **L. Daws:** None.

Poster

503. Serotonin and GABA Transporters

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Topic: B.05. Transporters

Support: MH078028 and MH094527 (RDB),

NS007491 (MJR)

NARSAD YI Award and Vanderbilt Conte Center Pilot Grant (NLB)

Title: Contributions of interleukin-1β signaling to the enduring effects of early-life stress: A serotonin connection?

Authors: *N. L. BAGANZ[1,2], J. T. SMITH[1], L. J. HARBOM[1], M. J. ROBSON[1,2], R. D. BLAKELY[1,2,3]
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Abstract: Early-life stress during childhood, if sustained, can have an enduring impact on the quality of life and social interactions, and lead to lifelong risk for cognitive disability and mood disorders, such as major depression. A growing awareness of the role of neuroimmune signaling in the adaptive and maladaptive responses to stress-mediated behaviors encouraged us to explore the contribution of inflammatory signaling in the risk for depressive symptoms in a mouse maternal separation (MS) model. In our MS model, we imposed a daily 3 hr separation of mouse pups from their mother over a 2-week period. As others have demonstrated, we detected long-lasting consequences on behavior, including heightened anxiety- and depressive-like behavior of adult animals subjected to MS. Additionally, we found that MS lead to adult elevations in the expression of multiple inflammatory signaling molecules (e.g. IL-1β and IL-6) in both the brain and periphery. Remarkably, when animals deprived of IL-1β signaling via a constitutive IL-1 receptor KO (IL-1R KO) were assessed for enduring behavioral effects of MS, these animals were found to lack the anxiety and depressive symptoms of their wild type, MS treated littermates. Moreover, the MS-induced elevations in inflammatory cytokine levels were absent. As IL-1β signaling can modulate serotonin (5-HT) transporter (SERT) activity (Zhu et al, 2006), effects that are also lost in the IL-1R KO (Zhu et al, 2010), and SSRI treatments can reverse MS-induced changes in behavior (El Khoury et al, 2006; Levine et al, 2012; Couto et al, 2012; Yoo et al, 2013) we hypothesize that one determinant of MS-induced behavioral changes may involve IL-1β alterations in SERT expression and/or function. Finally, using conditional IL-1R KO mice,

we are investigating whether early and/or later-life IL-1β signaling is required for MS-induced behavioral changes and to what degree these changes depend on IL-1β modulation of 5-HT signaling.

Disclosures: **N.L. Baganz:** None. **J.T. Smith:** None. **L.J. Harbom:** None. **M.J. Robson:** None. **R.D. Blakely:** None.

Poster

504. Dopamine Transporters

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Program#/Poster#: 504.01/D51

Topic: B.05. Transporters

Support: Intramural Research Program NIMH

Title: Molecular basis for interactions of the dopamine transporter with G protein βγ subunits

Authors: ***J. GARCIA-OLIVARES**[1], D. TORRES-SALAZAR[1], S. G. AMARA[1], G. E. TORRES[2]
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Abstract: Proper function of reward circuitry within 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. We recently reported a novel mechanism describing the regulation of DAT by heterotrimeric G-proteins. We found that Gβγ subunits bind directly to the C-terminus of DAT (residues 582-620), and upon G-protein activation, the release of Gβγ results in a decrease in DA uptake. To explore the molecular basis of this interaction, we generated a peptide library containing a series of sequential alanine and/or glycine substitutions from residues 582 to 620 of the sequence of the human DAT. Using an *in vitro* binding assay with this peptide library, we identified the putative binding site in the region 582 to 595. We measured DA uptake and efflux in HEK-293 cells expressing mutated DAT to corroborate the functional impact of the elimination of the putative Gβγ subunit binding site. The fusion of the potential binding site with a TAT-peptide to generate a cell permeant peptide, blocked the effect of the Gβγ activator mSIRK. We also explore how other known regulators of DAT, such as PKC and CamKII, play a role in the Gβγ binding and functional regulation. Taken together, our results

suggest that Gβγ subunits directly regulate DA transport through DAT. This novel mechanism could have important implications for the actions of amphetamine and other psychostimulants as well as for the regulation of the dopaminergic tone within the brain.

Disclosures: J. Garcia-Olivares: None. **D. Torres-Salazar:** None. **S.G. Amara:** None. **G.E. Torres:** None.

Poster

504. Dopamine Transporters

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Topic: B.05. Transporters

Support: MBRS/RISE funding 2 R25 GM059994-13

NINDS NS071122

NIDA DA026947

Title: Chronic methamphetamine exposure leads to diminished short-term memory

Authors: *A. NORTH[1], S. GOODWIN[2], H. KHOSHBOUEI[3]
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Abstract: Methamphetamine (METH) is a highly addictive and abused psychostimulant known for its long-lasting euphoric and neurotoxic effects. Its use in humans is often associated with addiction, neurocognitive impairment, and a disruption in brain function. Despite the negative consequences of METH use, it is used in FDA approved drugs that include Ritalin® and weight loss supplements. Recently, we have shown that METH increases the baseline synaptic transmission and diminishes LTP in the hippocampal CA1 region of young mice (Swant et al, 2011). In this study, we tested the hypothesis that a repeated neurotoxic regimen of METH exposure in adolescent mice decreases hippocampal synaptic plasticity and produces a deficit in short-term memory. Contrary to our prediction, after 14 days of METH exposure, we found no change in the hippocampal plasticity or short-term memory. However, we found that following 21 days of drug abstinence, METH-exposed mice displayed a deficit in the spatial memory and a decrease in hippocampal plasticity. Our findings support the hypothesis that the negative cognitive consequences of neurotoxic regiment of METH exposure can persist long after drug

abstinence. Thus, short-term and long-term consequences of methamphetamine exposure need to be studied in order to develop novel therapeutic approaches for methamphetamine use.

Disclosures: **A. North:** None. **S. Goodwin:** None. **H. Khoshbouei:** None.

Poster

504. Dopamine Transporters

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Topic: B.05. Transporters

Support: NIH Grant DA035224

Title: Dopamine transporter endocytic braking requires the non-receptor tyrosine kinase Ack downstream of cdc42 activation

Authors: ***S. WU**[1], H. E. MELIKIAN[2]
[1]Grad. Program in Neurosci., [2]Brudnick Neuropsychiatric Res. Institute, Dept. of Psychiatry, Univ. of Massachusetts Med. Sch., Worcester, MA

Abstract: Dopaminergic neurotransmission is essential for movement, reward and cognition, and dopamine (DA) signaling is implicated in Parkinson's disease, addiction and schizophrenia. The presynaptic Na^+/Cl^--dependent DA transporter (DAT) recaptures released DA, thereby temporally and spatially sculpting DAergic neurotransmission. DAT is potently inhibited by addictive (cocaine, amphetamine) and therapeutic (methylphenidate) psychostimulants. Thus, intrinsic cellular mechanisms modulating DAT availability are likewise predicted to robustly impact DAergic neurotransmission. DAT surface levels are acutely modulated by endocytic trafficking, and both PKC activation and AMPH exposure rapidly decrease DAT surface expression. DAT surface stability is governed by an endocytic braking mechanism that requires both the DAT amino and carboxy termini, but whose mechanistic underpinnings are not defined. We recently reported that 1) Rin GTPase activity is required for PKC-stimulated release of the DAT endocytic brake and 2) DAT endocytic recycling relies on a dynamin/actin-dependent mechanism. Both Rin and the actin cytoskeleton are intimately coupled to the Rho GTPase cdc42, suggesting that cdc42 may play a critical role in DAT endocytic trafficking. In the current study we used small molecule cdc42 inhibitors to test this possibility. Acute cdc42 inhibition with small molecules pirl1 and casin significantly decreased DAT surface expression in SK-N-MC cells and in mouse striatal slices. DAT surface losses were due to a striking and significant

increase in the DAT endocytic rate, suggesting that cdc42 activity is required to maintain the DAT endocytic brake. However, acute cdc42 inhibition did not alter SERT basal internalization rate, indicating a DAT-specific mechanism. Recent studies indicate that the non-receptor tyrosine kinase Ack is directly activated by cdc42 and is a negative regulator of endocytosis, raising the possibility that Ack participates in DAT endocytic braking downstream of cdc42. Studies examining Ack activation via Y284 autophosphorylation revealed that both PKC activation and cdc42 inhibition robustly diminish pY284-Ack levels. To test whether Ack activation impacts DAT activity and trafficking, we took advantage of a highly specific Ack inhibitor, AIM-100. AIM-100 treatment decreased both DA uptake and Y284 Ack autophosphorylation levels in a dose-dependent manner. Moreover, Ack inhibition increased constitutive DAT internalization rates, consistent with release of the DAT endocytic brake. Studies using cdc42 mutants and Ack mutants will elucidate the role of these proteins in regulated DAT endocytic trafficking.

Disclosures: S. Wu: None. **H.E. Melikian:** None.

Poster

504. Dopamine Transporters

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Topic: B.05. Transporters

Support: ERA/MRI Ontario

University of Toronto internal grant

Title: Pharmacological chaperone activity of bupropion on monoamine transporters

Authors: *P. BEEREPOOT, A. SALAHPOUR
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Abstract: Monoamine transporters are essential for recycling monoamine neurotransmitters and regulating extracellular neurotransmitter concentrations. Recently we have discovered that the commonly prescribed dopamine/norepinephrine transporter blocker bupropion can increase dopamine transporter (DAT) protein and function in HEK293 cells. Furthermore, chronic bupropion treatment can increase DAT protein and amphetamine response in mice. By using inhibitors of different steps in the DAT lifecycle, we have determined that the effect of

bupropion is post-translational. We hypothesize that bupropion modulates interaction between DAT and ER-resident chaperone proteins, and are currently exploring this hypothesis by immunoprecipitation and bioluminescence resonance energy transfer approaches. We have also tested bupropion on cells expressing other transporters, and have found that bupropion has similar activity on the serotonin, but not the GABA transporter. Lastly, we are investigating the activity of a number of bupropion analogues to determine structure-activity relationships. So far we have discovered that the primary metabolite of bupropion, hydroxybupropion, also increases DAT surface expression. Our data show that bupropion may have activity that has previously not been appreciated, which could be of significance for the clinical use of this drug. Furthermore, the action of bupropion and related compounds as pharmacological chaperones of monoamine transporters could be exploited therapeutically for pathological conditions involving reduced monoamine transporter function.

Disclosures: P. Beerepoot: None. **A. Salahpour:** None.

Poster

504. Dopamine Transporters

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Topic: B.05. Transporters

Support: RO1-Ey00925622

Title: Dopamine transporter deregulation impacts retinal physiology

Authors: *H. DAI[1], C. JACKSON[1], G. DAVIS[2], R. BLAKELY[3], D. MCMAHON[1]
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Abstract: Retinal dopamine reconfigures retinal circuits for light adaptation and high-resolution vision .Retina-specific disruption of dopamine synthesis leads to deficits in light-adapted electroretinogram (ERG) responses, contrast sensitivity and acuity. In addition to the synthesis pathway, extracellular dopamine levels are regulated by dopamine reuptake, which is mediated by dopamine transporter (DAT). To gain insight into the role of DAT in the retinal dopaminergic system and visual function, here we used a mouse strain carrying a human DAT (SLC6A3 gene) coding variant, Ala559Val (A559V), originally described in human ADHD subjects.This DAT variant exhibits an anomalous outward “leak” of cytoplasmic dopamine when pre-loaded with dopamine. We measured retinal mass electrical responses to light stimuli by electroretinogram

(ERG) in WT, heterozygous, homozygous DAT A559V littermates. Homozygous animals showed increased light-adapted b-wave amplitudes, but dark-adapted responses did not differ, compared to WT and heterozygotes. This elevated amplitude of the photopic ERG could be mimicked by applying D1 and D4 dopamine receptor agonists by i.p. injection to WT mice, suggesting that is due to increased light-adapted retinal dopamine levels in DAT homozygous mutant mice. However, visual acuity and contrast sensitivity, as measured by optokinetic tracking, and tissue dopamine levels in the dark, as measured by HPLC, were maintained at normal levels in the homozygous mice. Taken together these data suggest that alteration of dopamine clearance by DAT can influence light-adapted retinal responses.

Disclosures: H. Dai: None. **C. Jackson:** None. **G. Davis:** None. **R. Blakely:** None. **D. McMahon:** None.

Poster

504. Dopamine Transporters

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Topic: B.05. Transporters

Support: NIH Award MH078028 (RDB)

Title: Anomalous response to psychostimulants in a construct_valid mouse model of adhd dopamine dysfunction

Authors: *G. L. DAVIS[1], M. MERGY[2], R. GOWRISHANKAR[2], P. GRESCH[3], G. STANWOOD[3], M. HAHN[1,3], R. BLAKELY[3,4]
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Abstract: With ADHD being the most common childhood psychiatric disorder, and one that, left untreated, is associated with low socio-economic status, addiction and/or incarceration, a better understanding of ADHD etiology is critical. ADHD has long been associated with disruptions in dopamine (DA) signaling. Moreover, the main pharmacological treatments for ADHD (Ritalin (methylphenidate, MPH)) and Adderall (amphetamine, AMPH)) target the DA transporter (DAT). As current genetic models of ADHD lack construct validity, we screened subjects for the presence of functional DAT coding variation, identifying the variant DAT Val559 in two boys and small kindred, a variant previously identified in a girl with bipolar disorder. *In vitro* studies

that demonstrated anomalous DA efflux (ADE), as well as an anomalous response to AMPH, encouraged us to explore the impact of the variant *in vivo* via the generation of the DAT Val559 knock-in mouse. The DAT Val559 knock-in mouse has normal transporter protein expression and DA uptake *in vivo*, as predicted from *in vitro* studies. Striatal microdialysis studies reveal an elevation in basal extracellular DA levels in DAT Val559 animals and a blunted ability of AMPH to elevate these levels. Although DAT Val559 animals are not spontaneously hyperactive, they exhibit elevated escape speed with imminent handling (darting). Moreover, Val559 mice show a blunted locomotor response to both AMPH and MPH compared to wild-type mice. DAT Val559 animals also display a blunted rearing response to AMPH. Ongoing studies seek to determine what cellular and circuit level plasticities derive from lifelong expression of the DAT Val559 variant and whether other phenotypes, e.g. impulsivity, attention deficits, social behavior disruption, arise in these animals and respond to chronic psychostimulant treatments. Supported by NIH Award MH078028 (RDB)

Disclosures: G.L. Davis: None. **M. Mergy:** None. **R. Gowrishankar:** None. **P. Gresch:** None. **G. Stanwood:** None. **M. Hahn:** None. **R. Blakely:** None.

Poster

504. Dopamine Transporters

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Topic: B.05. Transporters

Support: NIH Award MH095055 (RDB).

Title: Five in a million: Analysis of changes in dopamine transporter function in dat-1 coding variants derived from the *C. elegans* million mutation project

Authors: *P. FREEMAN[1,3], S. M. WHITAKER[1], S. B. ROBINSON[1], R. D. BLAKELY[2]
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Abstract: The dopamine (DA) transporter (DAT) is critical for the termination of extracellular DA signaling as well as presynaptic recapture of DA for re-release. The orthologous *Caenorhabditis elegans* protein (DAT-1) plays an equivalent role at worm DA synapses, presenting an opportunity to gain a better understanding of the transporter's structure, function and regulation by use of the powerful forward and reverse genetic tools available with the worm

model. Recently, The Million Mutation Project (MMP, http://genome.sfu.ca/mmp/) represents a library of ~2,000 mutagenized worm strains where sequencing at a depth of 15X genome coverage reveals the presence of, on average, ~9 new non-synonymous alleles per gene, whose characterization can reveal novel links to protein structure and function. Thirteen such coding variants are present in the MMP. We have initiated a functional characterization of these variants, focusing initially on five lines that bear amino acid changes (D8N, P9L, M20T, P596S, P609S) at highly conserved locations and/or that are associated with regions suspected to confer/support DAT activity or regulation based on mammalian DAT studies. To date, we have confirmed behavior of four of these lines consistent with DAT-1 loss of function, assessed by the presence of Swimming-induced paralysis (Swip). Additionally, we observe differential changes in the ability of amphetamine to include Swip. Following successful backcrossing of these mutants, we will pursue additional worm and mammalian cell studies to expand the opportunity to understand the impact of structural changes in DAT for DAT trafficking, function and contributions to DA-linked behaviors *in vivo*. Supported by NIH award MH095055 (RDB).

Disclosures: P. Freeman: None. **S.M. Whitaker:** None. **S.B. Robinson:** None. **R.D. Blakely:** None.

Poster

504. Dopamine Transporters

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Topic: B.05. Transporters

Support: NIH Grant DA035559

NIH Grant MH093102

NIH Grant MH095044

Title: Discovery of a novel, conserved MAP kinase required for dopamine neuron function in *C. elegans*

Authors: *D. BERMINGHAM[1,2], J. A. HARDAWAY[2], S. WHITAKER[3], S. SNIDER[1], R. BLAKELY[3,4]
[2]Neurosci., [3]Pharmacol., [4]Psychiatry, [1]Vanderbilt Univ., Nashville, TN

Abstract: The dopamine (DA) transporter (DAT) acts across phylogeny to modulate DA signaling and behavior. We performed a forward genetic screen based on a hyperdopaminergic phenotype, “Swimming-induced paralysis” (Swip) that is displayed by animals with loss of *the C. elegans* DAT ortholog, DAT-1. One mutant that we recovered in this effort, *vt32*, was localized by SNP mapping and whole genome sequencing to an uncharacterized gene, here designated *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. Importantly, DA neuron-specific expression of the wild-type *swip-13* gene restores normal swimming behavior in *swip-13* animals, consistent with expression of SWIP-13 protein by DA neurons as key to kinase-modulation of DA signaling. Fluorescently-tagged, functional *swip-13* protein localizes to DA terminals, consistent with a presynaptic contribution to DA signaling. Implementation of an *in vivo*, FRAP-based approach reveals that *swip-13* animals display normal basal rates of vesicle DA release, whereas epistasis studies suggest that *swip-13* and *dat-1* function in the same pathway, possibly via kinase-modulation of DAT-1 trafficking or function. SWIP-13 is highly conserved, likely representing the nematode ortholog of a mammalian atypical MAP kinase, designated ERK7 in mouse), ERK8 in humans. Excitingly, ERK8/DAT co-expression in human neuroblastoma cells increases DA uptake capacity. Ongoing efforts seek to uncover the mechanism(s) by which SWIP-13 and ERK7/8 modulate DA signaling with an eye towards how our findings may provide insights into disorders linked to perturbed DA signaling.

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

Poster

504. Dopamine Transporters

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Program#/Poster#: 504.09/D59

Topic: B.05. Transporters

Support: MH096972

MH090738

Title: Abnormal dopaminergic signaling in mice expressing the ADHD-associated dopamine transporter variant DAT Val559

Authors: *R. GOWRISHANKAR[1], M. A. MERGY[1], S. C. GANTZ[2], P. J. GRESCH[1], G. L. DAVIS[1], J. T. WILLIAMS[2], M. K. HAHN[1], R. D. BLAKELY[1]
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Abstract: Attention-Deficit/Hyperactivity Disorder (ADHD) is a widespread developmental neuropsychiatric disorder that has been associated with perturbations in dopamine (DA) signaling pathways. The presynaptic DA transporter (DAT) is the target for the most common pharmacological therapies for ADHD, amphetamine (AMPH) and methylphenidate (MPH) and is responsible for terminating DA signaling via reuptake of DA into the presynaptic terminal. Previous work in the lab revealed the presence of a heritable, functional DAT coding variant (DAT Val559) in subjects with ADHD and demonstrated that cells expressing DAT Val559 exhibit anomalous basal outward DA efflux (ADE) that can be blocked by AMPH and MPH. Owing to the striking phenotype of DAT Val559 *in vitro*, and the lack of ADHD mouse models with good construct validity, we engineered knock-in mice expressing the DAT Val559 variant. *In vivo* microdialysis studies of DAT Val559 mice revealed a pronounced elevation in basal, extracellular DA levels along with a significantly blunted efflux of DA evoked by locally infused AMPH, supportive of our heterologous expression studies. To probe the neuronal impact of the DAT Val559 variant, we first interrogated the cellular properties of DA neurons in the substantia nigra (SN) and found a prolongation of the decay of evoked, somatodendritic DA D2 autoreceptor (D2AR)-mediated IPSCs (D2-IPSCs), indicative of disruptions in DA homeostasis. We also observed a reduction in the amplitude of D2-IPSCs in AMPH. In striatal slices, we observed a near-complete loss of DAT-dependent AMPH-induced [3H]-DA release as well as a significant reduction in depolarization-evoked vesicular [3H]-DA release. The latter effect appears to derive from constitutive presynaptic D2AR activity, sustained by ADE, as the effects of the D2R agonist quinpirole to reduce [3H]-DA release is lost in slices from DAT Val559 mice, whereas release can be normalized with pre-treatment of slices with the D2R antagonist raclopride. Together, our studies confirm a functional impact of the DAT Val559 variant on DA neuron physiology and on both vesicular and AMPH-mediated DA release, providing potential insights into ADHD etiology and its treatment.

Disclosures: R. Gowrishankar: None. **M.A. Mergy:** None. **S.C. Gantz:** None. **P.J. Gresch:** None. **G.L. Davis:** None. **J.T. Williams:** None. **M.K. Hahn:** None. **R.D. Blakely:** None.

Poster

504. Dopamine Transporters

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Support: NIH Grant DA13975

NSF Fellowship DGE0909667

NIH Grant F31 DA 035535-01

Title: An autism-derived de novo mutation in the dopamine transporter displays anomalous function that can be improved upon exposure to zinc

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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. We have previously reported a functional, structural, and behavioral characterization of this ASD-associated de novo mutation in the hDAT. We demonstrate that the hDAT T356M displays anomalous function, characterized as a reduction in DA uptake (substrate influx), a reduction in the amphetamine(AMPH)-induced DA efflux, and a persistent reverse transport of DA (substrate efflux). 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 *Drosophila* melanogaster, expression of hDAT T356M in DA neurons lacking *Drosophila* DAT leads to hyperlocomotion, a trait associated with DA dysfunction and ASD. Here, we have demonstrated that exposure to micromolar quantities of Zn2+ improves the function of the hDAT T356M in terms of DA uptake. Importantly, Zn2+ also partially restores the ability of AMPH to cause DA efflux in hDAT T356M. Taken together, our findings demonstrate that alterations in DA homeostasis, mediated by aberrant hDAT T356M functions may stem from altered conformational equilibrium of the hDAT protein, possibly supporting risk for ASD and related neuropsychiatric conditions.

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Poster

504. Dopamine Transporters

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Topic: B.05. Transporters

Support: NIH Grant P01 DA12408

Danish Medical Research Council

Lundbeck Foundation

Title: Maybe not a story about the dopamine transporter: A novel role of PICK1 in regulating dopamine D1 receptor signaling in response to cocaine?

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Abstract: Psychostimulants such as cocaine and amphetamine exert their action through their high affinity interaction with the presynaptic dopamine transporter (DAT), thereby increasing extracellular levels of dopamine. DAT belongs to the family of neurotransmitter:sodium symporters and plays a key role in maintaining dopamine homeostasis by mediating reuptake of dopamine from the synaptic cleft. DAT contains a C-terminal PDZ-domain binding sequence that can bind the PDZ domain of the scaffolding protein PICK1, as well as conceivably other yet unidentified PDZ-domain proteins. Our recent analyses of DAT knock-in mouse strains expressing DAT mutants incapable of interacting with PDZ domain proteins supported an essential role of PDZ domain interactions for distribution of DAT to striatal structures. However, a detailed analysis of PICK1 knock-out (KO) mice provided no evidence for a role of PICK1 in DAT regulation except from around 20% reduction in synaptosomal dopamine uptake.

Compared to WT mice, we found no change in DAT expression or distribution according to immunohistochemistry and western blotting experiments. In addition, surface biotinylation experiments showed no change in surface expression of DAT, amperometry experiments in striatal slices showed no change in amphetamine-induced dopamine efflux and sucrose density gradients centrifugation of striatal extracts showed no evidence for altered membrane raft association. Nonetheless, PICK1-KO mice were characterized by markedly reduced locomotor response to acute cocaine and amphetamine stimulation. Moreover, we observed impairment of behavioral sensitization as well as decreased self-administration of cocaine. Interestingly, stimulation with a selective dopamine D1 receptor agonists suggested that the reduced response to psychostimulants is caused by impaired postsynaptic dopamine D1 receptor signaling. A role of PICK1 in postsynaptic D1 receptor signaling was further supported by altered phosphorylation of DA- and cAMP regulated phosphoprotein (DARPP-32) in PICK1 KO mice. Summarized, our data suggest a hitherto unknown role of PICK1 in regulating downstream signaling in dopamine D1 receptor positive neurons.

Disclosures: **M. Rickhag:** None. **M. Rathje:** None. **G. Sørensen:** None. **A.H.R. Thomsen:** None. **D. Denkcer:** None. **I. Ammendrup-Johnsen:** None. **K. Erreger:** None. **A. Galli:** None. **A. Fink-Jensen:** None. **G. Wörthwein:** None. **K.L. Madsen:** None. **U. Gether:** None.

Poster

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Topic: B.05. Transporters

Support: NIH Grant DA13975

Title: A novel dopamine transporter (DAT) variant (ΔN336) associated with autism ablates dopamine transport function

Authors: ***N. CAMPBELL**, A. N. BELOVICH, P. J. HAMILTON, A. SHEKAR, K. ERREGER, A. GALLI, J. S. SUTCLIFFE
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Abstract: Autism spectrum disorder (ASD) is a neuropsychiatric condition affecting approximately 1% of the population and is characterized by a spectrum of impairments in social interactions and communication, and patterns of rigid-compulsive behaviors. ASD risk is

predominantly determined by genetic factors, however, this etiological architecture is highly complex. Rare, functional genetic variation (both de novo and inherited) is widely accepted as a significant contributor to susceptibility, and that multiple alleles within a particular gene can harbor coding or structural variants predisposing an individual to disease risk. Our recent discoveries on the functional consequences of a de novo missense mutation (T356M) within the dopamine (DA) transporter (DAT) gene (SLC6A3) in an ASD proband have strongly implicated disruption of DA transport as a potential biological risk factor in ASD. Here we report a novel, SLC6A3 variant, also identified in an ASD proband from whole exome sequencing (WES). The genetic variant is an in-frame deletion of three nucelotides resulting in a deletion of amino acid N336. Located in the third intracellular loop, N336 is conserved from human to *Drosophila*, and in silico algorithms predict a functionally damaging effect. Expression of ΔN336 DAT in Chinese hamster ovary (CHO) cells revealed a near absence of DAT-dependent DA uptake relative to wildtype DAT, yet surface expression was not affected. These results, when taken together with prior association of abnormal DAT function with attention deficit-hyperactivity disorder (ADHD), may inform the link between ASD and ADHD, which co-occurs in ~40% of people with ASD. Most importantly, this work adds to the growing body of literature implicating altered regulation of DA homeostasis/transport as a potential biological mechanism underlying liability to ASD.

Disclosures: **N. Campbell:** None. **A.N. Belovich:** None. **P.J. Hamilton:** None. **A. Shekar:** None. **K. Erreger:** None. **A. Galli:** None. **J.S. Sutcliffe:** None.

Poster

504. Dopamine Transporters

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Topic: B.05. Transporters

Support: NIH DA13975

Title: SLC6A3 coding variant Ala559Val found in two autism probands alters dopamine transporter function and trafficking

Authors: E. BOWTON[1], C. SAUNDERS[2], I. A. REDDY[1], N. G. CAMPBELL[1], P. J. HAMILTON[1], L. K. HENRY[4], H. COON[5], D. SAKRIKAR[2], J. M. VEENSTRA-VANDERWEELE[3], R. D. BLAKELY[2], J. S. SUTCLIFFE[1], H. J. MATTHIES[1], *K. ERREGER[1], A. GALLI[1]

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Abstract: Emerging evidence associates dysfunction in the dopamine (DA) transporter (DAT) with the pathophysiology of autism spectrum disorder (ASD). The human DAT (hDAT; SLC6A3) rare variant with an Ala to Val substitution at amino acid 559 (hDAT A559V) was previously reported in individuals with bipolar disorder or attention-deficit hyperactivity disorder (ADHD). We have demonstrated that this variant is hyper-phosphorylated at N-terminal serine (Ser) residues and exhibits an anomalous DA efflux (ADE) phenotype. Here, we report the novel identification of hDAT A559V in two unrelated ASD subjects and provide the first mechanistic description of its impaired trafficking phenotype. DAT surface expression is dynamically regulated by DAT substrates including the psychostimulant amphetamine (AMPH), which causes hDAT trafficking away from the plasma membrane. The integrity of DAT trafficking directly impacts DA transport capacity and therefore dopaminergic neurotransmission. Here, we show that hDAT A559V is resistant to AMPH-induced cell surface redistribution. This unique trafficking phenotype is conferred by altered protein kinase C β (PKCβ) activity. Cells expressing hDAT A559V exhibit constitutively elevated PKCβ activity, inhibition of which restores the AMPH-induced hDAT A559V membrane redistribution. Mechanistically, we link the inability of hDAT A559V to traffic in response to AMPH to the phosphorylation of the five most distal DAT N-terminal Ser. Mutation of these N-terminal Ser to Ala restores AMPH-induced trafficking. Furthermore, hDAT A559V has a diminished ability to transport AMPH, and therefore lacks AMPH-induced DA efflux. Pharmacological inhibition of PKCβ or Ser to Ala substitution in the hDAT A559V background restores AMPH-induced DA efflux. Although hDAT A559V is a rare variant, it has been found in multiple probands with neuropsychiatric disorders associated with imbalances in DA neurotransmission, including ADHD, bipolar disorder, and now ASD. These findings provide valuable insight into a new cellular phenotype (altered hDAT trafficking) supporting dysregulated DA function in these disorders. They also provide a novel potential target (PKCβ) for therapeutic interventions in individuals with ASD.

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Poster

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Topic: B.05. Transporters

Support: NIH/NIDA Grant DA13975

Title: An autism-associated variant at position 346 of the dopamine transporter impairs dopamine uptake while increasing transporter membrane expression

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Abstract: The dopamine (DA) transporter (DAT) acts to recycle released DA back into terminals and thus regulates extracellular DA levels. The DAT is also the primary target of the psychostimulant drugs amphetamine and cocaine. A small number of rare variants in the DAT gene (SLC6A3) have recently been linked to autism spectrum disorder (ASD). Here we report on a novel, SLC6A3 ASD variant identified via high-throughput exome sequencing in an affected individual belonging to the ARRA Autism Sequencing Consortium. This variant results in an alanine to a valine missense substitution at position 346 (A346V). We found that Chinese hamster ovary (CHO) cells expressing human DAT (hDAT) A346V exhibit a significant reduction in the Vmax of [3H]DA uptake but unaltered Km with respect to hDAT expressing cells. Somewhat surprisingly, this reduction in uptake was associated with a 44.3% increase in hDAT A346V membrane surface expression ($p<0.05$). Given that A346 is located within a conserved region of transmembrane domain 7 thought to interact with cholesterol, even a conservative amino acid change at this position could be responsible for increasing basal surface expression through altered interactions with the plasma membrane. Altered surface expression patterns might, in turn, disrupt DAT function and responsiveness to psychostimulants. Continuing work to identify the mechanisms behind such dysfunction will allow us to create new molecular models of disease and therapeutics for ASD.

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Poster

504. Dopamine Transporters

Location: Halls A-C

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Topic: B.05. Transporters

Support: NIH Grant DA13975

Title: Dopamine transporter variant associated with autism spectrum disorder displays impaired interaction with plasma membrane phospholipids

Authors: *A. N. BELOVICH[1], N. G. CAMPBELL[2], P. J. HAMILTON[2], A. M. POE[3], K. ERREGER[3], H. J. G. MATTHIES[3], J. S. SUTCLIFFE[4], A. GALLI[3]
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Abstract: Autism spectrum disorder (ASD) represents a group of developmental disorders characterized by deficits in social and communication skills, patterns of rigid-compulsive behaviors, and in many instances, an array of impaired motor functions. ASD is often comorbid with several other neuropsychiatric conditions, including ADHD and schizophrenia. ASD has devastating effects on the affected individuals and their caretakers, and represents a tremendous societal concern. The dopamine (DA) transporter (DAT) is a presynaptic membrane protein that clears extracellular DA through an active reuptake mechanism. Recently, we have demonstrated that a de novo mutation in the human DAT (hDAT) gene (SLC6A3) affects function of the hDAT and is associated with ASD (Thr to Met substitution at site 356: hDAT T356M). This suggests that DA neurotransmission can play an important role in the neural underpinnings of ASD pathology. Here, we show that a private, novel, heritable, ASD-associated genetic variation in hDAT (Arg to Trp substitution at site 51: hDAT R51W), displays significant functional deficits in sustaining reverse transport DA, as assessed using amperometry. However, the ability of the hDAT R51W to uptake DA is indistinguishable from wild type hDAT, suggesting this novel variant selectively impacts reverse DA transport. Using co-immunoprecipitation experiments, we demonstrate that the hDAT R51W has a significantly impaired interaction with phosphoinositol (4,5)-bisphosphate (PIP2), which has been previously demonstrated to be a key player in AMPH-induced DA efflux. hDAT R51W may therefore shed light on the importance of the N-terminus of hDAT and its interaction with the plasma membrane for neuropathologies, such as ASD. Taken together with our work on several novel ASD hDAT variants, these results strongly suggest that altered regulation of DA transport is a biological liability for ASD risk. A.N. Belovich and N.G. Campbell contributed equally to this work. J.S. Sutcliffe and A. Galli contributed equally to this work.

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Poster

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Topic: B.05. Transporters

Support: NIH R01-GM081054 (LDJ)

VCU start-up fund (LDJ)

Title: P38 mitogen-activated protein kinase mediated norepinephrine transporter regulation modulates cocaine sensitization and conditioned place preference

Authors: *P. MANNANGATTI[1], K. NARASIMHA NAIDU[2], M. I. DAMAJ[2], S. RAMAMOORTHY[2], L. D. JAYANTHI[2]
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Abstract: The noradrenergic and p38 mitogen activated protein kinase (p38 MAPK) systems have been implicated in behavioral effects of cocaine. The psychostimulant drug cocaine targets the presynaptic norepinephrine transporter (NET), and in part contributes to cocaine-mediated behaviors. Previously, we demonstrated a role for p38 MAPK mediated NET-T30 phosphorylation in cocaine upregulation of NET (Mannangatti et al, 2011 JBC). The present study explored the functional interaction between p38 MAPK mediated T30-phosphorylation dependent NET regulation and cocaine-induced behaviors. *In vitro* cocaine treatment of mouse prefrontal cortex (PFC) synaptosomes upregulated NET function, surface expression and phosphorylation. These cocaine-mediated effects are sensitive to both p38 MAPK inhibition and manipulation of NET-T30 phosphorylation suggesting the involvement of p38 MAPK mediated NET phosphorylation in cocaine upregulation of mouse NET. *In vivo* administration of p38 MAPK inhibitor SB203580 completely blocked cocaine-induced NET upregulation and p38 MAPK activation in the mouse PFC. Furthermore, *in vivo* administration of TAT-NET-T30 WT peptide but not the T30A mutant peptide completely abolished cocaine-induced NET upregulation. When tested for cocaine-induced locomotor sensitization and conditioned place preference (CPP), mice receiving SB203580 exhibited reduced cocaine-mediated locomotor sensitization and CPP compared to those receiving the vehicle. Similarly, mice receiving TAT-NET-T30 WT peptide but not the T30A mutant peptide exhibited blunted locomotor sensitization and CPP in response to cocaine. These findings indicate that selective interference of cocaine regulation of NET by either p38 MAPK inhibition or TAT-NET-T30 peptide strategy

attenuates cocaine locomotor sensitization and CPP revealing an important role for p38 MAPK mediated NET regulation in cocaine-elicited behaviors.

Disclosures: P. Mannangatti: None. **K. Narasimha Naidu:** None. **M.I. Damaj:** None. **S. Ramamoorthy:** None. **L.D. Jayanthi:** None.

Poster

504. Dopamine Transporters

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

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Topic: B.05. Transporters

Support: NIMH-MH083928

Title: Modulation of serotonin transporter function by kappa opioid receptor ligands

Authors: *S. SUNDARAMURTHY[1], S. RAMAMOORTHY[1], L. JAYANTHI[2]
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Abstract: Kappa opioid receptor (KOR) agonists produce dysphoria and psychotomimesis. While KOR agonists produce pro-depressant like effects, KOR antagonists produce anti-depressant-like effects in rodent models. The cellular mechanisms and downstream effector(s) by which KOR ligands produce these effects are not clear. KOR agonists modulate serotonin (5-HT) transmission in brain regions implicated in the regulation of mood and motivation. Presynaptic serotonin transporter (SERT) activity is critical in the modulation of synaptic 5-HT and hence in mood disorders. Detailing the molecular events of KOR-linked SERT regulation is important in view of the postulated role of this protein in mood disorders. The present study used heterologous expression systems and native tissue preparations to determine the cellular signaling cascades linked to KOR-mediated SERT regulation. KOR agonists U69,593 or U50,488 produced a concentration dependent, KOR antagonist-reversible decrease in SERT function. KOR-mediated functional down-regulation of SERT is sensitive to CamKII and Akt inhibition. The U69,593-evoked decrease in SERT activity is associated with decreased Vmax, increased SERT phosphorylation and cell surface expression. Furthermore, activation of KOR increases SERT-PP2Ac association, while SERT-syntaxin 1A association is decreased. These data demonstrate that KOR activation decreases SERT function. The decrease in function is likely due to alterations in SERT phosphorylation, trafficking and protein-protein interactions.

Interestingly, KOR-agonists exhibit differential influence on amine transporters in that they increase DAT function and decrease SERT function while having no effect on NET function. We hypothesize that the opposing effects of KOR agonists on SERT and DAT function may contribute to the pro-depressant and psychotomimetic effects of these agents.

Disclosures: S. Sundaramurthy: None. **S. ramamoorthy:** None. **L. jayanthi:** None.

Poster

504. Dopamine Transporters

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Topic: B.05. Transporters

Support: Start-up fund from VCU

Title: Role of neurokinin 1 signaling in amphetamine mediated norepinephrine transporter regulation

Authors: *L. D. JAYANTHI, P. MANNANGATTI, S. RAMAMOORTHY
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Abstract: The functional expression of norepinephrine (NE) transporter (NET) is rapidly coupled to the dynamics of NET regulatory mechanisms. Post-translational mechanisms phosphorylation, trafficking, and protein-protein interactions have been the focus of our investigations addressing NET regulation. Our previous work has provided evidence linking Substance-P/Neurokinin-1 receptor (SP-NK1R) mediated NET downregulation to PKC-induced phosphorylation of NET-T258/S259 motif. Very recently, we have demonstrated that raft-mediated protein-protein interactions play an important role in facilitating NK1R mediated NET regulation. Previously, we have also shown that the PKC-resistant T258/S259 motif is required for amphetamine (AMPH) mediated NET downregulation. However, the physiological significance of NK1R mediated NET regulation as well as the involvement of T258/S259 trafficking motif in both NK1R and AMPH mediated NET down regulation are unclear. Interestingly, AMPH is known to facilitate *in vivo* release of SP in the rodent nucleus accumbens. In addition, it has been shown that SP enhances amphetamine induced motor behavior and NK1R antagonists decrease psychostimulant-induced locomotor activity. Given the fact that NK1R modulates NET, and that NET is effective in clearing synaptic NE, it is possible that the interaction of NET and NK1R signaling may contribute to the physiological responses

mediated by AMPH. Here we report that *in vivo* administration of selective NK1R agonist, GR73632 results in reduced NE transport and plasma membrane expression of NET in the rat VST synaptosomes. Administration of NK1R antagonist, aprepitant prior to GR73632 administration prevents these NK1R-mediated effects. We also show that *in vivo* administration of AMPH results in reduced NET function and expression in the rat VST synaptosomes. Immunoprecipitation experiments revealed stable physical complexes containing abundant NET and NK1R proteins in the ventral striatum. Studies are underway examining the role of SP-NK1R signaling in AMPH mediated NET regulation and the significance of such regulation in AMPH-elicited behavioral effects. Results from these studies will provide new insights into the role of neurokinins in AMPH-mediated NET regulation and its contribution to behavior.

Disclosures: L.D. Jayanthi: None. **P. Mannangatti:** None. **S. Ramamoorthy:** None.

Poster

504. Dopamine Transporters

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Program#/Poster#: 504.19/D69

Topic: B.05. Transporters

Support: Virginia Commonwealth University

Title: Akt mediated regulation of the serotonin transporter function, expression and phosphorylation

Authors: *J. RAJAMANICKAM[1], B. ANNAMALAI[3], L. D. JAYANTHI[2], S. RAMAMOORTHY[2]
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Abstract: The serotonin (5-HT) transporter (SERT) controls serotonergic neurotransmission in the brain by rapid clearance of 5-HT from the synaptic cleft into presynaptic neurons. Altered SERT function is associated with psychiatric disorders and drug addiction. SERTs are primary targets for antidepressants and psychostimulants. The molecular basis for the alterations in SERT function in the mental illnesses or in drug abuse is largely unknown. Our previous studies have identified the involvement of several signaling pathways and protein kinases in regulating SERT function and expression. In this study, we investigated on the role of protein kinase B/Akt in regulating SERT function and expression. Treatment of human embryonic kidney (HEK 293)

cells expressing SERT with Akt phosphorylation inhibitor 'Akt inhibitor X' (AktX) significantly reduced 5-HT uptake in a time- and concentration- dependent fashion. AktX treatment reduced the levels of endogenously phosphorylated Akt. Furthermore, RNA interference targeted to Akt reduced total Akt, phospho-Akt levels as well as 5-HT uptake, confirming the involvement of active Akt in SERT regulation. Kinetic assay revealed reduced SERT Vmax and biotinylation experiments revealed reduced cell surface expression of SERT following AktX treatment that correlated with reduced SERT function. AktX treatment also reduced SERT exocytosis and SERT basal phosphorylation suggesting that reduced cell surface expression could arise from reduced plasma membrane delivery of the transporter protein that may be linked to regulation of SERT phosphorylation. These results collectively suggest that constitutively active Akt maintains functional expression of SERT and that any changes in the activation of Akt could alter SERT mediated 5-HT clearance and subsequently serotonergic neurotransmission.

Disclosures: J. Rajamanickam: None. **B. Annamalai:** None. **L.D. Jayanthi:** None. **S. Ramamoorthy:** None.

Poster

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Topic: B.05. Transporters

Support: Intramural Research Program of the NIMH

ARRA-funded NIH grant DA07595

Title: Mechanism of internalization of the dopamine transporter by amphetamine

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Abstract: Amphetamine (AMPH) and its derivatives are useful therapeutic agents, but also pose a danger as a consequence of their addictive properties. Acute AMPH exposure elevates extracellular dopamine by a variety of mechanisms including an increase in the rate of internalization of the plasma membrane dopamine transporter (DAT). In this study, we

investigated the mechanism of AMPH-mediated DAT endocytosis. We report here that AMPH-mediated DAT internalization is clathrin independent but requires dynamin and activation of the small GTPase RhoA. GFP-tagged clathrin light chain does not colocalize with mCherry-tagged DAT after AMPH treatment indicating a clathrin independent process. The dynamin inhibitors, Dynasore and Dynole-34-2, both attenuate AMPH-induced endocytosis of DAT. We also found that AMPH activates both of the small GTPases Rho and Rac and in recombinant expression systems, primary cultures and midbrain slices, activation of Rho and the downstream Rho-kinase (ROCK) triggers endocytosis of DAT. Inhibition of RhoA activity with a dominant negative mutant or introduction of the botulinum neurotoxin C3 prevents AMPH-induced DAT internalization. However, Rac1 inhibition has no significant effect on DAT trafficking. The ROCK inhibitor Y27632 also prevents AMPH-induced DAT internalization further supporting Rho activation in this phenomenon. Intriguingly, we found that AMPH must enter the cell to initiate this cascade, implicating an intracellular target in this event. Intracellular AMPH also increases cAMP leading to the serine phosphorylation of Rho by protein kinase A (PKA) that inactivates Rho and serves as a brake on DAT internalization, thus demonstrating an important interaction between PKA- and RhoA-dependent signaling in mediating the actions of AMPH. In agreement with our observations in cell lines and brain slices, we also found that the activation of D1/D5 receptors that couple to PKA in dopamine neurons within the brain *in vivo* can antagonize the DAT trafficking and behavioral effects of AMPH in mice. These observations reveal a novel intracellular target that mediates the effects of AMPH on Rho and cAMP signaling and suggest new pathways to target in order to better understand the mechanisms of action of AMPH.

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Poster

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Topic: B.05. Transporters

Support: DA021471/DA/NIDA

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NS071122/NS/NINDS

Title: The sigma-1 receptor interacts with the dopamine transporter and regulates its activity

Authors: *D. O. SAMBO[1], M. LIN[1], D. ANGOLI[1], B. RICHARDSON[1], E. CARTIER[1], M. SCHWENDT[1], B. BLOUGH[2], J. KATZ[3], H. KHOSHBOUEI[1]
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Abstract: The primary mechanism for terminating dopaminergic signaling in the brain is reuptake of dopamine (DA) via the dopamine transporter (DAT). This transporter is implicated in a variety of neurological disorders and is one of the main targets for highly addictive psychostimulants cocaine and methamphetamine (METH). METH acts as a substrate for DAT, increasing synaptic DA levels by competing with DA at the transporter as well as inducing efflux of DA into the synapse. Previous studies indicate that METH exposure causes up-regulation of an endoplasmic reticulum chaperone protein called the sigma-1 receptor in DAT-expressing regions of the brain. Upon activation, this protein can translocate to the plasma membrane where it has been shown to modulate the activity of various receptors and channels. We have shown that DAT and the sigma-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/sigma-1 receptor interaction on the activity of the transporter. These results show that the up-regulation of the sigma-1 receptor reduces the METH blockade of DA uptake, decreases METH-induced DA efflux, and inhibits METH-induced firing activity in dopaminergic neurons. These data suggest that up-regulation of the sigma-1 receptor may provide compensatory mechanisms to reduce the effects of METH. Understanding the mechanism of sigma-1 receptor regulation of DAT activity may reveal a novel therapeutic target for the treatment of METH addiction.

Disclosures: D.O. Sambo: None. **M. Lin:** None. **D. Angoli:** None. **B. Richardson:** None. **E. Cartier:** None. **M. Schwendt:** None. **B. Blough:** None. **J. Katz:** None. **H. Khoshbouei:** None.

Poster

504. Dopamine Transporters

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 504.22/D72

Topic: B.05. Transporters

Title: Imaging the cholesterol-dependent dopamine transporter nanodomains in the plasma-membrane

Authors: *T. RAHBEK-CLEMMENSEN, S. ERLENDSSON, J. ERIKSEN, F. VILHARDT, T. NYGAARD JØRGENSEN, U. GETHER
Dept. of Neurosci. and Pharmacol., Univ. of Copenhagen, Copenhagen N, Denmark

Abstract: The dopamine transporter (DAT) is an integral membrane protein that regulates dopamine homeostasis by mediating reuptake of released dopamine. Here we employ super-resolution microscopy techniques to investigate the cellular distribution of DAT at a resolution exceeding that of conventional fluorescence microscopy. Application of stochastic optical reconstruction microscopy (STORM), using an antibody directed against the DAT N-terminus, showed that DAT was not uniformly distributed in the plasma membrane of the neuronal extensions, varicosities and growth cones in cultured dopaminergic neurons but localized to discrete nanoscale domains with diameters of ~100-500 nm. The DAT signal appeared on either side of the extensions, permitting separation of two plasma membrnes sheets <95 nm apart. STORM imaging of catecholaminergic CAD cells transiently expressing DAT revealed a similar nanodomain distribution of the transporter. Moreover, a comparable nanodomain phenotype was seen with photoactivated localization microscopy (PALM) in CAD cells using photoswitchable Dronpa fused to the DAT N-terminus. Cholesterol-depletion with methyl-β-cyclodextrin decreased the number of DAT nanodomains in CAD cells while disrupting DAT clustering only in the varicosities and growth cones of the cultured neurons. The cytoskeleton disrupting agent cytochalasin D, however, showed a global effect on DAT clustering in dopaminergic neurons but only little or no effect in CAD cells. Summarized, we provide the first insight into the cellular distribution of DAT at subdiffracting resolution and obtain evidence for localization of DAT to discrete cholesterol-sensitive and cytoskeleton dependent nanodomains in dopaminergic neurons.

Disclosures: T. Rahbek-Clemmensen: None. **S. Erlendsson:** None. **J. Eriksen:** None. **F. Vilhardt:** None. **T. Nygaard Jørgensen:** None. **U. Gether:** None.

Poster

504. Dopamine Transporters

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 504.23/E1

Topic: B.05. Transporters

Support: NIH R01 DA09397

Title: Phosphorylation of PKC residues on the N-terminal of the dopamine transporter regulates amphetamine-stimulated dopamine efflux

Authors: *Q. WANG, N. BUBULA, P. VEZINA
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Abstract: Previously we showed that inhibiting PKC in the nucleus accumbens attenuates the ability of amphetamine to induce dopamine (DA) overflow in this site (Loweth et al., 2009). A role for the phosphorylation by PKC of the dopamine transporter (DAT) in this effect has been suggested by others showing that truncation of the first 22 amino acids of the N-terminal of the DAT also reduces amphetamine-induced DA efflux. This segment of the DAT N-terminal contains a number of putative PKC related residues including S4, S7, S12, and S13. Here we assessed the contribution of these residues to amphetamine-induced DA efflux by using serine to alanine (S-A) point mutations at each site on the DAT N-terminal. DATs with different S-A point mutations or combinations of S-A point mutations were transfected into HEK-293 cells and these were used to assess amphetamine-induced (10μM) DA efflux with an incubation procedure. WT-DATs were used as controls. In an initial experiment, we confirmed that inhibiting PKC with Go6976 (130nM) significantly reduced amphetamine-induced DA efflux. In follow-up experiments, cells transfected with the S12A, S13A, and S4,7,13A mutants showed a reduction of amphetamine-induced DA efflux similar to that observed with Go6976. The greatest reductions were observed with the S13A, and S4,7,13A mutants. Interestingly, cells transfected with the S7A mutant, identified by some as a PKC-PKA residue showed WT-DAT levels of amphetamine-induced DA efflux. These results suggest that multiple serine residues, either alone (S13) or in combination (S4,7,13), are targets for PKC phosphorylation during amphetamine-induced DA efflux.

Disclosures: Q. Wang: None. **N. Bubula:** None. **P. Vezina:** None.

Poster

504. Dopamine Transporters

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Program#/Poster#: 504.24/E2

Topic: B.05. Transporters

Support: NIH Grant NS071122

NIH Grant DA026947

Title: Characterization of human like dopaminergic neurons containing physiologically and pathologically relevant alpha-synuclein levels

Authors: *B. R. BUTLER, D. ANGOLI, H. KHOSHBOUEI
Univ. Of Florida, Gainesville, FL

Abstract: Dysregulation of dopaminergic signaling along with significant loss of midbrain dopaminergic neurons are pathological characteristics of Parkinson's disease (PD). Alpha-synuclein is a small neuronal protein whose physiological function has yet to be discovered. Alpha-synuclein has been implicated in the sporadic PD as it is a major component of the Lewy bodies, and point mutations of the protein along with locus triplication and duplication of the SNCA gene have shown to result in Familial PD. Here we seek to characterized the influence of alpha-synuclein overexpression in the dopamine neurons obtained from induced pluripotent stem cells (IPSCs) derived from control patients and those with triplication of alpha-synuclein that have been differentiated to dopaminergic neurons. Our preliminary results reveal that increased alpha-synuclein level in the human-like dopamine neurons 1) alters the resting membrane potential, where these neurons rest at a more depolarized state as compared with the neurons containing physiological level of alpha-synuclein. 2) Pathological level of alpha-synuclein influences the excitability of these neurons, 3) decreases the inward sodium current at voltages tested and 4) alters the amplitude of the action potential. These preliminary results suggest that the early stage alterations in the excitability of dopamine neurons mediated by increase alpha-synuclein may describe alpha-synuclein mediated pathologies in the long-term.

Disclosures: B.R. Butler: None. **D. Angoli:** None. **H. Khoshbouei:** None.

Poster

504. Dopamine Transporters

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 504.25/E3

Topic: B.05. Transporters

Title: Indole based structural analogs of modafinil inhibit the dopamine transporter

Authors: *C. EARLES OCHSNER, J. RUSSEL
Chem., St Norbert Col., DE PERE, WI

Abstract: Inhibition of the human dopamine transporter (hDAT) expressed in human embryonic kidney (HEK) by synthesized structural analogs of modafinil (2-[diphenylmethyl) sulfinyl] acetamide) was measured using rotating disk electrode voltammetry (RDEV). Our previous results using this technique show modafinil competitively inhibits the human dopamine

transporter in a manner similar to amphetamines. However, modafinil functionalized with reduced sulfur and an isopropylamide linkage inhibits the dopamine transporter in an uncompetitive manner similar to cocaine. These results lead us to functionalize the reduced sulfur isopropylamide arm with an indole instead of diphenylmethyl. The resulting compound did not inhibit transport, however, use of hydrophobic N-methyl in place of N-H indole inhibits the dopamine transporter in a dose dependent manner and appears to show competitive inhibition.

Disclosures: C. Earles Ochsner: None. **J. Russel:** None.

Poster

504. Dopamine Transporters

Location: Halls A-C

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Program#/Poster#: 504.26/E4

Topic: B.05. Transporters

Support: NIH Grant P01 DA12408

Title: The interaction between the dopamine transporter (DAT) and the SNARE protein syntaxin 1A is regulated by N-terminal phosphorylation of DAT

Authors: *K. L. MADSEN, T. FAURSCHOU ANDREASSEN, U. GETHER
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Abstract: Dopaminergic signaling serves multiple important cognitive functions including motor control and reward prediction. Correct regulation of DA homeostasis through tight temporal and spatial regulation is achieved by the release of stored DA containing vesicles and the subsequent reuptake into the presynaptic neuron by DAT. Improper regulation can lead to disease states such as Parkinson's disease, ADHD and schizophrenia. Moreover DAT is the principal target for multiple drugs of abuse including cocaine and amphetamine, which block the uptake of DA leading to increases the extra-synaptic concentration of DA as well as cellular responses such as trafficking and phosphorylation. The SNARE protein syntaxin 1A (STX1A) is a nervous system-specific; membrane bound protein implicated in the docking of synaptic vesicles to the plasma membrane. In addition to the role in synaptic release of neurotransmitters it has previously been implicated in regulating the function of DAT as co-expression of STX1A with DAT in heterologous cell cultures increases the AMPH induced efflux of DA. Despite the functional connection between STX1A and DAT the interaction site between the two proteins

are currently unresolved. Here we show, using a combination of FRET based assays in HEK293 cells and GST-pulldown that STX1A binds to the N-terminus of DAT and that specific phosphorylation sites on DAT are sufficient to regulate the interaction. Our results compliment previous findings on this interaction and provide a basis for the notion that proteins involved in the regulated exocytosis of neurotransmitters may regulate the reuptake in a dynamic, phophoregulated manner.

Disclosures: K.L. Madsen: None. **T. Faurschou Andreassen:** None. **U. Gether:** None.

Poster

505. LTP: Kinases and Intracellular Signaling

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 505.01/E5

Topic: B.08. Synaptic Plasticity

Support: PAPIIT IN 212013

Title: Role of CaMKII in the persistence of *in vivo* hippocampal mossy fiber synaptic plasticity

Authors: *L. RAMOS-LANGUREN, Y. JUÁREZ, A. RIVERA-OLVERA, M. L. ESCOBAR
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Abstract: Calcium/calmodulin-dependent protein kinase II (CaMKII) has been proposed as a suitable molecular substrate for long-term memory storage due to the capacity of this enzyme to maintain an active autophosporylated state even after the decay of the external stimuli. Recent work has demonstrated that CaMKII is necessary for the maintenance phase of long-term potentiation (LTP) in the CA1 synapses of hippocampal slices. The hippocampal mossy fiber-CA3 pathway (MF-CA3) is considered as a relevant area for acquisition and storage of different learning tasks. MF-LTP has been characterized by a slow initial increase in the EPSP slope that has been related to the independence of NMDA receptors activation. However, the molecular mechanisms that underlie the maintenance of long-lasting enhancement of synaptic transmission at MF pathway remain to be elucidated. Therefore, the aim of the present study was to evaluate the participation of CaMKII on the persistence of MF-LTP. Thus, we administrated acute microinfusions of the noncompetitive inhibitor of CaMKII (Myr-CaMKIINtide) in the hippocampal CA3 area of adult rats during the late-phase of *in vivo* MF-CA3 LTP. Our results show that CaMKII inhibition reverses MF potentiation in a persistent manner. These findings

support the idea that CaMKII is a key molecular substrate for the long-term synaptic plasticity maintenance.

Disclosures: L. Ramos-Languren: None. **Y. Juárez:** None. **A. Rivera-Olvera:** None. **M.L. Escobar:** None.

Poster

505. LTP: Kinases and Intracellular Signaling

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 505.02/E6

Topic: B.08. Synaptic Plasticity

Support: NIH Grant MH067229

Title: Ca2+-permeable AMPA receptors mediate functional compensation of cyclic GMP-dependent protein kinase II knockout in the hippocampus

Authors: *S. KIM[1], R. TITCOMBE[2], L. KHATRI[2], F. HOFMANN[3], E. B. ZIFF[4]
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Abstract: Activity-dependent trafficking of GluA1-containing AMPA receptors (AMPARs) is an important mechanism for synaptic plasticity. Cyclic GMP-dependent protein kinase II (cGKII) is activated by the NMDA receptor (NMDAR)-nitric oxide (NO)-cGMP pathway, and promotes surface expression of GluA1 and LTP (long-term potentiation) through phosphorylation of GluA1 serine 845 [pGluA1(S845)]. While acute cGKII inhibition causes a robust reduction in LTP at CA1 synapses in the hippocampus, initial studies of the cGKII knockout (KO) mouse found no impairment of LTP. Thus, we hypothesize that cGKII KO, unlike acute inhibition, induces compensatory mechanisms that maintain normal hippocampal LTP. Here, we describe a functional compensation present in the cGKII KO based on the downregulation of phosphatase activity. The Ca2+-dependent phosphatase, calcineurin, dephosphorylates pGluA1(S845), which enables GluA1-containing AMPARs to be endocytosed from the plasma membrane during long-term depression. Reduction of calcineurin activity in cultured cortical neurons induces synaptic expression of Ca2+-permeable AMPARs (CPARs) during synaptic scaling. Furthermore, LTP is enhanced when CPARs are present at synapses. Thus, we hypothesized that cGKII KO maintains normal hippocampal LTP via expression of CPARs induced by a cGKII KO-mediated decrease in calcineurin activity. We determined

spontaneous synaptic transmission by measuring mEPSCs in DIV14-17 cultured hippocampal neurons obtained from cGKII KO mice. We found that the mEPSC amplitude was significantly increased in KO neurons, and this increase was inhibited by naspm (1-naphthyl acetyl spermine), a blocker of CPARs, suggesting synaptic CAPR expression in cGKII KO neurons. GluA1 and pGluA1(S845) levels were increased in PSD (post synaptic density) of cGKII KO hippocampus while GluA2 levels were decreased. Furthermore, surface GluA1 was increased but GluA2 was decreased in cultured cGKII KO neurons. Therefore, CPARs were expressed at synapses of cGKII KO hippocampus. We also determined that calcineurin activity was lower in mutant neurons, accounting for the increased pGluA1(S845). Using GCaMP5, a genetically encoded Ca2+ indicator, we confirmed that Ca2+ activity was lower in cGKII KO. Taken together, decreased Ca2+ activity in cGKII KO hippocampal neurons, reduces calcineurin activity, which in turn increased GluA1 phosphorylation and expressed synaptic CPARs, account for normal LTP in the mutant hippocampus. These findings suggest that genetic deletion of cGKII causes functional compensation at a molecular level that increases CPARs at synapses, which maintains normal hippocampal LTP.

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Poster

505. LTP: Kinases and Intracellular Signaling

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 505.03/E7

Topic: B.08. Synaptic Plasticity

Support: Supported by the Research Council of Norway and K.G. Jebsen Foundation

Title: Post-translational regulation of Arc/Arg3.1 by Erk2

Authors: O. NIKOLAIENKO, M. S. ERIKSEN, *C. R. BRAMHAM
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Abstract: The immediate-early gene ARC (aka ARG3.1) is required for long-lasting synaptic plasticity and memory formation. Arc expression is precisely regulated in space and time by activation of extracellular signal-regulated kinase (Erk/MAPK). Here, we found that Erk2 also acts as a post-translational regulator of Arc. Using in silico tools Arc was predicted to bind Erk

family kinases and to contain several potential Erk phosphorylation sites. GST-fused Arc of rat origin was able to pull down endogenous Erk1 and Erk2 from rat hippocampal lysates. Using a cellulose-bound peptide array covering the Arc/Arg3.1 sequence, we mapped the binding site of purified Erk2 on Arc. Activated Erk2 phosphorylated bacterially expressed Arc *in vitro* at different sites, as confirmed by phospho-specific protein staining and subsequent LC-MS/MS analysis. We also raised rabbit polyclonal antibodies that specifically recognize S206-phosphorylated Arc and show that this residue is modified *in vivo* and that treatment with the mitogen-activated protein kinase kinase (MEK) inhibitor U0126 affects Arc S206 phosphorylation. The results identify Arc as an Erk substrate and further suggest a dual role for Erk signaling in regulating Arc expression and function.

Disclosures: O. Nikolaienko: None. **C.R. Bramham:** None. **M.S. Eriksen:** None.

Poster

505. LTP: Kinases and Intracellular Signaling

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Topic: B.08. Synaptic Plasticity

Support: Grants-in-Aid for Specially Promoted Area (No. 2000009 to H.K.) from the Ministry of Education, Culture, Sports, Science, and Technology (MEXT).

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Title: A critical time window for dopamine actions on the structural plasticity of dendritic spines

Authors: *S. YAGISHITA[1], A. HAYASHI-TAKAGI[1], G. C. R. ELLIS-DAVIES[2], H. URAKUBO[3], S. ISHII[3], H. KASAI[1]
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Abstract: Animal behaviors are reinforced by subsequent rewards that follow the behaviors within a narrow time window. The reward is coded by the phasic activity of dopaminergic neurons in the ventral tegmental area (VTA), which densely innervates the medium spiny neurons (MSNs) in the nucleus accumbens of the striatum, a key brain area for reinforcement

learning in mammals. Glutamatergic synapses on dendritic spines of the MSNs also receive sensorimotor inputs that evoke action potentials (APs). These concurrent neuronal activities of synaptic inputs and APs can induce spike-timing-dependent plasticity (STDP), a major Hebbian learning mechanism. Although dopamine may modulate the STDP, the slow kinetics of dopamine-regulated kinase signaling contradicts the ability to detect the narrow time sequence, and its mechanisms remain unknown. Here, we selectively stimulated dopaminergic and glutamatergic inputs on MSNs by optogenetic stimulation of dopaminergic fibres and two-photon uncaging of caged-glutamates paired with APs (STDP) in MSNs in acute slices of mouse brain. We found that dopamine markedly potentiated spine enlargement, but this only occurred within a narrow time window (0.3-2 s) closely following STDP, which is consistent with behavioural conditioning findings. FRET imaging of Ca2+/calmodulin-dependent protein kinase II (CaMKII) and protein-kinase A (PKA) revealed that the sequence detection involved molecular signaling upstream of PKA activation: Sufficient generation of cAMP for PKA activation occurred only when spikes preceded dopamine to prime adenylyl-cyclase (AC1), otherwise cAMP was effectively removed by a potent phosphodiesterase (PDE) activity in thin distal dendrites of MSNs due to a large surface-to-volume ratio. Therefore, PKA was activated only within the specific timing for reinforcement, which then activated CaMKII through the dopamine- and cAMP-regulated phosphoprotein 32 kDa (DARPP-32). Thus, we have clarified the molecular basis of reinforcement plasticity which is induced at the level of single dendritic spines.

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Poster

505. LTP: Kinases and Intracellular Signaling

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Topic: B.08. Synaptic Plasticity

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Title: CaMKIIα is necessary and sufficient for NMDA receptor-mediated long-term potentiation at the hippocampal mossy fiber-to-CA3 pyramidal cell synapse

Authors: *P. A. HAEGER[1,2], T. J. YOUNTS[2], R. LUJAN[3], S. J. TAVALIN[4], J. W. HELL[5], P. E. CASTILLO[2]

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Abstract: NMDA receptors (NMDARs) are heterotetrameric ligand-gated ion channels classically associated with controlling the induction of long-term plasticity of AMPA receptor-mediated synaptic transmission. Beyond this traditional role, our group recently identified a form of long-term potentiation (LTP) of NMDAR-mediated synaptic transmission expressed at the hippocampal mossy fiber (mf)-to-CA3 pyramidal cell (mf-CA3) synapse, termed NMDAR-mfLTP. To date, however, the molecular mechanisms underlying this form of plasticity are incompletely understood. NMDARs are typically composed of two GluN1 and two GluN2 (GluN2A or GluN2B) subunits. NMDAR subunit composition confers distinct functional properties to the channel, and imparts interaction specificity between the NMDAR and effector proteins like the Ca2+-sensitive enzyme Ca2+/calmodulin-dependent protein kinase IIα (CaMKIIα). CaMKIIα is a major constituent of excitatory synapses, interacts with the GluN2B subunit, and is central to the regulation and induction of classical AMPAR-LTP. In this study, we first examined NMDAR subunit expression at mf-CA3 synapses using electron microscopy and selective pharmacology. We then investigated the role of CaMKIIα in NMDAR-mfLTP using *in vitro* electrophysiology in rat and mouse hippocampal slices along with molecular manipulations that interfere with CaMKIIα signaling and the CaMKIIα-GluN2B interaction. We found that both GluN2A and GluN2B subunits are expressed at the mf-CA3 synapse. We also observed that the selective CaMKIIα inhibitor KN-93 blocked NMDAR-mfLTP whereas the inactive analog KN-92 had no effect. Likewise, when CA3 pyramidal neurons were loaded through the patch-pipette with a CaMKIIα inhibitory peptide (CaMKII-Ntide), which operates by a distinct mechanism from that of KN-93, we found that CaMKII-Ntide strongly inhibited NMDAR-mfLTP. Furthermore, we took advantage of a recently developed GluN2B knock-in mouse where the CaMKIIα-GluN2B interaction is impaired by two point mutations: L1298A and R1300Q. NMDAR-mfLTP was severely compromised in GluN2B-KI mice compared with littermate controls. Finally, by loading a constitutively active version of CaMKII(1-290) directly into CA3 pyramidal cells via the patch pipette, we found that CaMKIIα is sufficient to potentiate NMDAR-mediated mf-CA3 synaptic transmission. Together, these findings indicate CaMKIIα is

both necessary and sufficient for long-term plasticity at the mf-CA3 synapse and that the CaMKII-GluN2B interaction is a key step required for NMDAR-mfLTP. Our study supports the notion that CaMKIIα is an activity-dependent coordinator of synaptic NMDAR plasticity.

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Poster

505. LTP: Kinases and Intracellular Signaling

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 505.06/E10

Topic: B.08. Synaptic Plasticity

Title: Intracellular membrane association of *Aplysia* phosphodiesterase long- and short-form via different targeting mechanisms

Authors: Y.-W. JUN[1], J.-A. LEE[3], B.-K. KAANG[4], *D.-J. JANG[2]
[1]Kyungpook Natl. Univ., Sang-Ju, Korea, Republic of; [2]Dept. of Applied Biol., Kyungpook Natl. Univ., Sangju-si/gyeongsangbuk-Do, Korea, Republic of; [3]Hannam Univ., Daejeon, Korea, Republic of; [4]Seoul Natl. Univ., Seoul, Korea, Republic of

Abstract: Phosphodiesterases (PDEs) play key roles in the compartmentalization of cAMP in intracellular signaling processes through specific subcellular targeting. Previously, we have shown that *Aplysia* PDE4 (ApPDE4) long- and short-form, which were localized to distinct subacellular membrane organelles, play key roles in 5-HT-induced synaptic facilitation in *Aplysia* sensory to motor synapses. However, the molecular mechanism of the isoform-specific distinct subcellular membrane targeting was not clear. In this study, we further deciphered the molecular mechanism of the membrane targeting of ApPDE4 long- and short-form. We found that the membrane targeting of the long-form was mediated by hydrophobic interactions mainly via 16 amino acids at the N-terminal, whereas the short-form was targeted solely to the plasma membrane mainly by nonspecific electrostatic interactions between the N-terminal region and negatively charged lipids including PI4P and PI(4,5)P_2 in the plasma membrane. Moreover, oligomerization of the short-form by interaction of a truncated upstream conserved region 1 (UCR1) domain and a UCR2 domain enhanced the plasma membrane targeting of the short-form. These results suggest that ApPDE4 long- and short-form can be distinctly targeted to the intracellular membranes by direct membrane association via hydrophobic and electrostatic interactions, respectively.

Disclosures: **Y. Jun:** None. **J. Lee:** None. **B. Kaang:** None. **D. Jang:** None.

Poster

505. LTP: Kinases and Intracellular Signaling

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Program#/Poster#: 505.07/E11

Topic: B.08. Synaptic Plasticity

Support: NIH Grant R01NS066583

Title: Phosphorylation and degradation of the CREB repressor ATF4 during long-lasting long-term potentiation

Authors: ***K. A. HAYNES**[1], W. XU[2], A. N. HEGDE[1]
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Abstract: Activating transcription factor 4 (ATF4), the murine ortholog for human cAMP-response element binding protein 2 (CREB2), is a negative regulator of transcription. Previous work by others has shown that ATF4 phosphorylation at serine-219 is necessary for ubiquitination and degradation by the ubiquitin-proteasome pathway (UPP) (Lassot et al. 2001; Mol. Cell Biol. 21(6):2192-202). Using long-lasting chemically-induced long-term potentiation (LTP) and immunofluorescent labeling in coronal hippocampal slices from C57BL/6 mice, we endeavored to determine the time-course for ATF4 phosphorylation at serine-219 during LTP and the kinase responsible for this phosphorylation. We found that ATF4 phosphorylation at serine-219 peaks at 20 minutes after LTP induction, returning to control levels by 30 minutes. Furthermore, we determined that inhibition of the proteasome with beta-lactone blocks the decrease in ATF4 to baseline suggesting that the phosphorylation of ATF4 at serine-219 during LTP is indeed leading to the degradation of the transcriptional repressor via the UPP. Finally, we found that the serine-219 phosphorylation could be blocked with the general kinase inhibitor, staurosporine, suggesting a subset of likely candidates for the kinase responsible for marking ATF4 for ubiquitination and degradation by the UPP. Because de novo transcription is necessary for the maintenance of LTP, these results provide novel insight into the molecular mechanisms behind LTP regulation by the UPP through the degradation of transcriptional repressors such as ATF4.

Disclosures: **K.A. Haynes:** None. **W. Xu:** None. **A.N. Hegde:** None.

Poster

505. LTP: Kinases and Intracellular Signaling

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Program#/Poster#: 505.08/E12

Topic: B.08. Synaptic Plasticity

Support: MH071739

GM058234

Title: CaM Kinases are critical for activity-dependent gene expression in parvalbumin positive inhibitory interneurons

Authors: *S. M. COHEN, R. W. TSIEN
NYU Sch. of Med., New York, NY

Abstract: Transcriptional regulation via excitation-transcription (E-T) coupling is essential for the long-term adaptive changes that occur during brain development, learning and memory, and drug addiction. Recent work has demonstrated that experience induces plastic changes in parvalbumin (PV) positive interneurons to regulate adult learning. Environmental enrichment and fear conditioning induce opposite changes in levels of PV expression, with direct implications for memory consolidation and retrieval, excitatory-to-inhibitory synaptic-density ratios, and structural synaptic plasticity {{Donato, 2013}}. We study mechanisms of E-T coupling in PV+ interneurons, employing pharmacological, imaging, and immunocytochemical techniques on cultured rat cortical neurons. Depolarization of PV+ neurons for 2 min with a 40mM K+ solution resulted in a marked increase in PV and c-fos expression in PV+ cells. This increase was blocked by CaV1 (L-Type) blocker Nimodipine or Ca2+/CaM dependent Kinase (CaMK) blocker KN93. MEK1 inhibitor PD98059 partially inhibited this increase. To understand the mechanism underlying this activity dependent increase in PV, we focused on the steps leading to Ser133 phosphorylation of the transcription factor CREB (pCREB). Regulation of this key signaling event remains relatively unexplored in inhibitory interneurons. We showed that PV+ cells can employ CaMKs to trigger nuclear CREB phosphorylation. Nimodipine, KN93 and CaMKK inhibitor Sto609 prevented increases in pCREB after 30 seconds of K+-depolarization. Depolarization-induced Ca2+ entry via CaV1 channels triggered increases in the nuclear CaM in a manner correlated with increases in pCREB. However, interneurons displayed little immunoreactivity for CaMKIV (the CREB kinase in excitatory neurons). Our preliminary results suggest that CaMKI, which shares a high degree of homology with CaMKIV, translocates to the nucleus. CaMKI could serve as a CaM shuttle, or may itself phosphorylate CREB.

Interestingly, evidence points to the dysfunction of GABAergic transmission and of proteins involved in E-T coupling (CaV1, γCaMKII, βCaMKII, and Calcineurin) in numerous neuropsychiatric diseases. Specifically, disrupted expression of the activity-dependent genes GAD67 and PV is characteristic of schizophrenia. Understanding mechanisms of E-T coupling in PV+ cells will likely shed light on the cell biology underlying the experience regulated network plasticity important for learning, as well as the pathophysiology of neuropsychiatric disease.

Disclosures: S.M. Cohen: None. **R.W. Tsien:** None.

Poster

505. LTP: Kinases and Intracellular Signaling

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 505.09/E13

Topic: B.08. Synaptic Plasticity

Support: AHA Award #11POST7580006

NIH Grant T32NS007083

NIH Grant R01NS040701

Title: Palmitoylation of AKAP79/150 by the palmitoyl acyltransferase DHHC2 controls synaptic potentiation

Authors: *K. WOOLFREY, J. L. SANDERSON, M. L. DELL'ACQUA
Pharmacol., Univ. of Colorado Denver, Aurora, CO

Abstract: Glutamate receptor trafficking is a key plasticity mechanism for excitatory neurons found in the mammalian forebrain. Covalent modification of AMPA-type glutamate receptors by kinases and phosphatases is thought to play an essential role in controlling the channel properties and synaptic incorporation of these receptors during forms of synaptic plasticity such as LTP. Such signaling is coordinated by the synaptic scaffolding protein AKAP79/150 through its ability to bind and target PKA, PKC, calcineurin, receptors, ion channels and other scaffolding proteins. Posttranslational modification of AKAP79/150 by palmitoylation is a critical step in its targeting to recycling endosomes. These endosomes have recently been identified as important intracellular AMPA receptor repositories. Here, we explore mechanisms of AKAP79/150 palmitoylation and the cellular consequences of disrupting this signaling in neurons. We find that

the recycling endosome-resident palmitoyl acyltransferase DHHC2 is an enzyme critical for AKAP79 palmitoylation. RNAi-mediated knockdown of DHHC2 disrupts recycling endosome exocytosis, altering regulated delivery of AMPA receptors to synapses in hippocampal neurons. Accordingly, DHHC2 signaling through AKAP79/150 is crucial for the NMDA receptor-dependent synaptic strengthening that mediates LTP. Importantly, a palmitoylation-independent lipidated AKAP79 mutant is capable of restoring aspects of normal plasticity in DHHC2-deficient neurons. Thus, we demonstrate that DHHC2-AKAP79/150 signaling is an essential regulator of synaptic potentiation.

Disclosures: **K. Woolfrey:** None. **J.L. Sanderson:** None. **M.L. Dell'Acqua:** None.

Poster

505. LTP: Kinases and Intracellular Signaling

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 505.11/E15

Topic: B.08. Synaptic Plasticity

Title: Activated CaMKII immobilized in spines both at the PSD and away from the PSD as resolved by single-molecule tracking PALM

Authors: ***H. LU**[1,2], H. D. MACGILLAVRY[3,2], N. A. FROST[4,2], T. BLANPIED[2]
[1]Univ. Maryland, Baltimore, BALTIMORE, MD; [2]Physiol., Univ. of Maryland, Baltimore, Baltimore, MD; [3]Dept. of Biol., Utrecht Univ., Utrecht, Netherlands; [4]Dept. of Neurol., UCSF, San Francisco, CA

Abstract: Calcium/calmodulin-dependent kinase II (CaMKII) is essential for synaptic plasticity underlying memory formation. With stimuli that induce LTP, activated CaMKII translocates to the PSD and phosphorylates synaptic proteins including AMPA receptors. However, though many known CaMKII substrates that could regulate synaptic transmission lie away from the PSD, it is not likely that PSD-bound CaMKII could phosphorylate such targets. To clarify this, we sought evidence of potential subpopulations of bound CaMKII away from the synapse by examining its mobility within spines. Confocal imaging does not achieve the resolution necessary to distinguish subpopulations within the small volumes of a spine. Therefore, we used

live-cell photoactivated localization microscopy (PALM) to track single CaMKII molecules at a resolution of ~25 nm, mapping the spatial and kinetic heterogeneity of CaMKII within single, living spines. Cultured rat hippocampal neurons were transfected with mEos2-CaMKIIα and single molecules were imaged and localized. Molecules were tracked as they moved within the cell, and their effective diffusion coefficient, Deff was calculated. This showed that CaMKIIα exhibits at least three kinetic subpopulations even within spines. The Deff distribution could be altered by CaMKIIβ overexpression or by acute latrunculin application, demonstrating that at least one kinetic subpopulation is regulated by the actin cytoskeleton. To determine the spatial distribution of these subpopulations, we constructed maps of Deff across neurons and showed that CaMKII with slow Deff is typically enriched in spines compared to dendrites, consistent with the presence of a higher density of potential binding partners. Importantly, activation of CaMKII following NMDA receptor stimulation prompted the immobilization and presumed binding of CaMKII not only at PSDs (marked by PSD-95-Cerulean3) but also at other points in spines but outside of the PSD, suggesting that the activated kinase does not act only at the PSD. Finally, by visualizing immunostained phospho-T286 CaMKII at nanometer resolution via stochastic optical reconstruction microscopy (STORM), we found that endogenous, activated CaMKII concentrated in spines including points quite distant from the PSD. In conclusion, our results suggest that CaMKII mobility within spines is determined by association with multiple interacting proteins, even outside the PSD, suggesting diverse mechanisms by which CaMKII may regulate synaptic transmission.

Disclosures: **H. Lu:** None. **H.D. MacGillavry:** None. **N.A. Frost:** None. **T. Blanpied:** None.

Poster

505. LTP: Kinases and Intracellular Signaling

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Program#/Poster#: 505.12/F1

Topic: B.08. Synaptic Plasticity

Support: NIH Grant R01 MH053576

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Title: Constitutive and conditional PKMζ KO mice display distinct spatial long-memory deficits

Authors: *P. TSOKAS[1], C. HSIEH[2], E. J. C. WALLACE[2], Y. YAO[2], A. A. FENTON[4], T. C. SACKTOR[3]
[1]Furchgott Ctr. for Neural & Behav. Sci., Physiol. & Pharmacol., Anesthesiol., [2]Furchgott Ctr. for Neural & Behav. Sci., Physiol. & Pharmacol., [3]Furchgott Ctr. for Neural & Behav. Sci., Physiol. & Pharmacol., Neurology, Anesthesiol., SUNY Downstate Med. Center, Brooklyn, NY, BROOKLYN, NY; [4]Ctr. for Neural Sci., New York Univ., New York, NY

Abstract: Using pharmacological inhibitors or a dominant negative mutation, many studies have indicated that the persistent kinase activity of PKMζ--a brain-specific, constitutively active atypical protein kinase C isoform--is crucial for the maintenance of the protein synthesis-dependent phase of long-term potentiation (late-LTP or L-LTP) and for the consolidation of spatial long-term memory (LTM). However, recent genetic evidence from PKCζ/PKMζ null mice has questioned this hypothesis (Lee et al., Nature, 493:416, 2013; Volk et al., Nature, 493:420, 2013). Here we show that although the PKC/PKMζ null mice do initially learn, they consistently display an abnormal and inefficient behavioral strategy. Whereas wild-type mice rapidly move to the safest location in an active place avoidance arena, the KOs remain in the most dangerous region next to the shock zone, even though they show equivalent sensitivity to shock as the wild-type. This altered pattern of behavior in the KOs allows for effective learning and memory for ~2 days. When memory reaches asymptotic performance, however, further training and testing reveal a deficit compared to wild-type mice, as demonstrated by a significant difference for the KOs in errors on memory testing without shock. Prior studies on the PKCζ/PKMζ null mice had not reported an increased activation of PKCι/λ, the other ZIP-sensitive atypical PKC. But PKCι/λ activation had been examined in lysates from the whole brain of the KO. Using a "split-brain" preparation, we confirmed this result in lysates from KO hemibrains. But the contralateral isolated dorsal hippocampus from the same animals showed a ~400% increase in activated PKCι/λ. To avert this compensation, we used a conditional knockout approach, which involved AAV-Cre recombinase injections into the hippocampus of PKC/PKMζ-floxed mice. Conditional knockout showed no compensation by PKCι/λ and resulted in the disruption of both late-LTP and spatial LTM consolidation. Thus, under physiological conditions or when compensation is averted, PKMζ is crucial for consolidation of L-LTP and spatial LTM.

Disclosures: P. Tsokas: None. **C. Hsieh:** None. **E.J.C. Wallace:** None. **Y. Yao:** None. **T.C. Sacktor:** None. **A.A. Fenton:** None.

Poster

505. LTP: Kinases and Intracellular Signaling

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Program#/Poster#: 505.13/F2

Topic: B.08. Synaptic Plasticity

Support: NIH grant RO1 AA 018060

HFSP grant RGSP/2005

Title: A presynaptic role for PKA in synaptic tagging and memory

Authors: *A. J. PARK, R. HAVEKES, J. CHOI, V. LUCZAK, T. NIE, T. HUANG, T. ABEL
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Abstract: Protein kinase A (PKA) and other signaling molecules are spatially restricted within neurons by A-kinase anchoring proteins (AKAPs). Although studies on compartmentalized PKA signaling have focused on postsynaptic mechanisms, presynaptically anchored PKA may contribute to synaptic plasticity and memory because PKA also regulates presynaptic transmitter release. Here, we examine this issue using genetic and pharmacological application of Ht31, a PKA anchoring disrupting peptide. At the hippocampal Schaffer collateral CA3-CA1 synapse, Ht31 treatment elicits a rapid decay of synaptic responses to repetitive stimuli, indicating a fast depletion of the readily releasable pool of synaptic vesicles. The interaction between PKA and proteins involved in producing this pool of synaptic vesicles is supported by biochemical assays showing that synaptic vesicle protein 2 (SV2), Rim1, and SNAP25 are components of a complex that interacts with cAMP. Moreover, acute treatment with Ht31 reduces the levels of SV2. Finally, transgenic mouse lines expressing Ht31 in excitatory neurons at the Schaffer collateral CA3-CA1 synapse highlight a requirement for presynaptically anchored PKA in pathway-specific synaptic tagging and long-term contextual fear memory. These results suggest that a presynaptically compartmentalized PKA is critical for synaptic plasticity and memory by regulating the readily releasable pool of synaptic vesicles.

Disclosures: A.J. Park: None. **R. Havekes:** None. **J. Choi:** None. **V. Luczak:** None. **T. Nie:** None. **T. Huang:** None. **T. Abel:** None.

Poster

505. LTP: Kinases and Intracellular Signaling

Location: Halls A-C

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Program#/Poster#: 505.14/F3

Topic: B.08. Synaptic Plasticity

Title: GDPβs unsilences silent synapses in CA1 pyramidal cells by disinhibiting the protein kinase A activating pathway

Authors: *M. OUARDOUZ
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Abstract: During early brain developmental stages, excitatory synapses mediated by AMPA receptors are weak or silent. Activity dependent insertion of AMPA receptors into synapses depends on the activation of protein kinase A. In this work, we investigated the effect of GDPβS on excitatory and inhibitory synaptic currents in CA1 pyramidal cells of Wistar rats at postnatal days 9-12, using whole-cell patch-clamp recordings. GDPβS applied through the recording electrode induces an increase in excitatory synaptic current amplitude, but not in inhibitory synaptic current amplitude. An analysis of the change in excitatory synaptic current amplitude in the presence of GDPβS revealed a progressive increase, which is blocked by the protein kinase A inhibitor Rp-cAMP, suggesting that GDPβS inhibits G-protein with a tonic negative control on a protein kinase A activating pathway. To further characterise the mechanism of excitatory synaptic current amplitude increase, we monitored the amplitude and frequency of spontaneous excitatory synaptic currents in the presence of GDPβS. Although the frequency increased, no change in the amplitude was observed. In addition, GDPβS has no effect on paired-pulse facilitation, suggesting that the probability of glutamate release is not affected. Moreover, as GDPβS was applied to postsynaptic neurons, the increase in spontaneous excitatory postsynaptic current frequency could be related to changes at the postsynaptic side associated with turning on silent synapses. In conclusion, the results indicate that in juvenile hippocampal CA1 pyramidal cells, the tonic negative modulation of a protein kinase A activating pathway through the activation of a G-protein prevents the activation of silent or weak synapses.

Disclosures: M. Ouardouz: None.

Poster

505. LTP: Kinases and Intracellular Signaling

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 505.15/F4

Topic: B.08. Synaptic Plasticity

Support: NIH Grant R01 NS073974

Title: Simulations suggest pharmacological methods for rescuing long-term potentiation

Authors: *P. D. SMOLEN, D. A. BAXTER, J. H. BYRNE
Neurobio. and Anat., The Univ. of Texas Med. Sch., Houston, TX

Abstract: Congenital cognitive dysfunctions are frequently associated with deficits in molecular pathways that underlie the induction and/or maintenance of synaptic plasticity. For example, Rubinstein-Taybi syndrome (RTS) is commonly due to a mutation in *cbp*, which encodes the histone acetyltransferase CREB-binding protein (CBP) (Alarcon et al., 2004; Bourtchouladze et al., 2003). CBP is a transcriptional co-activator that binds to phosphorylated CREB, and the consequent induction of CREB-dependent transcription plays a key role in long-term memory formation. Here, we illustrate a strategy for the rational design of molecular therapeutic approaches for improving memory formation. The strategy uses computational models of the molecular processes that correlate with impairment of learning to simulate the effects of the underlying molecular lesions. We extended our previous model of long-term synaptic potentiation (LTP) to describe CBP-mediated histone acetylation, and simulated LTP impairment due to *cbp* mutation. By altering parameters, molecular targets were identified that may serve as pharmacological targets to restore RTS-mediated deficits in LTP. Single parameter variations of moderate amplitude (~30%) did not restore simulated LTP. However, a pair of parameter changes, that together simulated the effects of a phosphodiesterase inhibitor and a deacetylase inhibitor, was effective in restoring LTP while maintaining basal synaptic weight near its normal value. A pair that simulated the effects of a phosphodiesterase inhibitor and an acetylase activator was similarly effective. For both pairs, additive synergism was present, in that the effect of the combination was greater than the summed effect of the separate parameter changes. These results suggest that the strategy of combining modeling and empirical studies may lead to effective therapies for improving long-term synaptic plasticity and learning and memory in cognitive disorders.

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Poster

505. LTP: Kinases and Intracellular Signaling

Location: Halls A-C

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Program#/Poster#: 505.16/F5

Topic: B.08. Synaptic Plasticity

Support: National Honor Scientist Program

POSCO TJ Park foundation

Title: Mindbomb homolog 2 is involved in hippocampal synaptic plasticity

Authors: *T. KIM, S. KIM, H.-R. LEE, Y.-Y. KONG, B.-K. KAANG
Dept. of Biosci., Seoul Natl. Univ., Seoul, Korea, Republic of

Abstract: Mindbomb homolog 2(Mib2) is a protein strongly expressed in adult brain, heart, skeletal muscle and brain. Previous studies reported that Mib2 is down-regulated in malignant melanoma. However, the role of Mib2 in brain tissue has not been yet identified. Here, using Mib2 knockout mouse, we report the role of Mib2 in hippocampal synaptic plasticity using field recording and contextual fear conditioning. Mib2 KO animals showed less freezing behavior (WT: 34.87±2.4% n=8, KO: 23.47%±2.5, n=11) in contextual fear conditioning (0.4mA shock). Hippocampal slices from Mib2 KO mice showed impairment in long-term potentiation. With TBS protocol, impairment was seen in early-phase long-term potentiation (WT: 198.3%±16.4, n=13 slices, KO: 157.5%±6.7, n=14 slices) For basal synaptic transmission at SC-CA1 synapses, there was no change in Mib2 KO hippocampal slices(WT: n=12 slices, KO: n=5 slices). These results suggest that Mib2 protein is essential for contextual fear memory and synaptic plasticity in adult mouse brain. (T. Kim and S. Kim both contributed equally to this work)

Disclosures: **T. Kim:** None. **S. Kim:** None. **H. Lee:** None. **Y. Kong:** None. **B. Kaang:** None.

Poster

505. LTP: Kinases and Intracellular Signaling

Location: Halls A-C

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Program#/Poster#: 505.17/F6

Topic: B.08. Synaptic Plasticity

Title: The integrin-regulated Abl2/Arg kinase modulates NMDA receptor activity and NMDAR-dependent plasticity

Authors: *X. XIAO, A. LEVY, S. WARREN, A. KOLESKE
MB&B, Yale Univ., New Haven, CT

Abstract: Integrin α3β1 adhesion receptor signaling through the Abl2/Arg nonreceptor tyrosine kinase is critical for dendrite and dendritic spine stability in excitatory forebrain neurons. Arg acts through p190RhoGAP to stabilize dendrite arbors, but the mechanism by which Arg regulates spine stability is unclear. N-methyl-D-aspartate receptors (NMDARs) are critical

determinants of dendritic spine stability and NMDAR antagonists suppress spine destabilization in Arg-deficient neurons, suggesting that Arg interacts functionally with NMDARs. We show here that arg-/- mice exhibit increased NMDAR function beginning at postnatal day 21 (P21), before spines are lost, and that the increased NMDAR currents are driven by GluN2B subunit-containing NMDARs and are regulated by Src family kinase signaling. While NMDAR subunit levels are unaltered, tyrosine phosphorylation of GluN2B, which increases NMDAR currents, is significantly increased in arg-/- hippocampi, suggesting the involvement of a phosphatase downstream of Arg. Finally, we demonstrate that NMDAR-dependent LTP and LTD are significantly altered by P42 in arg-/- mice. These plasticity changes are also governed by the modulation in GluN2B phosphorylation through Src family kinase. Together, these data demonstrate that the integrin-regulated Arg kinase modulates GluN2B phosphorylation to regulate NMDAR function and NMDAR-dependent synaptic plasticity.

Disclosures: **X. Xiao:** None. **A. Levy:** None. **S. Warren:** None. **A. Koleske:** None.

Poster

505. LTP: Kinases and Intracellular Signaling

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Topic: B.08. Synaptic Plasticity

Support: Korea Research Foundation 2012R1A1A2006838

Title: miRNA-mediated regulation of Eph/ephrin signaling plays a role in synaptic plasticity control in aged mouse hippocampus

Authors: ***K. KIM**[1,2], C. P. D. MOHAMMED[3], H. LEE[2], H. LEE[4], B. PHEE[2], J. PARK[4], S. PARK[3], H. NAM[1,2]
[1]New Biol., [2]DGIST, Daegu, Korea, Republic of; [3]POSTECH, Pohang, Korea, Republic of; [4]Macrogen, Seoul, Korea, Republic of

Abstract: Hippocampal learning and memory function deteriorates with aging. MicroRNAs (miRNAs) are small noncoding endogenous RNA molecules that regulate multiple biological processes including neuronal regulation and synaptic plasticity mainly through post-transcriptional silencing of their mRNA targets. In order to gain an insight into how changes in expression patterns of miRNAs affect hippocampal function during aging, we performed small RNA profiling at seven different life stages of mouse hippocampus. From deep sequencing and

subsequent bioinformatical analysis of these small RNAs, we found that 54 (35 upregulated and 19 downregulated) miRNAs are differentially expressed showing more than two fold change between young (2M) and old (18M) mouse hippocampus, and predicted that their potential target mRNAs are important components in neuronal regulation pathways including axon guidance. Here we report that Eph/ephrin signaling pathway is significantly downregulated during adult normal aging in hippocampus. mRNA levels of key Eph/ephrin signaling molecules including EphB2, decreased and mRNA level of its inhibitory molecule, RhoA, increased between young(2M) and old(18M) stages as evidenced by qPCR validation. We found more pronounced differential expression at their protein levels by performing Western Blot analysis on three independent hippocampal tissues. One of upregulated miRNAs in old hippocampus specifically targets EphB2 and causes a reduced protein expression in hippocampal neurons, resulting in significantly decreased spine density of hippocampal cultured neurons. The spine density deficit was recovered to near normal level by specific inhibition of the miRNA targeting EphB2. These results suggest that the miRNA plays an important role in age dependent regulation of Eph/ephrin signaling pathway by targeting key receptor molecule EphB2 and provides a potential for therapeutic intervention for improved cognitive function in aged humans.

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Poster

505. LTP: Kinases and Intracellular Signaling

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Topic: B.08. Synaptic Plasticity

Title: miR-26a and miR-384-5p are required for LTP maintenance and spine enlargement

Authors: *Q. GU, H. SHEN
NIH, Bethesda, MD

Abstract: Long-term potentiation (LTP) is a form of synaptic plasticity leading to enhanced synaptic strength. It is associated with the formation and enlargement of dendritic spines, tiny protrusions accommodating excitatory synapses. Both LTP and spine remodeling are crucial processes for brain development, cognition, and the pathophysiology of neurological disorders. However, the mechanisms underlying their long-term maintenance are largely unclear. Using next-generation sequencing to profile miRNA transcriptomes, we found that miR-26a and miR-

384-5p specifically regulate the maintenance but not induction of LTP, and different stages of spine enlargement. Both miRNAs affects LTP by regulating the expression of RSK3. Using bioinformatics, we also determined the global effect of miRNA transcriptome changes during LTP on gene expression and cellular activities. This study reveals a novel miRNA-mediated mechanism for gene-specific regulation of translation in LTP, identifies two miRNAs required for long-lasting synaptic and spine plasticity, and presented a catalog of candidate "LTP miRNAs".

Disclosures: Q. Gu: None. **H. Shen:** None.

Poster

505. LTP: Kinases and Intracellular Signaling

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Topic: B.08. Synaptic Plasticity

Support: Thompson Center for Autism Research and Translation

NINDS NS45260

Title: Marked developmental changes in actin dynamics accompany the termination of growth and precede the emergence of adult forms of plasticity in hippocampus

Authors: *J. D. RICE[1], E. KRAMAR[1], J. LIU[1], C. KARSTEN[1], C. M. GALL[1], G. S. LYNCH[1,2]
[1]Anat. and Neurobio., [2]Psychiatry and Human Behavior, Univ. of California, Irvine, Irvine, CA

Abstract: Why dendritic growth, synaptogenesis, axon sprouting, and Long Term Potentiation (LTP) in rat hippocampus all undergo pronounced changes in the third post-natal week is poorly understood. There is, however, good agreement that actin management plays a key role in synapse formation, spine growth, and the generation of stable LTP. Accordingly, we carried out the first tests of whether the activity of actin regulatory proteins and actin dynamics change during the critical week. Western blot analyses of several such proteins indicated that concentrations of phosphorylated (inactive) cofilin, a factor that blocks actin polymerization when active, decreased precipitously during the third week. Conversely, phosphorylation of ARP2/3, a protein that elaborates and stabilizes actin networks, increased during the same period. Consonant with the cofilin results, filamentous actin (F-actin) within dendritic spines

decreased from post natal day 11 forward. The high levels of F-actin in the immature hippocampus were eliminated by a toxin that disrupts dynamic actin filaments and this was accompanied by depression of synaptic potentials, an effect not seen in adult slices. Theta burst stimulation triggered actin polymerization in spines, an event critical to LTP consolidation, at 21 days post-natal but not earlier. We propose that cofilin activation (dephosphorylation) during week three of post-natal development depresses the formation of dynamic actin filaments and thereby terminates spine formation and synaptogenesis. This, along with a rise in p-ARP2/3, are suggested to open the way for the synapse specific cytoskeletal changes required for consolidation of LTP and memory. Data relating to the causes of the opposing changes in cofilin vs. ARP2/3 phosphorylation are described.

Disclosures: J.D. Rice: None. **E. Kramar:** None. **J. Liu:** None. **C. Karsten:** None. **C.M. Gall:** None. **G.S. Lynch:** None.

Poster

505. LTP: Kinases and Intracellular Signaling

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Shenzhen Peacock Plan

Y. Shen is a recipient of MSD R&D China Postdoctoral Research Fellowship Award

Title: Melanocortin-4 receptor regulates hippocampal synaptic plasticity through a PKA-dependent mechanism

Authors: *Y. SHEN[1,2,3], W.-Y. FU[1,2,3], E. Y. L. CHENG[1,2,3], M. TIAN[1,2,3], A. K. Y. FU[1,2,3], N. Y. IP[1,2,3]
[1]Div. of Life Sci., [2]Mol. Neurosci. Ctr., [3]State Key Lab. of Mol. Neurosci., The Hong Kong Univ. of Sci. and Technol., Hong Kong, China

Abstract: Learning and memory require the orchestrated regulation of both neuronal connections and synaptic strength in the hippocampus. While neuropeptide alpha melanocyte-stimulating hormone (α-MSH) is implicated in memory acquisition and retention, the functional role of its cognate receptor, melanocortin-4 receptor (MC4R), in the hippocampus remains unclear. Our recent findings revealed that MC4R activation is important for hippocampal synaptic plasticity via the regulation of dendritic spine morphology as well as the abundance of postsynaptic glutamate receptors. While knockdown of MC4R in rat hippocampal neurons resulted in a reduction of mature dendritic spines, activation of the receptor stimulated the surface trafficking of AMPA receptor subunit GluA1 in a PKA-dependent manner. Blockade of the PKA signaling abolished the MC4R-stimulated enhancement of neurotransmission and formation of long-term potentiation (LTP) in hippocampal slices. Importantly, deletion of MC4R in the mouse hippocampal CA1 region led to the impairment of LTP, whereas *in vivo* delivery of MC4R agonist such deficit. These findings collectively demonstrate that postsynaptic MC4R signaling is critical for the regulation of structural and functional synaptic plasticity.

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Poster

506. Spike Timing Dependent Plasticity

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Program#/Poster#: 506.01/F11

Topic: B.08. Synaptic Plasticity

Support: ISRAEL SCIENCE FOUNDATION Grant 722/10

Title: STDP induced phase tuning in an oscillatory feed forward network

Authors: *Y. LUZ, M. SHAMIR
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Abstract: Oscillations in neuronal activity are common in the brain, ranging from the Delta frequencies (below 4 Hz) and up to the Gamma frequencies (above 30 Hz). A population of neurons oscillating in synchrony induces an oscillating LFP with same frequency, and amplitude that depends on the amplitude and phase distribution of individual neurons in the population. It is yet unknown how this population oscillatory activity interacts with synaptic learning. Spike Timing Dependent Plasticity (STDP), is an experimentally observed learning rule that

strengthens or weakens synaptic connectivity based on the temporal distance of pre and post synaptic firing. Hence, one would expect the oscillations' frequency and phase to interact with the mechanisms responsible for STDP. Here, we study a possible role of STDP learning as a mechanism for input selectivity by phase tuning. For the case of learning a single synapse, we derived analytical mean field solution in the limit of weak coupling and extend our analytical results using numerical simulations. In this case our model demonstrates that STDP induces phase tuning, i.e., the fixed point of the learning dynamics (the asymptotic synaptic strength) as a function of the relative phase between pre and post synaptic firing is bell shaped. Next, we studied the case of a population of excitatory synaptic inputs with uniform phase distribution. Our simulations revealed that the STDP produced spontaneous symmetry breaking with bell shaped input selectivity by the input phase. However, we found that the arbitrary preferred phase is not stable and drifts in time due to the intrinsic delay between presynaptic activity and its effect on post synaptic firing. Our model so far could not find a stable solution, but rather suggests a limit cycle. We further investigate potential stabilizing mechanism to compensate for this drifting effect.

Disclosures: **Y. Luz:** None. **M. Shamir:** None.

Poster

506. Spike Timing Dependent Plasticity

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Topic: B.08. Synaptic Plasticity

Support: NSF Grant IOS-1050701

NIH Grant DC012452

Title: Spike-timing-dependent plasticity shapes interval selectivity of electrosensory midbrain neurons

Authors: *C. A. BAKER, X. MA, B. A. CARLSON
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Abstract: Spike-timing-dependent plasticity (STDP), in which the connection strength between two neurons can change based on the relative timing of pre- and postsynaptic spikes, has been implicated in visual, somatosensory, auditory, and electrosensory processing. Here we show that

STDP influences processing of communication signals among a network of midbrain electrosensory neurons. Mormyrid fish vary the interpulse intervals (IPIs) between electric pulses to communicate, and neurons in the posterior exterolateral nucleus (ELp) exhibit a wide range of IPI tuning. We previously found a dense excitatory network in ELp in which similarly tuned neurons are more likely to be connected and with stronger connections. Here we ask whether STDP contributes to this network topology in *Brevimyrus niger* and *Brienomyrus brachyistius*. Using a whole-brain *in vitro* preparation, we varied the delay between extracellular presynaptic fiber stimulation and a postsynaptic spike induced by intracellular current injection into an ELp neuron. The largest increases in postsynaptic potentials (PSPs) in ELp neurons occurred when presynaptic spikes led postsynaptic spikes by ~20 ms, and the largest decreases in PSPs occurred when presynaptic spikes trailed postsynaptic spikes by ~10 ms. The NMDA receptor antagonist APV eliminated both effects, indicating that STDP in ELp is NMDA-dependent. To test whether STDP can influence IPI tuning, we stimulated afferent inputs with different IPIs, but paired postsynaptic spikes at a potentiating delay with only one particular IPI. This led to a consistent shift in IPI tuning in the direction of the paired IPI. Next, to test whether natural patterns of spiking in this circuit can influence IPI tuning, we repeatedly stimulated afferent input using constant IPI trains without postsynaptic current injection. This caused the IPI tuning of ELp neurons to shift in the direction of the repeated IPI. Finally, we tested whether natural patterns of spiking can influence tuning *in vivo* by recording evoked potentials from ELp before and after presenting electrosensory pulses of constant IPI. In contrast to the *in vitro* results, IPI tuning shifted away from the repeated IPI. We hypothesize that the different observed effects *in vivo* versus *in vitro* reflect differential effects of inhibition. Whereas the afferent stimulation *in vitro* follows natural temporal patterns, it likely does not capture natural spatial patterns. This could result in stimulation of different amounts of excitation and inhibition *in vitro* versus *in vivo*. Collectively, our results suggest that STDP may alter the topology of this network to optimize the coding of changes in stimulus statistics.

Disclosures: C.A. Baker: None. **X. Ma:** None. **B.A. Carlson:** None.

Poster

506. Spike Timing Dependent Plasticity

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 506.03/G1

Topic: B.08. Synaptic Plasticity

Support: MEXT-Supported Program for the Strategic Research Foundation at Private Universities, 2013-2017

Title: Interneuron effects on cholinergically-induced STDP in hippocampal CA1 network

Authors: *E. SUGISAKI[1,2], Y. FUKUSHIMA[3], T. AIHARA[2]
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Abstract: One of the neurotransmitters in diffuse modulatory system is an acetylcholine (ACh), and the cholinergic fibers from the medial septum are projected to the neurons in hippocampal CA1 region. It is known that the ACh is released from cholinergic terminals when in arousal and /or in attentional state *in vivo*. While the multimodal sensory inputs, such as visual, auditory, olfactory et cetera, are received in hippocampus via entorhinal cortex to play a crucial role in information processing. Previous study has shown that the synaptic plasticity induced by injection of spike timing-dependent plasticity (STDP) stimulation in CA1 region was influenced by ACh in GABAA receptor-blocked network. In order to investigate the ACh effect on STDP in CA1 network in more natural condition, the interneuron-activated rat hippocampal slices were used. As the results, ACh receptors (AChRs) not only on pyramidal neurons but also on interneurons were co-activated and the STDP was significantly enhanced both by positive and negative timing of STDP protocols in the presence of eserine, a cholinesterase inhibitor. According to these results, the question was raised which type of AChRs, muscarinic (mAChR) or nicotinic (nAChR), on pyramidal or interneuron was more effective to the STDP enhancement. The results brought in both timing in the GABAA-blocked network showed that the activation of mAChRs on pyramidal neuron were more effective to the enhancement of STDP than nAChR activation on pyramidal neuron. In addition, the similar experiments were performed in the interneuron-activated network, and the activation of mAChRs on interneuron was more sensitive than nAChRs on interneuron for preventing the expansion of cholinergically-induced STDP. These observations suggest that the calcium, which is important substance for the synaptic plasticity, is additionally influx due to the activation of mAChRs and nAChRs on pyramidal neuron. While the mAChRs and nAChRs on interneuron regulate the cholinergically-induced STDP by reducing the excitability of pyramidal neuron. We conclude that ACh is a critical neurotransmitter for learning and memory, furthermore, the appropriate magnitude of synaptic plasticity is decided by co-activation of AChRs not only on pyramidal neurons but also on interneurons.

Disclosures: E. Sugisaki: None. **Y. Fukushima:** None. **T. Aihara:** None.

Poster

506. Spike Timing Dependent Plasticity

Location: Halls A-C

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Program#/Poster#: 506.04/G2

Topic: B.08. Synaptic Plasticity

Support: Mol Cell Biol presidium of RAS

RFBR 13-04-00352 A

RFBR 13-04-40332-H

Title: Presynaptic calcium entry into proximal boutons and 1st Ranvier node shapes backpropagating action potentials in L5 pyramidal neurons of visual cortex

Authors: *E. S. NIKITIN, P. M. BALABAN, M. ROSHCHIN
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Abstract: It is well documented that nonsynaptic as well as synaptic plasticity can be a substrate for long-term memory. Synaptic plasticity is widely regarded as the primary mechanism. Recently it has been demonstrated that global nonsynaptic changes can be linked to compartmentalized synaptic changes and increase in synaptic efficacy [Nikitin et al. 2013]. Little is known, however, about how the activation of efficient synapses can change the neuronal excitability of the presynaptic neuron in short-term at subcellular level. Large boutons (≥1 mkm) of axonal collaterals of L5 pyramidal neurons are typically regarded as presynaptic parts of functionally mature cortical synapses [Romand et al. 2011]. Also, a strong correlation between the size of active zones and the number of docked vesicles and postsynaptic density was reported in a number of publications [Schikorski, Stevens 1997]. In our experiments in rat visuial cortex (P18-P24) a morphological examination of L5 pyramidal neurons with an intracellularly loaded fluorescent morphological tracer demonstrated that the very first synaptic boutons emerged on the first collaterals at 7-12 mkm from the 1st node of Ranvier. We performed simultaneous calcium imaging of the first synaptic bouton and node with a line-scanning fast confocal microscope and a calcium sensor delivered through the patch pipette. Somatically induced action potentials (APs) evoked significantly larger calcium transients in the first boutons suggesting that there is a gradient of calcium elevation in the proximal part of axonal collaterals towards the node with peaks in the proximal boutons. Than we induced additional calcium elevation in the first proximal boutons close to the 1st node and in the node itself by Ca2+ uncaging with a blue laser under the confocal microscope. Ca2+ uncaging produced broadening of the APs recorded in the soma when prior calcium elevation was induced either in the bouton or node comparing to control flashing of background regions. There is evidence that axonal calcium-sensitive potassium channels (BK-type) can regulate the AP width in L5 pyramidal neurons of visual cortex [Yu 2010]. Presynaptic calcium elevation initiates release of neurotransmitters at synapses, and regulation of presynaptic Ca2+ channels has a powerful influence on synaptic

strength. Emerging evidence suggests a critical role of Ca2+ regulation in control of neurotransmission and in presynaptic plasticity [Catterall, Few 2008]. We propose a novel mechanism of how the presynaptic plasticity and changes in calcium elevation in proximal boutons can regulate the backpropagation of action potentials and tune the excitability of the presynaptic neuron.

Disclosures: E.S. Nikitin: None. **P.M. Balaban:** None. **M. Roshchin:** None.

Poster

506. Spike Timing Dependent Plasticity

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Program#/Poster#: 506.05/G3

Topic: B.08. Synaptic Plasticity

Support: CIHR grant

Title: LTP induced by pairing subthreshold basal dendritic excitation with backpropagating apical dendritic evoked spike *in vivo*

Authors: C. LAW[1], T. FUNG[1], *L. LEUNG[2]
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Abstract: Hippocampal CA1 pyramidal cells receive excitation of their basal and apical dendrites from different cells in CA3, and synaptic integration and plasticity may depend on the interaction between basal and apical dendritic excitation. In order to study the properties of long-term plasticity associated with spike timing dependent plasticity (STDP), subthreshold basal dendritic population excitation (E) in hippocampal CA1 was paired with a strong apical dendritic excitation that evoked a population spike (S) in urethane-anesthetized adult rats *in vivo*. Average field excitatory postsynaptic potentials were recorded in CA1 at 50 micron intervals by a 16-channel silicon probe, and analyzed as current source density. A strong stratum radiatum stimulus evoked a population spike that backpropagated into the basal dendrites. We hypothesize that excitation-spike (ES) pairing at positive intervals- generating a synaptic excitation before a spike - results in long-term potentiation (LTP), while pairing at a negative ES interval results in long-term depression (LTD), as has been shown for different preparations *in vitro*. ES-pairing (50 pairs at 5 Hz given in 5 segments each of 10 pairs) at ES interval of -10 ms resulted in significant LTP of the slope and amplitude of the basal dendritic excitatory sink for 2 h, while

pairing at +10 or +20 ms ES interval did not induce LTP or LTD. At 1 h after pairing, the basal-dendritic sink was 1.29 ± 0.12, 0.97 ± 0.07, and 0.96 ± 0.12 times of the pre-pairing baseline for ES interval of -10, +10 and +20 ms, respectively. The apical-dendritic sink was not significantly changed by pairing. Similar basal dendritic LTP was found for ES interval of 0 ms (synchronous) or -10 ms if the 50 pairs were given continuously. No LTP or LTD resulted from 50 stimuli of 5 Hz, evoking only an apical dendritic spike or only subthreshold basal dendritic excitation. No LTP was observed after pairing at ES -10 ms if the rats were pretreated with NMDA receptor antagonist CPP (10 mg/kg i.p.). Contrary to our hypothesis, we found that pairing at negative or zero ES interval resulted in LTP, while no LTP or LTD resulted from pairing at ES intervals of +10 and +20 ms. Pairing at ES interval of -10 ms may involve the coincidence of the weak basal dendritic excitation with the depolarizing afterpotential or spike burst following a backpropagated spike, thus opening NMDA receptor channels and resulting in subsequent LTP.

Disclosures: C. Law: None. **T. Fung:** None. **L. Leung:** None.

Poster

506. Spike Timing Dependent Plasticity

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Program#/Poster#: 506.06/G4

Topic: B.08. Synaptic Plasticity

Support: SFB 779/B06

CBBS FKZ1211080005

Title: Timing-dependent LTP can be induced by six repetitions of single pre- and postsynaptic spikes at CA3-CA1 synapses in acute hippocampal slices

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Abstract: Long-term potentiation is thought to be a cellular correlate of learning and memory formation, which is often studied in field potential recordings by means of high frequency stimulation protocols. Here we analyse timing dependent (t-) LTP at the level of single postsynaptic CA1 neurons using few repeats of synaptic stimulation in a spike timing-dependent plasticity (STDP) paradigm, which most likely approximates more physiological synaptic

activation patterns. The canonical form of STDP is commonly induced with high repeat numbers (50-100 times) of precisely timed pre- and postsynaptic spike pairs at higher pairing frequency (up to 5 Hz) to induce timing-dependent long-term potentiation (t-LTP). Nevertheless, the STDP rules vary between synapses in different brain regions and are dependent on multiple factors. Our study now aimed at evaluating requirements of canonical STDP at CA3-CA1 synapses in acute hippocampal slices using minimal numbers of repetitions. Using patch clamp experiments in acute hippocampal slices of juvenile rats and mice, t-LTP at CA3-CA1 synapses was induced with different numbers of spike pairings of one presynaptically induced excitatory postsynaptic potential (EPSP) in combination with one postsynaptic AP (1EPSP/1AP) at positive delays between pre- and postsynaptic activation and at low pairing frequency. We achieved successful t-LTP by 1EPSP/1AP spike pairings, which were repeated 6 to 70 and 12 to 100 times in mice and rats, respectively. Efficiency of t-LTP amplitudes seemed to be inversely correlated to repeat numbers, but nevertheless all protocols produce reliable t-LTP. Lower repeat numbers such as 3x in mice (1.06±0.2) or 6x in rats (0.80±0.1) did not show any t-LTP and the resulting EPSP amplitudes were comparable to the values obtained from unpaired control experiments (mice: 0.95±0.1 or rats: 0.80±0.1, respectively). The 6-repeat t-LTP protocol which was successful at short intervals, did not elicit successful t-LTP at longer positive pairing delays, which fits to the asymmetric STDP curve described for 1EPSP/1AP pairings. Our results reveal a novel canonical STDP paradigm, which induced robust t-LTP in hippocampal CA1 region, generated by only six repeats of 1EPSP/1AP pairings at low frequency and follows the rules of classical t-LTP.

Disclosures: **E. Cepeda-Prado:** None. **E. Edelmann:** None. **V. Lessmann:** None.

Poster

506. Spike Timing Dependent Plasticity

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Topic: B.08. Synaptic Plasticity

Support: NIH Grant R01MH093665

Title: Stable reinforcement learning via temporal competition between LTP and LTD traces

Authors: M. A. HUERTAS[1], S. SCHWETTMANN[2], *H. Z. SHOUVAL[1]
[1]Neurobiol & Anat, Univ. Tex Medl Schl Houston, HOUSTON, TX; [2]Computat. and Applied Math, Rice Univesity, Houston, TX

Abstract: Although synaptic plasticity is responsible for reinforcement learning in the brain, theories of reinforcement learning are generally abstract and involve neither neurons nor synapses. Here we describe a biophysically based theory of reinforcement-modulated synaptic plasticity. We postulate the existence of two eligibility traces, one corresponding to the induction of LTP, and the other to the induction of LTD. The traces have different temporal profiles: the LTP-associated eligibility trace, with a slow decay time constant on the order of several seconds, is larger than the corresponding decay time constant for the LTD-associated trace. Such assumptions are consistent with recent experimental results by He and Kirkwood (SFN abstracts 2013). The magnitude of these traces, at the synapse, depends on the activity of pre- and post-synaptic neurons. The traces decay individually over time, and their magnitude at the time of reward determines if synaptic modification will correspond to LTP or LTD. Due to the difference in their temporal profiles, the LTP and LTD traces can exhibit temporal competition at the reward time. We suggest that this competition provides a mechanism for stable reinforcement learning. To test this novel reinforcement-learning rule, we applied it to a model of a recurrent cortical network designed to reproduce the experimentally observed responses of neurons in the rodent visual cortex. In these experiments a visual stimuli is paired with delayed reward signals, resulting in neural responses that last significantly beyond the visual stimulation and correlated well with the expected time of reward. Previously we proposed a model to account for this phenomenon by using a plastic recurrent neural network with a learning rule that uses only a single eligibility trace that potentiates the synapses and a mechanism for inhibiting the reward signal in order to obtain stability. Our new approach uses two Hebbian eligibility traces with different time constants, which we show can also serve as a more biophysically plausible stability mechanism. Initially when the network responds primarily to the stimuli the LTP trace dominates at the time of reward due to its longer time constant, potentiating synapses and resulting in prolonged network activity. As activity is prolonged and approaches the reinforcement signal, the relative level of the LTD trace increases, until it completely balances the LTP trace. The proposed learning rule produces a stable network, with prolonged transient activity that terminates near the expected reward time.

Disclosures: **M.A. Huertas:** None. **H.Z. Shouval:** None. **S. Schwettmann:** None.

Poster

506. Spike Timing Dependent Plasticity

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Program#/Poster#: 506.08/G6

Topic: B.08. Synaptic Plasticity

Support: LO591506

Title: Non-genomic action of estradiol on a spinal cord neuromuscular reflex in female rat

Authors: *O. D. LARA GARCIA[1], M. LARA GARCÍA[1,2], Y. CRUZ[2], E. CUEVAS[2], M. MARTÍNEZ-GÓMEZ[2], P. PACHECO[3,1]
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Abstract: Pubococcygeus muscle (Pcm) reflex activity evoked by mechanical stimulation of clitoris (Clit) in female rat, results an interesting model to explore hormonal effects on its components. It is known that dermatome size of pudendal nerve which innervates the Clit, is increased by estrogen treatment or during the receptivity period of the estrous cycle. Here, we explored the characteristics of this reflex in diestrous (DE), proestrous (PE) and ovariectomized (Ovx) animals as well as the immediate estradiol action on this reflex after its subcutaneous injection. Methods: In DE, PE and Ovx animals deeply anesthetized with intraperitoneal urethane, the electromyography (EMG) characteristics of Pcm reflex were recorded using intramuscular stainless steel bipolar electrodes. Mechanical stimulation (pressure) was applied to Clit during 5-15 sec, before and after estradiol benzoate administration (40mg/kg). Results: EMG from Pcm fibers was compound of regular rhythmic firing of its corresponding motoneurons. Animals in DE and PE, during stimulation evoked a discharge rate of 15-20 pulses per second. After the end of stimulation, PE animals showed an afterdischarge of 20-100 sec. Ovx animals did not present reflex activity during or after stimulation; however, after 5-10 min of estradiol injection, the reflex activity appeared during and after Clit stimulation. In DE animals, after estradiol injection the afterdischarges also appeared. Discussion: Mammalian motoneurons including rat, display a repetitive rhythmic firing; then, it is likely that the EMG discharges of Pcm here described are in accordance with this motoneuronal property. Summation of currents from soma, dendrites and the initial axon segment had been implicated in the production of an afterhyperpolarization current which is supposed to trigger the motoneuronal repetitive firing that we have indirectly recorded in Pcm EMG. These motoneuronal areas can be activated by clitoral receptors which through interneurons evoke motoneuronal firing. Thus, neural components involved in Pcm reflex activity are modulated by estradiol. The short-time effect of estradiol here described, suggests the idea of a non-genomic modulatory effect which is rich, when blood levels of estradiol are high (PE) or poor when these are low (DE), or absent when there is a lack of estradiol (Ovx). Conclusions: Clitoral receptors are connected with their corresponding Pcm motoneurons. The EMG reflex activity evoked in the Pcm by mechanical stimulation of Clit is blocked by ovariectomy; however, it is immediately restored by the non-genomic effect produced after subcutaneous injection of estradiol.

Disclosures: **O.D. Lara Garcia:** None. **M. Lara García:** None. **Y. Cruz:** None. **E. Cuevas:** None. **M. Martínez-Gómez:** None. **P. Pacheco:** None.

Poster

506. Spike Timing Dependent Plasticity

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Topic: B.08. Synaptic Plasticity

Support: NEI

Title: Eligibility traces in cortical synapses

Authors: ***K. HE**[1], A. KIRKWOOD[1,2]
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Abstract: Eligibility trace has been proposed as the transient synaptic 'tag' that allows the brain reinforcement signal to identify the target neural activity underlying reward-based learning. Previously we have established an experimental approach to prove for the first time that the eligibility trace does exist and can be generated by correlated neuronal spiking activity in a polarity-specific manner. We have described eligibility trace that are transient, synapse-specific and can be consolidated by distinct catecholamines into long-term potentiation or depression. This novel neuromodulators-mediated consolidation of eligibility trace could be the candidate brain substrate for reinforcement learning. Here we extended our investigation to further understand the universality of this novel learning rule and the properties of the eligibility trace. Similar to what we have shown previously in the primary visual cortex, we found dopamine and serotonin respectively consolidate LTP and LTD eligibility traces in the medial prefrontal cortex. We are currently evaluating whether these elegibility traces are determined by kinase/phosphatase activity.

Disclosures: **K. He:** None. **A. Kirkwood:** None.

Poster

506. Spike Timing Dependent Plasticity

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NIH grant P41-GM103712

Title: Highly precise hippocampal synaptic plasticity

Authors: *C. BROMER[1,2,3], T. M. BARTOL[1], J. KINNEY[2], K. HARRIS[3], T. SEJNOWSKI[1]
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Abstract: Changes in the strengths of synapses drive changes in neuronal computation. The size of a dendritic spine head is highly correlated with the size of the presynaptic axon active zone and both are directly related to the strength of the synapse. In an electron microscopic reconstruction of the hippocampal neuropil, we analyzed the dimensions of the spine heads, the areas of postsynaptic densities, and the availability of presynaptic vesicles of neurotransmitter at synapses on CA1 apical dendrites and found that pairs of dendritic spines on the same dendritic branch receiving input from a single presynaptic axon are nearly identical in size (CV = 0.083). Despite a small sample size, the relationship is highly significant over a factor of 60 in spine head sizes, which implies 6 bits of accuracy. This does not occur for dendritic spines located on different dendrites but synapsing with the same presynaptic axon. Although there are numerous sources of variability in the responses of synapses, including low neurotransmitter release probabilities, the precision indicated here suggests that the biochemical pathways inside excitatory hippocampal synapses is tightly controlled and in particular must be able to average the impact of input spike trains over many minutes. If similar precision is found at excitatory synapses on pyramidal neurons in the cerebral cortex then the capacity of the brain to store information may be much higher than previously thought.

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Poster

506. Spike Timing Dependent Plasticity

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Topic: B.08. Synaptic Plasticity

Support: NIA grant SC1AG046907

NINDS grant S11NS055883

Title: E-S potentiation in CA1 rat hippocampal neurons is selectively induced during the lifespan and is dependent on L-type calcium channels

Authors: E. CARPENTER-HYLAND[1], E. BICHLER[2], *M. BENVENISTE[1]
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Abstract: Under certain conditions hippocampal LTP, a neurophysiological correlates to learning and memory, has been shown to abate with normal aging. Currently, little is known about alterations in non-synaptic forms of plasticity in normal aging, and if neurons in the adult employ such mechanisms to enhance plasticity as LTP declines. One relatively unexplored mechanism of non-synaptic plasticity involves the potential change in ability of a postsynaptic neuron to produce action potentials as a result of specific EPSP input strength . This would be a change in intrinsic excitability, or EPSP to spike (E-S) plasticity. E-S plasticity can be observed as an alteration in the probability of producing action potentials at given EPSP slopes. We have measured E-S plasticity in CA1 neurons from adult and juvenile rat hippocampal slices using whole-cell current clamp in conjunction with various LTP-induction protocols. We observed that E-S potentiation was more sensitive that LTP, being induced under conditions of weak stimulation when little or no LTP was produced (100 pairings of Schaffer collateral stimulation with spike-producing postsynaptic depolarization @ 0.3 Hz). E-S plasticity was differentiated from synaptic plasticity as it could be produced in conjunction with either LTP or LTD, although E-S potentiation and LTP become highly correlated under conditions when strong stimulation such as theta burst was used for LTP induction. Interestingly, E-S potentiation differed across the lifespan, appearing in adult rat CA1 (60-90 days) while being absent from young rats (13-26

days). Expression of E-S potentiation in adults was found to be unaffected by 100 μM APV and therefore NMDA receptor independent, but dependent on intracellular calcium since it was blocked when 30 mM BAPTA was present in the intracellular recording solution. In addition under strong stimulation induction using theta burst, L-type calcium blocker, nifedipine (10 μM), eliminated E-S potentiation in CA1 neurons in the adult, whereas robust LTP was fully preserved. These findings support a novel form of non-synaptic plasticity present in adult hippocampus that may eventually contribute to understanding memory deficits which can occur with normal aging.

Disclosures: E. Carpenter-Hyland: None. **M. Benveniste:** None. **E. Bichler:** None.

Poster

506. Spike Timing Dependent Plasticity

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Program#/Poster#: 506.12/G10

Topic: B.08. Synaptic Plasticity

Support: Wellcome Trust

BBSRC

Title: Coordinated distinct Ca2+ sources and mGluRs encode spike timing-dependent plasticity at mature hippocampal Schaffer collateral synapses

Authors: *C. M. TIGARET[1], J. H. L. SADOWSKI[1], T. J. O. GRIFFITH[2], K. TSANEVA-ATANASOVA[3], J. R. MELLOR[1]
[1]Sch. of Physiol. and Pharmacol., [2]Dept. of Engin. Mathematics, Univ. of Bristol, Bristol, United Kingdom; [3]Col. of Engineering, Mathematics and Physical Sci., Univ. of Exeter, Exeter, United Kingdom

Abstract: Induction of spike timing-dependent synaptic plasticity (STDP) at glutamatergic Schaffer collateral synapses onto CA1 hippocampal pyramidal neurons requires specific timing between synaptic input and post-synaptic action potentials (APs). The NMDA receptor-generated Ca^{2+} transients at the dendritic spines (EPSCaTs) are proposed to determine the direction of plasticity, with large Ca^{2+} transients elicited during near-coincident APs leading to potentiation, and small Ca^{2+} transients leading to depression (1,2). We evaluated STDP at Schaffer collateral to CA1 pyramidal neuron synapses in acute hippocampal slices from adult

rats, by pairing pre-synaptic stimuli delivered in the stratum radiatum with somatically-evoked APs. Using two-photon Ca^{2+} fluorescence imaging we recorded the EPSCaTs triggered by paired activity at apical dendritic spines and we correlated them with the ability of the pairing protocols to generate synaptic plasticity. Recordings were performed in whole-cell current clamp, at 36°C, under $GABA_A$ receptor inhibition. Single pre-synaptic stimuli paired with somatic APs elicited larger EPSCaTs when compared to unpaired stimuli. This effect was stronger when the pre-synaptic stimulus was paired with two back-propagated APs within 10 ms and was independent of the timing sign. In contrast, induction of NMDA receptor-dependent LTP required pairing of at least two back-propagated action potentials 10-50 ms following, but not preceding, a single pre-synaptic stimulus. Pairs of pre-synaptic stimuli at 100 Hz yielded larger EPSCaTs than the LTP-inducing pairing protocols but failed to induce plasticity, whereas pairing at 20 Hz induced LTD. Induction of spike timing-dependent LTP required the activation of T- and L-type voltage-sensitive Ca^{2+} channels, which contributed only partially to the EPSCaTs generated by the pairing protocol. LTP was specifically blocked by the $mGlu_1$ antagonist YM298198, which did not alter the magnitude of the EPSCaTs. Our data indicate that the coordinated activation of NMDA receptors, voltage-sensitive Ca^{2+} fluxes, and of mGlu1 receptors determine the induction of STDP at mature Schaffer collateral synapses, whereas the magnitude of EPSCaT is not sufficient to determine the direction of synaptic plasticity. References: 1. Buchanan, K.A., and Mellor, J.R. (2010) The activity requirements for spike timing-dependent plasticity in the hippocampus. Frontiers in Synaptic Neurocience, 2, doi: 10.3389/fnsyn.2010.00011 2. Shouval, H.Z., Bear, M. F., Cooper, L.N. (2002) A unified model of NMDA receptor-dependent bidirectional synaptic plasticity Proc. Natl. Acad. Sci. USA. 99(16): 10831-10836

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Poster

507. Cellular Mechanisms of Modulation of Neuronal Firing Properties

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 507.01/G11

Topic: B.10. Intrinsic Membrane Properties

Support: NIH R01 MH085074

Title: History-dependent changes in spiking patterns of cholinergic neurons in the medial septum

Authors: *E. D. MELONAKOS, J. A. WHITE, F. R. FERNANDEZ
Dept. of Bioengineering, Univ. of Utah, Salt Lake City, UT

Abstract: Hippocampal theta rhythms are sensitive to atropine, a muscarinic acetylcholine receptor antagonist. Cholinergic neurons from the medial septum target the hippocampus, and have thus been suggested as a neuromodulatory driver of hippocampal theta rhythms. An understanding of the role of medial septal cholinergic neurons in hippocampal theta rhythms requires a detailed description of their individual electrophysiological properties. An important electrophysiological characteristic of a neuron that influences spike phase-locking and rhythm generation is the neuronal sensitivity to current inputs or gain. In particular, changes in gain significantly influence spike phase-locking to weak sinusoidal inputs (Broicher et al., 2012). In our efforts to characterize cholinergic neurons, we found that these cells express a significant history- and voltage-dependent gain response. We found that changing the holding voltage from about -74 mV to about -44 mV led to a 5.5-fold increase in subsequent gain. Further investigation revealed that this effect resulted from a slow, likely potassium current with reversal potential around -80 mV. This current activates and inactivates at depolarized potentials and is deinactivated at hyperpolarized potentials. This unique property of cholinergic neurons in the medial septum may be important for determining the history-dependence of hippocampal cholinergic tone. Reference: Broicher, T., Malerba, P., Dorval, A. D., Borisyuk, A., Fernandez, F. R., & White, J. A. (2012). Spike Phase Locking in CA1 Pyramidal Neurons Depends on Background Conductance and Firing Rate. Journal of Neuroscience, 32(41), 14374-14388. doi:10.1523/JNEUROSCI.0842-12.2012

Disclosures: E.D. Melonakos: None. **J.A. White:** None. **F.R. Fernandez:** None.

Poster

507. Cellular Mechanisms of Modulation of Neuronal Firing Properties

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 507.02/G12

Topic: B.10. Intrinsic Membrane Properties

Title: Perineuronal nets modulate neuronal physiology in the mouse barrel cortex

Authors: *P. CHU[1], K. BUDHU[2], J. C. BRUMBERG[3,4]
[2]Psychology, [1]Queens College, City Univ. of New York, Flushing, NY; [3]Psychology, City Univ. of New York, The Grad. Ctr., NY, NY; [4]Psychology and Neurosci. Major, Queens College, CUNY, Flushing, NY

Abstract: Perineuronal nets (PNNs) are an aggregated form of extracellular matrix that exists in the central nervous system. Although the presence of PNNs was reported by Camillo Golgi in the late 19th century, their functions have only recently begun to be elucidated. For example, removing components of PNNs genetically and enzymatically results in enhanced plasticity in adult animals. However, the mechanism for the enhancement is not well understood. We sought to determine the role of PNNs in regulating neuronal physiology through enzymatic digestion and *in vitro* whole cell patch clamping in the mouse barrel cortex. Following enzymatic digestion with chondroitinase ABC (ChABC), we recorded from cells in layers 2/3, 4 and 5. In response to hyperpolarizing and depolarizing current pulses, the intrinsic properties of putative excitatory neurons (electrophysiological identified as "regular spiking", RS) and putative inhibitory neurons (fast spiking, FS) are altered following digestion of the PNN with chABC. However, there was no change in the intrinsic properties of another putative class of GABAergic inhibitory interneurons, low threshold spiking cells. FS interneurons showed a 10-20% reduction in action potential amplitude and a decrease in input resistance. RS neurons did not show any changes in their action potential amplitude but did show a reduction in input resistance that was less than that for FS cells. Other intrinsic properties of the cells were not affected in any cell class (e.g. spike frequency, resting membrane potential, spike thresholds, first spike delays) suggesting that the impact of PNN digestion was specific to those properties and not due to non-specific effects of the enzyme. The greater effect on FS cells is consistent with anatomical data showing that PNNs preferentially ensheath fast-spiking parvalbumin+ interneurons. The effect of chABC on RS cells, not typically thought to express PNNs in large proportions, may be due to modulation by the PNNs expressed on neighboring FS cells. Overall our results suggest that Perineuronal nets are capable of modulating the intrinsic properties of neurons.

Disclosures: P. Chu: None. **K. Budhu:** None. **J.C. Brumberg:** A. Employment/Salary (full or part-time):; Professor Queens College and the The Graduate Center, CUNY.

Poster

507. Cellular Mechanisms of Modulation of Neuronal Firing Properties

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 507.03/H1

Topic: B.10. Intrinsic Membrane Properties

Support: National Institute for Neurological Disorders (RO1 NS 065761 to David M. Ornitz and Jeanne M. Nerbonne)

Title: Intracellular fibroblast growth factor 14 in the regulation and dysregulation of purkinje neuron excitability

Authors: *J. L. RANSDELL, M. XIAO, D. M. ORNITZ, J. M. NERBONNE
Washington Univ., Saint Louis, MO

Abstract: Intracellular fibroblast growth factor 14 (iFGF14) is a regulator of neuronal intrinsic excitability that is known to bind and modulate voltage gated sodium channels. In addition, *FGF14* is the locus of mutations responsible for spinal cerebellar ataxia 27 (SCA27), an autosomal dominant disease characterized by progressive ataxia and cognitive decline. A number of SCA27 linked mutations in *FGF14* have now been identified, of these, the first was the missense mutation $FGF14^{145S}$, identified in an extended Dutch family. Recent studies have demonstrated that $Fgf14^{-/-}$ Purkinje neurons have attenuated intrinsic excitability, but, repetitive firing properties similar to wild type Purkinje neurons can be rescued by prior membrane hyperpolarization, suggesting that attenuation of excitability may be caused by a change in the transient sodium current, I_{Na}, steady-state inactivation. To test this hypothesis directly, we used a recently developed voltage-clamp technique to obtain recordings of I_{Na} in Purkinje neurons in acute slice preparations from $Fgf14^{-/-}$ and $Fgf14^{+/-}$ animals, and compared them with wild type. Results in these studies indicate that steady-state inactivation of I_{Na} in $Fgf14^{-/-}$ Purkinje neurons is significantly more hyperpolarized than in wild type Purkinje neurons (wild type $V_{1/2}$ = -55mV, $Fgf14^{-/-}$ $V_{1/2}$= -73mV, $p<.001$). These data demonstrate directly that iFGF14 has an effect on I_{Na} steady-state inactivation in Purkinje neurons. To investigate directly the effects of the $FGF14^{145S}$ human mutant protein on Purkinje neuron excitability we developed a knockin mouse model. Results to date reveal a marked decrease in spontaneous firing in $FGF14^{145S\ +/-}$, consistent with the autosomal dominant / haploinsufficient genetics of SCA27. We also found $Fgf14^{+/-}$ Purkinje neuron spontaneous firing is attenuated when compared to wild type, demonstrating that iFGF14 haploinsufficiency also affects Purkinje neuron firing properties.

Disclosures: J.L. Ransdell: None. **M. Xiao:** None. **D.M. Ornitz:** None. **J.M. Nerbonne:** None.

Poster

507. Cellular Mechanisms of Modulation of Neuronal Firing Properties

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 507.04/H2

Topic: B.10. Intrinsic Membrane Properties

Support: NIH Grant NS036855

NIH Grant NS080323

Title: Kv2 and BK channels in substantia nigra dopamine neurons differentially regulate spontaneous and burst-like firing

Authors: *C. KIMM, B. P. BEAN
Dept. of Neurobio., Harvard Med. Sch., Boston, MA

Abstract: Dopamine neurons of the *substantia nigra pars compacta* (SNc) have two major modes of activity: tonic slow pacemaking and higher-frequency bursts evoked by synaptic input. Substantial work has explored the ionic mechanisms underlying the spontaneous depolarization of these cells and the commencement and cessation of burst firing, but less is known about the voltage-dependent potassium currents contributing to spike repolarization and to the timing of action potentials within a burst. Performing action potential clamp experiments using acutely-dissociated mouse SNc dopamine neurons at 37°C, we found that the outward current flowing during spike repolarization included major contributions from BK calcium-activated potassium channels (sensitive to paxilline) and Kv2 channels (sensitive to GxTx-1E). Given the large BK current during action potentials, we were surprised that in current clamp experiments, application of paxilline had only a small effect on spike width (1.47 ± 0.14 ms in control to 1.89 ± 0.11 ms in 300 nM paxilline, mean ± S.E.M., n=16) and no clear effect on spontaneous firing frequency. Similarly, GxTx-1E also had small effects on spike width (1.60 ± 0.15 ms in control to 1.86 ± 0.17 ms in 100 nM GxTx-1E, n=18) and no clear effect on spontaneous firing frequency. However, co-application of paxilline and GxTx-1E produced a dramatic effect on spike width (1.57 ± 0.14 ms in control to 5.85 ± 0.87 ms in 300 nM paxilline + 100 nM GxTx-1E, n=24) and decreased firing rate from 7.4 ± 1.0 Hz to 4.2 ± 0.4 Hz (n=24). Further experiments revealed large “reserves” of both BK and Kv2 currents relative to the current activated during individual action potentials and showed that acute inhibition of either current led to additional recruitment of the other, minimizing changes in spike width. While inhibition of either Kv2 or BK channels alone had no effect on spontaneous firing frequency, higher-frequency firing evoked by current injection was differentially altered. Blocking BK channels resulted in reduction in maximal firing frequency and flattening of the F-I curve, while inhibiting Kv2 channels resulted in faster firing and steeper F-I curves. Thus, BK and Kv2 channels have a high degree of “redundancy” during spontaneous action potentials but differentially regulate evoked firing.

Disclosures: C. Kimm: None. **B.P. Bean:** None.

Poster

507. Cellular Mechanisms of Modulation of Neuronal Firing Properties

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 507.05/H3

Topic: B.10. Intrinsic Membrane Properties

Support: FONDECYT 1171140

ANILLO ACT-1109

CONICYT PhD scholarship

Title: Localization and characterization of the axon initial segment of *in vivo* labeled individual substantia nigra dopaminergic neurons in the mouse

Authors: *R. MEZA[1,2], A. OÑATE[1], N. M. DOIG[1], M. FAUNES[1], P. HENNY[1]
[1]Anatomy, Sch. of Med., [2]Physiological Sciences, Fac. of Biol. Sci., Pontificia Univ. Católica De Chile, Santiago, Chile

Abstract: Dopaminergic neurons of the substantia nigra compacta (SNc) play a key role in movement control, motivated behavior and learning. The electrical activity of dopaminergic neurons is characterized by low frequency, regular or irregular firing and is mostly controlled by intrinsic membrane properties. This tonic activity is punctuated by brief periods of high-frequency burst firing, as well as short pauses in firing, which are synaptically mediated and have been shown to encode behaviorally salient stimuli. In spite of these common firing characteristics, there exists a large variation in individual neurons activity and driven responses to stimuli. In most nigral dopaminergic neurons, the axon does not emerge from the cell body, but from primary or secondary dendrites. The most proximal region of the axon, the initial segment (AIS), is an unmyelinated structure and is where the action potentials are generated. The AIS is enriched in sodium channels and other voltage gated channels, as well as scaffolding proteins such as Ankyrin-G (Ank-G). The aim of this project is to examine whether the structural characteristics, including localization, of the AIS relate to the diverse activity patterns showed by these neurons. In order to address this question, dopaminergic SNc neurons were recorded in adult male mice under urethane anesthesia. Neurons were recorded during spontaneous basal activity and after the application of nociceptive somatosensory stimulation to the hind paw. Recorded neurons were labeled using the juxtacellular method with neurobiotin, after which animals were perfused and their brains removed and serially sectioned. Labeled neurons were revealed using streptavidin and identified as tyrosine hydroxylase positive using immunofluorescence. In order to reconstruct the neurons, Z-stacks of images from all sections containing labeled dendritic and local axon fragments were obtained using a confocal microscope. Fragments were traced and spliced to render a 3D reconstruction of the somato-dendritic and local axonal domain using Neurolucida (MBF). The localization of the AIS was inferred from reconstructions and further confirmed using immunofluorescence staining for Ank-

G. Preliminary results confirm a heterogeneous axon origin and AIS structure in these neurons, and analyses are being carried out to test a correlation of these parameters with spontaneous and driven firing patterns recorded *in vivo*.

Disclosures: **R. Meza:** None. **A. Oñate:** None. **N.M. Doig:** None. **M. Faunes:** None. **P. Henny:** None.

Poster

507. Cellular Mechanisms of Modulation of Neuronal Firing Properties

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 507.06/H4

Topic: B.10. Intrinsic Membrane Properties

Support: Okinawa Institute of Science and Technology

Human Frontiers Grant

Title: Burst-pause-burst pattern in striatal cholinergic interneurons: Can they pause without bursting?

Authors: ***A. ZUCCA**, S. AOKI, A. LIU, S. ZUCCA, J. WICKENS
Wickens Unit, Okinawa Inst. of Sci. and Technol., Kunigamigun, Japan

Abstract: Striatal cholinergic interneurons (sCINs) are local interneurons representing only 1-2% of neurons in the striatum of rodents. Although sparse, studies show that they have an important role in reinforcement-learning and movement. sCINs display tonic firing activity *in vivo* and electrophysiological recordings *in vivo* and *in vitro* reveal a burst-pause-burst pattern of firing. We are interested in understanding the functions of the pause for the rest of the striatum. Here we ask if we can alter and manipulate the burst-pause-burst pattern (i.e. Can we cause a pause without bursting?) and further examine rebound firing. Using transgenic rats that express cre-recombinase under the control of choline acetyltransferase (Witten, I. B. et al. Neuron 72, 721-733, 2011) and cre-dependent expression of opsins via AAV injection in the dorsal striatum, we analyzed the electrophysiological properties of sCINs. During hyperpolarization-induced rebound firing we manipulated their characteristic burst-pause-burst pattern with light in an acute slice preparation as a prequel to *in vivo* experimentation. In our condition, rebound firing of sCINs is dependent on several parameters: hyperpolarization potential, duration of hyperpolarization, resting membrane potential and shape of hyperpolarizing waveform.

Halorhodopsin and Archaerhodopsin hyperpolarize the membrane, creating a pause. This synchronous and strong hyperpolarization induces a robust burst after a square light pulse, possibly masking the effect of a pause alone. By being able to prevent firing after the pause, or induce a pause without inducing a burst, we hope to separately identify the role of the pause and burst on post-synaptic cells and during certain behaviors.

Disclosures: A. Zucca: None. **S. Aoki:** None. **A. Liu:** None. **S. Zucca:** None. **J. Wickens:** None.

Poster

507. Cellular Mechanisms of Modulation of Neuronal Firing Properties

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 507.07/H5

Topic: B.10. Intrinsic Membrane Properties

Support: AHA Grant

MU Research Board Grant

NIH IMSD Fellowship

Title: Coupling compartmental models to live neurons to investigate action potential mechanisms

Authors: *M. A. NAVARRO[1], S. L. DEBS[2], L. S. MILESCU[1]
[1]Biol. Sci., Univ. of Missouri-Columbia, Columbia, MO; [2]Whitman Col., Walla Walla, WA

Abstract: In mammalian central neurons, action potentials are initiated in the axonal initial segment (AIS) by Nav1.2 and Nav1.6 channels, and shaped and terminated by other voltage-gated ion channels. From the AIS, the AP travels down the axon towards the presynaptic site, but also back-propagates towards the soma. The role of axonal sodium channels in AP initiation and propagation is still incompletely understood, mostly because it is difficult to record from these channels at the AIS. Instead, most experimental evidence of axonal activity is obtained indirectly, from electrical recordings at the soma. To better understand these mechanisms, we developed a real-time computational procedure where a compartmental model of the axon is coupled to a live neuron using dynamic clamp. The properties of this computational model (e.g., spatial distribution and kinetics of ion channels) are varied until the firing activity of the hybrid construct (neuron + axon compartmental model) best matches the normal activity of the neuron.

Disclosures: **M.A. Navarro:** None. **S.L. Debs:** None. **L.S. Milescu:** None.

Poster

507. Cellular Mechanisms of Modulation of Neuronal Firing Properties

Location: Halls A-C

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Topic: B.10. Intrinsic Membrane Properties

Support: NIH Grant GM08016

NIH Grant MD007597

HUCM BFPSAP

Title: Toad venoms resibufogenin and cinobufagin activate central neurons through a ouabain-like action

Authors: Z.-J. WANG, L. SUN, *T. HEINBOCKEL
Anat., Howard Univ. Coll Med, Anat., WASHINGTON, DC

Abstract: Cinobufagin and resibufogenin are two major effective bufadienolides of Chan su (toad venom) which is a Chinese medicine obtained from the skin venom gland of toads. It is used as a cardiotonic and CNS respiratory agent, as analgesic and anesthetic and as remedy for ulcers. Many clinical cases showed that Chan su and Chan su-containing formulations have severe side-effects on the CNS, causing shortness of breath, breathlessness, seizure, coma and cardiac arrhythmia. We used whole-cell recordings from brain slices to determine the effects of bufadienolides on excitability of a principal neuron in the main olfactory bulb, mitral cells, and the cellular mechanism underlying the excitation. At higher concentrations, cinobufagin and resibufogenin induced irreversible over-excitation of mitral cells indicating a toxic effect. At lower concentrations, they concentration-dependently increased spontaneous firing rate, depolarized the membrane potential of mitral cells, and elicited inward currents. The excitatory effects were due to a direct action on mitral cells rather than an indirect phasic action. Bufadienolides and ouabain had similar effects on the firing of mitral cells which suggested that bufadienolides activated neurons through a ouabain-like effect, most likely by inhibiting Na+/K+-ATPase. The direct action of bufadienolide on brain Na+ channels was tested by recordings from stably Nav1.2-transfected cells. Bufadienolides failed to make significant changes of the main properties of Nav1.2 channels in current amplitude, current-voltage (I-V)

relationships, activation and inactivation, suggesting that bufadienolide-evoked activation of neurons is not mediated by the Na+ channels. Our results suggest that inhibition of Na+/K+-ATPase may be involved in both the pharmacological and toxic effects of bufadienolide-evoked CNS excitation.

Disclosures: Z. Wang: None. **T. Heinbockel:** None. **L. Sun:** None.

Poster

507. Cellular Mechanisms of Modulation of Neuronal Firing Properties

Location: Halls A-C

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Program#/Poster#: 507.09/H7

Topic: B.10. Intrinsic Membrane Properties

Support: CONACyT CB166241

CONACyT 243422

Title: Propylparaben decreases the neuronal excitability of Hippocampal CA1 pyramidal cells *in vitro*

Authors: *L. LARA, L. ROCHA, E. J. GALVÁN
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Abstract: Propylparaben (PPB) is an antimicrobial preservative widely used in food, cosmetics and pharmaceutics. Previous studies demonstrate that PPB blocks voltage-dependent sodium channels; virtual screening studies suggest that topological descriptors of PPB share similarities with certain antiepileptic drugs of clinical use. To further explore the effects of PPB, patch clamp experiments were carried out on acute hippocampal slices and changes in the intrinsic excitability of CA1 neurons were tested in the presence of PPB. In current clamp mode, cells were held at -65 mV and current steps (1 sec duration, 50 pA increase) were injected to generate burst of action potentials (APs). Increasing concentration of PPB (100, 200 and 500 μM) blocked the evoked APs in all the cells tested (n=34) in a concentration dependent manner. Systematically, PPB increased the rheobase current to evoke APs (Rheobase increase: 10 ± 9%; 80 ± 32% and 220 ± 23%, for 100, 200 and 500 μM, respectively) and decreased the input resistance of pyramidal cells (Rn decrease 14.6 ± 5.4% and 21.8 ± 5.6 for 200 and 500 μM, respectively). In another set of experiments, cells were clamped at -65 mV and slow voltage ramps (20 mV/s) from -98 to -28 mV were applied. In control ACSF, neurons elicited burst of

action currents (29 Hz). In the presence of PPB (200 μM), the action currents generated at -45 mV were abolished and conductance responses turned linear. Lastly, we assessed the antiepileptic potential of PPB. Gap-free activity was acquired in current clamp mode, and epileptiform activity was induced with 4-AP (100 μM) + Mg2+-free ACSF. Spontaneous APs were very rare during baseline (frequency was less than 0.01 Hz). The perfusion of 4-AP generated paroxysmal depolarizing shifts (PDS) with burst of APs (8.9 Hz). Both, PDS and AP burst were reverted by the perfusion of PPB (AP burst activity decreased to 0.7 Hz). We surmise that PPB blocks the voltage dependent sodium channels, therefore neuronal excitability decreases.

Disclosures: L. Lara: None. **L. Rocha:** None. **E.J. Galván:** None.

Poster

507. Cellular Mechanisms of Modulation of Neuronal Firing Properties

Location: Halls A-C

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Program#/Poster#: 507.10/H8

Topic: B.10. Intrinsic Membrane Properties

Support: ANR Grant ANR-10-IAIHU-06

Title: Impact of background synaptic activity on the excitability and integrative properties of somatosensory cortex neurons *in vivo*

Authors: *T. ALTWEGG-BOUSSAC[1], M. CHAVEZ[1], S. DEMERET[2], V.-H. NGUYEN MICHEL[3], V. NAVARRO[3,1], S. MAHON[1], S. CHARPIER[1]
[1]UPMC Univ. Paris 06, UMR S 1127, Inserm U1127, ICM, Paris, France; [2]Neurolog. Intensive Care Unit, AP-PH, Pitié-Salpêtriere Hôpital, Paris, France; [3]Epilepsy Unit, AP-PH, Pitié-Salpêtriere Hôpital, Paris, France

Abstract: In the absence of any environmental stimuli, the brain endogenously generates spontaneous electrical activity that can be recorded from the surface of the brain, as electroencephalographic (EEG) waves, and in individual neurons, as a complex barrage of excitatory and inhibitory synaptic potentials. This endogenous brain electrical activity varies as a function of vigilance states and can be dramatically altered during pathological states, such as epilepsies and comas. We have investigated by the means of surface EEG coupled with intracellular recordings in the rat *in vivo*, the impact of an oscillatory or tonic background synaptic activity on the membrane excitability and firing responses of somatosensory cortex

pyramidal neurons. The two network activities were induced by systemic injection of pentobarbital or fentanyl, allowing general analgesia and mimicking sleep- and waking-like cortical patterns, respectively. We compared the neuronal electrical properties in the two states to those acquired after complete removal of background activity by injection of high doses of pentobarbital, leading to isoelectric states in both neurons and EEG. Compared to the oscillatory state, the tonic spontaneous activity resulted in a more depolarized and less fluctuating membrane potential (Vm), a lower input resistance (Rm) and steeper relations of firing frequency versus injected current (F-I curves). Suppression of background synaptic activities caused a Vm hyperpolarization, without any change in Rm, and induced a rightward shift of F-I curves. Both types of synaptic drive generated a high variability in current-induced firing rate and pattern, which was much reduced after removal of spontaneous activity. These findings indicate that endogenous brain activity increases the sensitivity of cortical neurons to weak inputs and is responsible for a large variability in firing responses. The functional outcome of this differential modulation of cortical excitability as a function of background synaptic activities is currently investigated in our lab. We are analyzing and comparing, in the presence and absence of synaptic activity, the sensory responses evoked in somatosensory cortical neurons by the application of controlled air-puff stimuli to the whiskers. In collaboration with the Intensive Care Unit of the Hospital, we are also investigating the sensory reactivity of the isoelectric cortex in human patients suffering from “status epilepticus”, and maintained, for therapeutic purpose, in deep comatose with high doses of pentobarbital.

Disclosures: **T. Altwegg-boussac:** None. **M. Chavez:** None. **S. Demeret:** None. **V. Nguyen michel:** None. **V. Navarro:** None. **S. Mahon:** None. **S. Charpier:** None.

Poster

507. Cellular Mechanisms of Modulation of Neuronal Firing Properties

Location: Halls A-C

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Program#/Poster#: 507.11/H9

Topic: B.10. Intrinsic Membrane Properties

Support: NIH AG008796

AG017139

Title: Calbindin-D28K restores the intrinsic excitability properties of aged CA1 pyramidal neurons to young-like state

Authors: *D. SIMKIN, A. HOFFMAN, M. M. OH, J. F. DISTERHOFT
Physiology, Feinberg Sch. of Med., Northwestern Univ., Chicago, IL

Abstract: Aging- and Alzheimer's-related Ca2+ dysregulation is implicated in impaired synaptic plasticity and learning/memory (Khachaturian '94, Thibault, et al. '07). Activity-dependent Ca2+ influx is enhanced in CA1 pyramidal neurons from aged animals (Tombaugh, et al. '05) overwhelming endogenous Ca2+ binding/buffering proteins (CBP) leading to an enlarged Ca2+-dependent postburst-afterhyperpolarization (sAHP) in these neurons (Disterhoft and Oh '06, Tombaugh, et al. '05). Recently, we have discovered that the endogenous buffer capacity for Ca2+ is enhanced in aged CA1 neurons (Oh, et al. '13). Also, a previous report suggests that reductions in a classical CBP calbindin-D28K (CB) correlates with aging-related learning and memory deficits (Soontornniyomkij, et al. '12). Therefore, we hypothesize that enhancing endogenous Ca2+ buffer(s) will rescue aging-related intrinsic excitability and cognitive deficits. Although chemical Ca2+ chelators (e.g. BAPTA) have been shown to impact the intrinsic excitability of pyramidal neurons, it remains to be determined if increasing the endogenous CBP level will increase excitability of CA1 neurons by reducing the sAHP. Hence, we evaluated the intrinsic excitability properties (including: sAHP, spike frequency accommodation, and ability to fire trains of APs at θ-burst frequencies) of CA1 pyramidal neurons from young (2-4 mo) and aged (29-31 mo) rats after intracellular delivery (through internal pipette solution) of purified CBP (calbindin-D28K). We observed that CBPs reduce the sAHP and accommodation in aged CA1 pyramidal neurons to young-like levels. Therefore, these data suggest that aging-related intrinsic excitability and cognitive deficits could be ameliorated by increasing the endogenous CBPs by use of gene therapeutics (e. g. AAV-mediated gene transfer).

Disclosures: D. Simkin: None. **A. Hoffman:** None. **M.M. Oh:** None. **J.F. Disterhoft:** None.

Poster

507. Cellular Mechanisms of Modulation of Neuronal Firing Properties

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Program#/Poster#: 507.12/H10

Topic: B.10. Intrinsic Membrane Properties

Support: NIH Grant MH093412

Brain and Behavior Research Foundation

Title: Effects of oxytocin on intrinsic properties of pyramidal neurons in rat insular cortex

Authors: *J. A. VARELA, J. CHRISTIANSON
Psychology, Boston Col., Chestnut Hill, MA

Abstract: The neuropeptide oxytocin (OT) is a highly conserved neural modulator and is known to contribute to social, feeding, and fear behaviors. OT positive axons and OT receptors (OTR) are found in the insula but not in many adjacent cortical areas of adult male rats. The present study sought to describe the effects of OT on layer 5/6 pyramidal neurons of the insular cortex. Visualized whole cell patch clamp recordings were made from 300μm slices taken from 4 to 6 week old male Sprague Dawely rats (N=15, neurons N=30); cells were included for analysis if they were found in the deep layers of Agranular or Granular insular cortex 2.8mm (+/- .5mm) caudal to bregma and appeared to have pyramidal morphology (confirmed by biocytin staining). Active and passive membrane properties were determined first in conventional aCSF and 10 min after perfusion with aCSF containing 500nM OT. OT caused a number of significant changes(ps < 0.05), namely: Depolarization of the resting potential, increase in input resistance and rheobase, decrease in SAG ratio and membrane time constant, decrease in action potential (AP) amplitude and AP rise rate and increase in AP halfwidth. The input-output curve, measured as APs/s evoked by positive current injections was left-shifted. Simultaneous application of the phospholipase C (PLC) inhibitor, U 73122 (10 μM), prevented OT effects on AP amplitude, input resistance and the input-output curve. (N=5, neurons N = 5/group). Taken together these data suggest that the OT alters the intrinsic neuronal excitability in the insula through a GPCR/PLC mediated mechanism.

Disclosures: J.A. Varela: None. **J. Christianson:** None.

Poster

507. Cellular Mechanisms of Modulation of Neuronal Firing Properties

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Topic: B.10. Intrinsic Membrane Properties

Support: Wellcome Trust 087497

Lendület - Hungarian Academy of Sciences - LP/2009-009

Stephen W. Kuffler Research Foundation

Title: Functionally distinct populations within anatomically similar CCK-expressing hippocampal interneurons due to different availability of potassium conductances

Authors: V. J. OLAH, *J. SZABADICS
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Abstract: Specific functions of various voltage-dependent ionic channels contribute to the functional divergence of GABAergic cell types. The firing properties are specific for distinct cell types and are important for their function within the neuronal networks as a general determinant of their integrative properties. Furthermore, the firing properties are often used for the identification of the recorded cell types because these complex properties are determined by the cell-type specific combinations of ionic channels. Here we provide evidence for an exception for the cell-type specific firing patterns by showing that within anatomically and functionally identified same cell types, individual GABAergic cells can have markedly different firing properties. Using whole-cell patch clamp recordings and anatomical identification, we distinguished two populations of CCK-expressing GABAergic cells (CCK-IN) in the CA3 region of the hippocampal acute slices based on the presence or the absence of state-dependent firing properties. In the first subset of CCK-INs, action potentials are inhibited at the onset when the firing patterns were evoked from hyperpolarized membrane potentials (at -80mV). However, this transient outward rectification (TOR) was absent in the same cells at depolarized membrane potentials (-60mV). The second subset of morphologically indistinguishable CCK-INs did not show state-dependent inhibition of action potential generation (non-TOR cells). Interestingly, the presence or absence of the state-dependent firing characteristic did not correlate with any of the previously known anatomical subdivision of CCK-expressing GABAergic cells (including VGlut3, SATB1 and calbindin expression, axonal arborization), thus the state dependent firing provide an novel level of complexity within this cell group. Voltage-clamp recordings revealed that TOR CCK-INs have substantial A-currents with leftward shifted activation and inactivation voltages, which can explain the state dependent firing. Furthermore, the state-dependent inhibition of firing was inhibited by high concentration of 4-AP; whereas TEA and low concentration of 4-AP did not change the onset firing suggesting the involvement of specific potassium channels (potentially Kv4.3 and KChIP). Altogether, the results revealed that different availability of an A-type potassium conductance can results in different functionality among anatomically homogeneous CCK-INs in the CA3 region.

Disclosures: V.J. Olah: None. **J. Szabadics:** None.

Poster

507. Cellular Mechanisms of Modulation of Neuronal Firing Properties

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

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Topic: B.10. Intrinsic Membrane Properties

Support: MHLW Grant-in-Aid 11103605

KAKENHI 24240076

KAKENHI 24500269

Title: Effect of maternal bisphenol A exposure on network excitability in mouse hippocampal slices assessed by voltage-sensitive dye imaging

Authors: *Y. TOMINAGA[1], K. IGARASHI[2,3], M. I. OTSUKA[2,3], Y. FURUKAWA[3], J. KANNO[3], K. TANEMURA[4], T. TOMINAGA[1]
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Abstract: There are increasing concerns about disruption of brain function due to exposure to environmental chemical substances, especially in early development. We aimed to develop a method to assess such effects on neuronal circuit activity. Bisphenol A (BPA), which is widely used in the production of polycarbonate plastics and epoxy resins, is a potentially hazardous chemical substance. BPA can bind to estrogen receptors and affect the signaling cascade of endogenous estradiol. A number of reports have indicated that estrogen affects synaptic plasticity in the hippocampal (HP) circuit in rats and mice. In particular, maternal exposure to BPA is a great concern owing to the associated risk of delayed emotional and cognitive deficits in the offspring. Here, we aimed to assay the effects of maternal exposure to BPA on neural circuit activity after development. Pairs of male and female mice were transferred for mating into cages supplied with BPA-containing drinking water at four different concentrations (0, 0.1, 1, and 10 ppm). On confirmation of pregnancy, the male mouse was removed, and BPA-containing drinking water was supplied until weaning. Male offspring were randomly chosen from mothers maintained under the same conditions. HP slices were made by standard procedures and analyzed after staining with voltage-sensitive dye (VSD: Di-4-ANEPPS) after at least 8 weeks. Three main pathways, namely the perforant, mossy fiber, and Schaffer collateral pathways, were stimulated under wide-field optics (THT-macroscope, BrainVision LLC), and the neuronal activation patterns were recorded by a CMOS high-speed imager (MiCAM Ultima, BrainVision LLC). The neuronal responses of the HP slices of control (0 ppm) and BPA-exposed mice (0.1, 1, and 10 ppm BPA) were tested in normal and Gabazine (10 μM)-containing artificial cerebrospinal fluid. Gabazine increased the peak amplitude and duration of the response to

strong stimulation in the HP slices of both control and BPA-exposed mice. In the HP slices of control mice, even very weak stimulation was sufficient to induce large and long responses in the entire CA3-CA1, whereas the HP slices of BPA-exposed mice did not exhibit such responses. This clear difference may indicate an alteration in the threshold of excitability of the cells. These modifications in the HP circuit may be different from the deficits in valproate-treated mice reported last year. These results highlight the benefits of VSD imaging assays when assessing environmental risk factors.

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Poster

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IGSN Scholarship

Title: Switching between cholinergic-dependent mnemonic and epileptiform responses in individual hippocampal CA1 pyramidal neurons in acute rat brain slice preparations

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Abstract: The cholinergic system supports memory but is also involved in mesial temporal lobe epilepsy. We previously showed that individual CA1 pyramidal neurons may support short-term maintenance of information by persistent firing which is action potential generation that outlasts the stimulation. Other groups have shown that CA1 pyramidal neurons engage in persistent activity which was accompanied by depolarization block and is termed here as epileptiform response. Both forms of responses require the activation of muscarinic acetylcholine receptors (mAChRs). Preventing epileptiform activity, while allowing the neurons to exhibit memory-related forms of activity may be of clinical interest. However, it remains unclear what distinguishes these two responses. In this study, we aim to clarify what down-stream

mechanisms of mAChR stimulation determine whether persistent firing or an epileptiform response is induced. We used *in vitro* whole-cell patch clamp recordings from hippocampal CA1 pyramidal neurons in acute brain slices from P14-24 Long-Evans rats. While recording, slices were submerged in 35±1°C artificial cerebrospinal fluid with ionotropic synaptic blockers (2 mM kynurenic acid, 0.1 mM picrotoxin). We first present that stronger activation of mAChRs (5, 10, 20 μM carbachol (Cch)) increased the likelihood of epileptiform responses. One of the down-stream effects of mAChR activation is the gating of transient receptor potential (TRPC) channels. We show that elevated concentrations of intracellular ATP (0, 2, 4, 10 mM) and the blockade of TRPC4/5 channels (20 μM ML204) depressed both persistent firing and epileptiform responses. This suggests that the TRPC channels support depolarization in both responses. Another down-stream effect of mAChR stimulation is the reduction of post-burst afterhyperpolarization (AHP). In the presence of Cch, additional blockade of the SK- (100 nM apamin) and/or M-channels (20 μM XE-991) often switched persistent firing to epileptiform responses. Under blockade of SK- and M-channels but in the absence of Cch, we observed depolarization block during the current injection. Neither the persistent firing nor the epileptiform response that outlasted the current injection was observed in this condition. These results suggest that TRPC channels and AHP currents (SK- and M current) both contribute to the transition between persistent firing and epileptiform response. Our results indicate that individual neurons may switch between persistent firing and epileptiform responses by modulating single cell properties down-stream of mAChR activation which may affect network activity *in vivo* and may have clinical implications.

Disclosures: B. Knauer: None. **M. Yoshida:** None.

Poster

507. Cellular Mechanisms of Modulation of Neuronal Firing Properties

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Wisconsin Distinguished Rath Graduate Fellowship to C.M.F.

Title: Ketamine differentially affects neuronal activity in primary and high order sensory cortex in mice

Authors: *C. M. FUNK[1,2], S. HONJOH[1], A. V. RODRIGUEZ[1,2], C. CIRELLI[1], G. TONONI[1]
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Abstract: Ketamine anesthesia produces sensory disconnection from the environment that is associated with intense dreaming in humans. Ketamine induces varied effects on neuronal spiking both directly through antagonism of NMDA receptors and indirectly by disinhibition of excitatory neurons and alteration of the neuromodulatory milieu. These complex and often opposing effects undermine attempts to predict how ketamine may influence specific brain areas and necessitate *in vivo* recordings of neuronal activity in order to determine the overall response of a given brain region to ketamine. We performed laminar recordings in primary visual cortex (V1) and retrosplenial cortex (RS) in mice to examine whether ketamine differentially affects neuronal activity in primary and high order sensory cortex. We implanted adult male mice (n=4) with 16ch silicon laminar probes in V1 and/or RS. Following recovery from surgery, local field potentials and neuronal activity were recorded continuously along with epidural EEG and EMG. On experimental days (n=4, 1 per mouse), 100mg/kg ketamine was administered IP. Behavioral state was determined by assessing the righting reflex (RR) and other behaviors. Administration of ketamine led to two distinct behavioral phases. First, mice lost the RR and remained immobile for 821 +- 185 seconds. Upon recovery of the RR, mice exhibited locomotion that resembled crawling and a tendency to approach corners of the clear plastic cage, where they continued to perform crawling movements, as if attempting to "walk through" the wall. At the neurophysiological level, periods of neuronal silence (OFF periods) dominated throughout the first phase in both V1 and RS (54+-27% and 48+-9% time spent in OFF periods, respectively). However, neuronal firing levels during ON periods differed markedly between areas. In V1, spiking during ON periods was decreased by 80% compared to baseline wake ($p<0.05$), while in RS, ON period firing rates exceeded baseline levels by more than 100% ($p<0.005$). In the second phase, percent time OFF decreased in both areas ($p<0.05$ for both). In V1, ON period firing rates gradually increased toward baseline levels, with deep layers recovering more quickly than superficial layers. In RS, elevated ON period spiking persisted throughout the second phase. These findings reveal that ketamine attenuates V1 activity while triggering periods of intense firing in RS. This striking difference may reflect an imbalance in feedforward and feedback signaling, whereby weak feedforward activity in V1 is occluded by strong feedback signaling from RS, perhaps contributing to the sensory disconnection caused by ketamine.

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Poster

507. Cellular Mechanisms of Modulation of Neuronal Firing Properties

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Title: Effects of ambient neuromodulators in cerebrospinal fluid on neuronal activity in the hippocampus

Authors: *A. BJOREFELDT, U. ANDREASSON, J. DABORG, I. RIEBE, P. WASLING, H. ZETTERBERG, E. HANSE
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Abstract: Central neurons are surrounded by an extracellular fluid whose composition results from the continuous mixing of cerebrospinal fluid (CSF), occupying the ventricles and subarachnoid space, with interstitial fluid of the parenchyma. The CSF is known to harbor many different neuromodulatory substances at ambient levels, but whether they actually influence the activity of central neurons is still unknown. By using a matched artificial CSF as control, we show that human CSF (hCSF) strongly increases spontaneous excitatory, but not inhibitory, activity in the hippocampus. In CA1 neurons, hCSF lowers the action potential threshold and depolarizes the resting membrane potential to boost spontaneous firing. Clamping G-protein activity in CA1 neurons with GTPγS completely occluded these effects, suggesting that the neuromodulators in hCSF act specifically via G-protein coupled receptors to increase spontaneous excitatory activity. We also found an augmentation of evoked glutamatergic transmission that was associated with an increased release probability at CA3-CA1 synapses, and a slightly increased presynaptic axonal excitability. Taken together, these findings show that ambient neuromodulators in brain extracellular fluid potently increases the activity of pyramidal neurons, but not interneurons, and thus promote spontaneous excitatory activity. Our results may help explain differences in the level of spontaneous activity displayed by pyramidal neurons in *in vivo* and *in vitro* recordings.

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Poster

507. Cellular Mechanisms of Modulation of Neuronal Firing Properties

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Topic: B.10. Intrinsic Membrane Properties

Title: Amyloid β-protein induces hippocampal neuron hyperexcitability through A-type K+ current inhibition mediated by caspases and GSK-3 activation

Authors: F. SCALA[1], S. FUSCO[1], C. RIPOLI[1], R. PIACENTINI[1], D. D. LI PUMA[1], M. SPINELLI[1], F. LAEZZA[2], C. GRASSI[1], *M. D'ASCENZO[1]
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Abstract: Amyloid β-protein (Aβ) pathologies have been linked to dysfunction of excitability in hippocampal neurons, but the molecular mechanisms underlying this process are still poorly understood. Here we applied whole-cell patch-clamp electrophysiology to primary hippocampal neurons and show that intracellular $A\beta_{42}$ delivery leads to increased spike discharge and action potential broadening through down-regulation of A-type K^+ currents. Pharmacological studies showed that caspases and GSK-3 activation are required for these $A\beta_{42}$-induced effects. Extracellular perfusion and subsequent internalization of $A\beta_{42}$ increase spike discharge and promote GSK-3-dependent phosphorylation of the Kv4.2 α-subunit, a molecular determinant of A-type K^+ currents, at Ser-616. In acute hippocampal slices derived from an adult triple-transgenic Alzheimer's mouse model (3xTg-AD), characterized by early intracellular accumulation of $A\beta_{42}$, CA1 pyramidal neurons exhibit hyperexcitability accompanied by increased phosphorylation of Kv4.2 at Ser-616. Collectively, these data suggest that intraneuronal $A\beta_{42}$ accumulation leads to an intracellular cascade culminating into caspases activation and GSK-3-dependent phosphorylation of Kv4.2 channels. Taken together these data provide novel insights into the toxic mechanisms triggered by intracellular $A\beta_{42}$ and offer potentially new therapeutic targets for Alzheimer's disease treatment.

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Poster

507. Cellular Mechanisms of Modulation of Neuronal Firing Properties

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Topic: B.10. Intrinsic Membrane Properties

Support: MRC

Title: Overexpression of tau augments afterhyperpolarizations in rat CA1 hippocampal neurons

Authors: *T. W. CHURCH[1], J. T. BROWN[3], N. V. MARRION[2]
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Abstract: Cognitive impairment is seen in a number of diseases involving the alteration and physiological dysfunction of the microtubule-associated protein tau. These disease states, known as tauopathies, are characterised by progressive neurodegeneration, but it is unknown whether changes in excitability precede cell death to underlie cognitive decline in the early stages of disease. To examine the physiological effects of tau overexpression, rat organotypic hippocampal slices were infected with the Sindbis virus encoding the human 4R0N isoform of tau. Whole-cell current-clamp recordings were made from CA1 pyramidal neurons labelled with co-expressed eGFP. Hippocampal CA1 neurons, overexpressing wildtype tau, displayed an augmented afterhyperpolarization (AHP) following a train of 15 action potentials (APs). Both components of the AHP (medium and slow AHP respectively) increased in amplitude compared with control by 62% and 100% respectively. The effect of tau overexpression on the medium and slow AHP was reproduced by overexpression of a mutant form of tau with a single point mutation (P301L) that is found in frontotemporal dementia patients, shown by a 63% and 93% augmentation on the mAHP and sAHP, respectively. Passive and active properties of hippocampal CA1 neurons were examined in cells overexpressing eGFP and eGFP + tau. A small but significant increase in the membrane time constant was observed in cells overexpressing tau, correlating with an apparent change in cell morphology, specifically in the structure of the distal dendritic and axonal arbours. Furthermore, AP threshold was shifted to more hyperpolarized potentials, but no change in the AP rate of rise or duration was observed. Evoking AP firing from a resting potential of -75 mV resulted in a mAHP driven by activation of both SK and H current. Bathing infected slices in Ca2+-free extracellular solution showed that tau overexpression augmented the SK channel component of the mAHP. The dependence of the sAHP on extracellular Ca2+ was maintained in cells expressing tau. These data, together with the finding that intracellular cAMP was able to abolish the augmented sAHP, suggests that tau overexpression does not change the ion channel subtype that underlies the sAHP. These data

indicate that the overexpression of wild-type tau reduced hippocampal pyramidal cell excitability by enhancing AHPs that regulate cell firing. This effect might occur in concert with a change in cell morphology and suggests that dysregulation of tau expression could result in changes in excitability that may underlie cognitive impairment observed in the early stages of tauopathies.

Disclosures: T.W. Church: None. **J.T. Brown:** None. **N.V. Marrion:** None.

Poster

507. Cellular Mechanisms of Modulation of Neuronal Firing Properties

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Topic: B.10. Intrinsic Membrane Properties

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ECNU Initiating Grant

Title: Activation of muscarinic receptors underlies cholinergic modulation of serotonergic neurons in the brainstem of ePet-EYFP mice

Authors: *Y. DAI[1], L. M. JORDAN[2]
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Abstract: 5-HT neurons in the parapyramidal region (PPR) of the medulla play an essential role in generating locomotion. It has been shown that stimulation of the PPR produces locomotion in the isolated neonatal rat brainstem-spinal cord preparation. However, little is known about the properties of these locomotor 5-HT neurons. Using brainstem slices from ePet-EYFP mice in which EYFP was expressed in 5-HT neurons we were able to perform whole cell patch-clamp recordings on EYFP+ neurons of the brainstem (PND 1 - PND 15). We had previously shown that the 5HT neurons received excitatory cholinergic input and displayed location-related responses to acetylcholine (ACh). Bath application of 15-30 μM ACh produced depolarization of the membrane potential in 76% of the 5-HT neurons (n=50) in PPR while 67% of 5-HT neurons (n=33) in the midline raphe nuclei (MRN) of the brainstem did not respond to ACh. In this study, we further investigated the cholinergic modulation of 5-HT neurons in the brainstem of ePet-EYFP mice with bath administration of muscarine (10-20 μM) and nicotine (10-15 μM). Muscarine could mimic the effects of ACh on 5-HT neurons in the medulla while nicotine did

not show any substantial effect on these neurons. Similar to ACh, muscarine induced depolarization of membrane potential in 70% of PPR 5-HT neurons (n=33) with decreases in rheobase (5.1±12 pA), input resistance (176.7±290 MΩ), action potential height (2.2±4 mV) and AHP depth (2.6±4 mV), whereas 72% of MRN 5-HT neurons (n=14) did not respond to muscarine. Similar to the observations with ACh, a small number of the PPR 5-HT neurons exhibited a hyperpolarization of membrane potential (6/33) or did not respond to muscarine (4/33), while a few MRN 5-HT neurons displayed depolarization (2/14) or hyperpolarization (2/14) in the presence of muscarine. Muscarine also induced or enhanced membrane oscillations in some of the 5-HT neurons. We further demonstrated that the effects of muscarine on PPR or MRN 5-HT neurons could be antagonized by 1-3 μM atropine (n=12), suggesting that the cholinergic modulation of 5-HT neurons in the brainstem of ePet-EYFP mice was mediated through activation of muscarinic receptors. Furthermore, we examined the subtype of muscarinic receptors with administration of antagonists of M1-M4 receptors (telenzepine 5-10 μM; methoctramine 5-10 μM; 4-DAMP 2-5 μM; PD102807 1-2 μM) in the presence of muscarine. Our data suggested that both M2 and M3 receptors were implicated in the muscarinic modulation of the 5-HT neurons. This study provides insight into the mechanisms underlying the cholinergic modulation of 5-HT neurons in the brainstem of ePet-EYFP mice.

Disclosures: Y. Dai: None. **L.M. Jordan:** None.

Poster

507. Cellular Mechanisms of Modulation of Neuronal Firing Properties

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Title: Hodgkin-Huxley model with fractional differentiation displays spike time adaptation

Authors: *W. W. TEKA, T. M. MARINOV, F. SANTAMARIA
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Abstract: The electrical activities of neurons have previously been described using biophysical models based on the Markov state of ion channels. Recent studies show that neuronal dynamics can be affected by the long term interactions between ion channels that have non-Markovian states. The temporal correlation and state distributions of the channels follow power law dynamics. Mathematical models with fractional (non-integer order) derivatives can be more effective than models with standard (integer order) derivatives in describing neuronal dynamics with power law distributions. In a previous paper, we showed that the emergent properties of spike time adaptation are modeled well by the Fractional Leaky Integrate- and-Fire (FLIF) model, which displays upward and downward spike adaptations without any additional adapting current. In the current study, we go beyond the functional model approach of the FLIF and apply fractional derivatives to actual biophysical models and develop the new Fractional Hodgkin-Huxley model (FHH). We find that spike time adaptation emerges from the fractional derivative of the biophysical models without any adapting potassium currents. From the Fractional Hodgkin-Huxley model emerges a temporal adapting process that is highly influenced by the integration of all the past activities (called memory trace). The effect of the memory trace on the adapting process becomes stronger when the fractional exponent is close to zero as seen in experimental data. The Fractional Hodgkin-Huxley model promises to be useful in the further study of spiking properties of neurons caused by the long term correlation of ion channels.

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Poster

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Title: Functional roles and noradrenergic modulation of calcium-dependent potassium conductances in identified subtypes of layer 5 pyramidal neurons from mouse somatosensory cortex

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

Abstract: We used BAC mouse lines that express EGFP in different subpopulations of layer 5 pyramidal neurons from somatosensory cortex to test whether functional differences between the two types of pyramidal cells were due to differential expression of calcium-dependent potassium channels. The mouse lines were (1) Tg(Etvl-EGFP)BZ192Gsat/Mmucd (*etv1*), where EGFP was only expressed in IT-type, layer 5A pyramidal neurons that had thin apical dendrites (Type 2 morphology); (2) Tg(Glt25d2-EGFP)BN20Gsat/Mmnc (*glt*), in which EGFP was expressed in PT-type, "thick-tufted" layer 5B pyramidal neurons (Type 1 morphology: Larkman and Mason, *J Neurosci* 10: 1407, 1990). We prepared acute brain slices from 2-4 week old mice. EGFP-positive cells were identified under epifluorescence and studied with whole-cell patch clamp current-clamp recordings (established using IR /DIC microscopy). E*tv1* pyramidal cells had significantly broader action potential half-width, lower dV/dt for spike polarization and repolarization, and larger slow afterhyperpolarizations as compared to *glt* pyramidal cells (Groh et al. *Cerebral Cortex* 20: 826, 2010). *Etv1* cells also exhibited greater spike frequency adaptation and reduced dc gain (lower f-I slope) for repetitive firing than *glt* cells. We found that calcium-dependent conductances contribute to functional differences between *glt* and *etv1* pyramidal cells. A large portion of the fast, medium and slow AHPs (fAHP, mAHP and sAHP, respectively) were Ca-dependent in both cell types. We did not find differences between *etv1* and *glt* cells in the effects of BK channel blockers. Whereas *glt* cells had significantly larger apamin-sensitive (sK-mediated) current, *etv1* cells had significantly larger slow AHP and I_{sAHP}. Consistent with the latter findings, apamin had a greater effect on repetitive firing in *glt* cells. The sAHP and I_{sAHP} were strongly inhibited by norepinephrine acting via β-receptors. Since the sAHP was much larger in *etv1* cells, NE had a dramatic effect on repetitive firing in *etv1* cells and almost no effect on firing in *glt* neurons. The f-I gain was increased and spike frequency adaptation reduced by NE. In the presence of NE, *etv1* cells fire similar to *glt* cells in the absence of modulation. Thus, in addition to pyramidal cells from layers 5A and 5B differing in terms of their morphology, thalamic inputs, cellular targets, and receptive field size (e.g. Manns et al. J Physiol 556: 601, 2004), they also differ in their response to neuromodulators such as NE. Additional experiments tested for differences in the expression of voltage-gated potassium conductances in *etv1* and *glt* neurons. Supported by NS44163 (to RCF).

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Poster

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Title: Small conductance calcium-activated potassium channels mediate nitric oxide effects on neuronal excitability via S-nitrosylation

Authors: *L. R. ARTINIAN, S. MCLEISH, J. EIDELMAN, V. REHDER
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Abstract: Small conductance calcium-activated potassium channels (SK channels) play an important role in controlling the neuronal firing frequency, intrinsic excitability and synaptic integration. The regulation of these channels by neurotransmitters and neuromodulators is of great importance to their function. Nitric oxide (NO) is a gaseous neuromodulator that controls neuronal excitability. In mature B5 neurons of the fresh water snail, Helisoma trivolvis, NO increases neuronal spiking by depolarizing the membrane potential via inhibition of SK channels1, and, using this mechanism, controls neuronal excitability. We demonstrated that this effect of NO was cGMP-independent 2, suggesting that the inhibition of SK channels might be mediated by S-nitrosylation, a selective and reversible posttranslational modification of cysteine residues by NO. To address this possibility, we first cloned the Helisoma SK channel (HeliSK channel) from the snail CNS. The channel protein, composed of 564 amino acids, demonstrated a high homology with mammalian SK channels, especially in the calmodulin-binding site and transmembrane domains. The channel was then heterologously expressed in HEK 293 cells. Whole-cell voltage-clamp recordings with 2μM [Ca2+] inside of the recording pipette demonstrated a functional channel that could be blocked with the SK channel specific blocker apamin. The NO donor NOC-7 (100 μM) inhibited the HeliSK channel-mediated current. To evaluate the possible regulation of the SK channel by NO via S-nitrosylation, cysteine residues in the channel protein were identified and subsequently mutated to alanine residues. A single-point mutation of one of the eight cysteine residues prevented the NO-mediated inhibition of the channel current. Interestingly, this cysteine residue is conserved among species. Taken together, our data suggest that S-nitrosylation of the HeliSK channel serves as a major regulatory mechanism by which NO controls neuronal excitability. 1. Artinian, L., Tornieri, K., Zhong, L., Baro, D. & Rehder, V. (2010) Nitric oxide acts as a volume transmitter to modulate electrical properties of spontaneously firing neurons via apamin-sensitive potassium channels. J Neurosci 30:1699-1711. 2. Artinian, L., Zhong, L., Yang, H. & Rehder, V. (2012) Nitric oxide as intracellular modulator: internal production of NO increases neuronal excitability via modulation of several ionic conductances. Eur J Neurosci 36: 3333-3343.

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Poster

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Topic: B.10. Intrinsic Membrane Properties

Support: NS027881

Title: Contributions of calcium influx and calcium-induced calcium release (CICR) to the orexin-enhanced afterhyperpolarization (oeAHP) in dorsal raphe neurons

Authors: M. ISHIBASHI, E. LYNN, *C. S. LEONARD
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Abstract: Orexin (hypocretin) neuropeptides critically regulate sleep and waking behavior along with other homeostatic processes via extensive projections to brainstem targets including serotonergic dorsal raphe (DR) neurons. Previously, we reported that orexin not only depolarizes DR neurons, but also powerfully *enhances* their Ca^{2+}-dependent afterhyperpolarizations (AHPs) resulting in slowed firing and decreased firing variability. Since the oeAHP is blocked by buffering $[Ca^{2+}]_i$ with BAPTA (10mM), and is mediated, in part, by prolonging the apamin-sensitive SK-current, we considered three factors by which orexin might influence SK currents using simultaneous whole-cell voltage-clamp and Ca^{2+} imagining in mouse brain slices. First, since SK2 currents are prolonged by de-phosphorylation, we intracellularly applied okadaic acid (25 nM), an inhibitor of the SK2-associated protein phosphatase 2A (PP2A). This did not reduce the oeAHP current, suggesting that prolongation of SK currents by orexin does not require this de-phosphorylation. Second, since the oeAHP depends on PLC, but not PKC activation, and is sensitive to depletion of Ca^{2+} stores with CPA (10 μM), we examined whether store involvement was mediated by IP3 and/or ryanadine receptors (RyRs). Intracellular application of the IP3 receptor antagonists 2-APB (50 μM) or xestospongin C (1 μM) did not reduce the oeAHP, while the RyR antagonist, ruthenium red (100 μM) significantly reduced the oeAHP. This suggests that CICR rather than IP3 receptor activation contributes to the oeAHP. We next re-examined the possibility that Ca^{2+} influx was increased by orexin, although previously we did not detect changes in somatic Ca^{2+} indicator signals. Indeed, the Ca^{2+} action currents (CACs) evoked by our depolarizing voltage trains were enhanced by orexin. To test if this resulted from increased Ca^{2+} current, we applied orexin while measuring Ca^{2+} current with a Cs^{+}-rich patch solution. Ca^{2+} currents were not increased, although the Cs^{+} solution occluded enhancement of the CACs by orexin. Since the K^{+} channel blocker 4-AP (5 mM, but not 0.5 mM) also occluded enhancement of the CACs, we examined the effect of orexin on A-type K^{+} current in DR

neurons and found that this current was inhibited by orexin. Collectively, these findings suggest that orexin-enhanced activation of SK current, which occurs as part of the oeAHP, results from suppression of A-current, spike broadening and increased Ca^{2+} influx which is then amplified by CICR in DR neurons.

Disclosures: **M. Ishibashi:** None. **E. Lynn:** None. **C.S. Leonard:** None.

Poster

507. Cellular Mechanisms of Modulation of Neuronal Firing Properties

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 507.25/I11

Topic: B.10. Intrinsic Membrane Properties

Support: Einstein Foundation Berlin

German Federal Ministry of Education and Research (01GQ0901, 01GQ1001A, 01GQ0972)

Title: Spikelets in cortical pyramidal neurons: Origin and functional consequences

Authors: ***M. MICHALIKOVA**[1], M. REMME[1], R. KEMPTER[1,2]
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Abstract: Spikelets are transient, all-or-none depolarizations of small amplitude (< 15 mV), which can be measured in somatic intracellular recordings. In hippocampal CA1 pyramidal neurons, spikelets were reported in awake behaving (Epsztein et al., Science, 2010) and anesthetized animals (Chorev & Brecht, J Neurophysiol, 2012) whereas they are rarely observed *in vitro*. Both the mechanism underlying spikelet generation and their functional significance remain unknown. Here, we investigate the emergence of spikelets using mathematical analysis and numerical simulations of simplified neuron models consisting of an axon, an axon initial segment (AIS), and somato-dendritic compartments. Somatic spikelets are produced in the model upon orthodromic (dendritic or somatic) stimulation. We find that the generation of spikelets requires the combination of two main properties of the model cell: 1) a voltage shift of several millivolts (~10 mV) of the activation of somatic versus axonal sodium channels, and 2) sufficient electrical segregation of the axonal spike initiation zone from the somato-dendritic current sink. In this way, weak orthodromic stimuli trigger APs at the AIS that fail to activate somatic sodium

channels and manifest as somatic spikelets. Stronger stimuli lead to full-size APs at the soma, either through axonal APs that backpropagate to the soma ('shouldered APs') or direct somatic AP generation ('full-blown APs'). Through analysis and simulations we isolated the cell parameters that allow for spikelet generation. We hypothesize that spikelets occur rarely *in vitro* because the difference in activation voltages between somatic and axonal sodium channels under in-vitro conditions might be smaller than under in-vivo conditions. More precisely, the activation voltage of sodium channels might be controlled by neuronal activity, which is typically much higher *in vivo* than *in vitro*. Physiological regulation of these activation voltages, e.g., through various neuromodulators (dopamine, serotonin) acting via channel phosphorylation, was shown to differentially affect axonal versus somatic sodium-channel subtype. In our model, somatic spikelets represent APs that are only propagated down the axon, but are not backpropagated to the soma and the apical dendrites. Consequently, such a mechanism might be involved in the control of dendritic plasticity and/or in the homeostatic regulation of somato-dendritic firing rates without affecting the axonal output of a neuron.

Disclosures: **M. Michalikova:** None. **M. Remme:** None. **R. Kempter:** None.

Poster

507. Cellular Mechanisms of Modulation of Neuronal Firing Properties

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Program#/Poster#: 507.26/I12

Topic: B.10. Intrinsic Membrane Properties

Support: NSF IOS 1257338

Title: Cyclic AMP increases excitability at warm temperatures and decreases excitability at cold temperatures through activation of the hyperpolarization-activated current (Ih) in neurons from the bullfrog

Authors: ***J. SANTIN**[1], L. HARTZLER[2]
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Abstract: Both endothermic and ectothermic animals experience changes in brain temperature that challenge the control of behavioral and physiological systems. Given the profound temperature-sensitivity neural signaling within the brain, changes in brain temperature pose a challenge to the normal-functioning CNS. Second messenger molecules like cyclic AMP (cAMP) play a critical role in modifying neuronal excitability, but limited consideration has been

given to the ability of second messenger systems to modulate neuronal firing at different temperatures. We hypothesized that increasing cAMP would exert temperature-specific effects on neuronal firing in locus coeruleus (LC) neurons of bullfrogs. Using whole-cell current clamp, we found that LC neurons containing 100μM cAMP had increased spontaneous firing rates compared to control LC neurons (0.78±0.13Hz *vs.* 2.79±0.47Hz; $P<0.05$). Intriguingly, as we have demonstrated in previous work, LC neurons increased firing during cooling from 20°C to 10°C (0.57±0.9Hz *vs*. 2.24±0.35Hz; $P<0.05$); however, in the presence of cAMP, firing was reduced by cooling (1.71±0.42Hz *vs.* 0.61±11Hz; $P<0.05$) in 80% (12/15) of neurons. cAMP, therefore, exerts opposite effects on excitability at 20°C and 10°C. Whole-cell voltage clamp experiments revealed that cAMP increased a hyperpolarization-activated current (I_h) at 20°C and 10°C by shifting voltage dependent activation to more depolarized potentials (20°C: $V_{0.5}$=-77.06±3.03 mV in control and -62.85±2.37mV with cAMP; 10°C: $V_{0.5}$=-95.09±2.35mV in control and -76.32±3.89mV with cAMP). In the presence of cAMP, inhibition of I_h using 2mM Cs^+ ([Cs^+] did not block K^+ currents) reduced firing at 20°C (2.16±0.48Hz *vs.* 0.4±0.19Hz; $P<0.05$), but converted firing decreases upon cooling into increases similar to those observed in neurons containing control intracellular solution. Finally, the 3 neurons that maintained cold-activated responses in the presence of cAMP had a lower I_h density compared to 5 neurons that reduced firing rates during cooling (-3.23±0.35pA/pF *vs.* -6.16±0.77pA/pF; $P<0.05$), but did not exhibit differences in voltage dependence of activation at 20° or 10°C, indicating that the ability of cAMP to modify excitability depends on the amount of I_h. I_h is carried by HCN channels that are expressed ubiquitously throughout the CNS and PNS in many animals that have been shown to undergo relatively large changes in temperature. These data provide evidence that activation of I_h by cAMP can exert opposite effects on neuronal excitability at different temperatures and therefore may have broad implications in normal and pathological neuronal function. Supported by NSF IOS 1257338.

Disclosures: J. Santin: None. **L. Hartzler:** None.

Poster

508. Astroglia Function and Reaction to Pathological Processes

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 508.01/J1

Topic: B.11. Glial Mechanisms

Support: NIH/NINDS NS066005

Title: Common changes in the expression of extracellular matrix-related genes via astrocytic TGFb signaling in different epileptogenesis models

Authors: *S. KIM[1], K. LIPPMANN[3], Y. MA[4], I. PRADA[4], D. A. PRINCE[4], U. HEINEMANN[3], A. FRIEDMAN[5], D. KAUFER[1,2]
[1]Dept. of Integrative Biol., [2]Helen Wilis Neurosci. Inst., Univ. of California Berkeley, Berkeley, CA; [3]Inst. of Neurophysiol., Charité Universitätsmedizin, Berlin, Germany; [4]Dept. of Neurol. and Neurolog. Sci., Stanford Univ. Sch. of Med., Stanford, CA; [5]Departments of Physiol. & Neurobiology, Cognitive & Brain Sciences, The Zlotowski Ctr. for Neuro, Ben-Gurion Univ. of the Negev, Beer-Sheva, Israel

Abstract: Although brain damage is one of the risk factors for acquired epilepsies, the underlying mechanism remains poorly understood. Various types of brain injuries often involve breakdown of blood-brain barrier. Albumin extravasation through compromised blood-brain barrier is known to be critical in the development of epilepsy, which is mediated by astrocytic uptake of albumin and in turn by the activation of transforming growth factor beta (TGF-β) signaling cascade. Since extracellular matrix (ECM) plays a key role in regulating synaptic plasticity and in control of excitatory/inhibitory neurotransmission balance, and astrocytes are a major source of ECM secretion, we hypothesize that astrocyte-induced ECM changes are a common phenomena in the pathophysiology of post-traumatic epileptogenesis. Here we compare transcriptome profiles following distinct types of damage-related epileptogenesis models - partial cortical isolation ("Undercut") and photothrombotic cortical stroke in rats. Using cDNA microarray analysis, we demonstrate that several ECM remodeling genes are commonly upregulated in both of the injury models. Similar changes were also found in rat brains following BBB disruption by deoxycholic acid, albumin treatment, and direct application of TGF-β. These genes include inhibitors of major proteases as well as chondroitin sulfate proteoglycans (CSPGs). In cortical astrocytes, albumin treatment increases transcription of various ECM related genes that can be blocked by TGF-β signaling inhibitors. These data suggest that albumin extravasation through compromised BBB may alter the provisional property of ECM around synapses via astrocytes, contributing to the development of epilepsy.

Disclosures: S. Kim: None. **K. Lippmann:** None. **Y. Ma:** None. **I. Prada:** None. **D.A. Prince:** None. **U. Heinemann:** None. **A. Friedman:** None. **D. Kaufer:** None.

Poster

508. Astroglia Function and Reaction to Pathological Processes

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 508.02/J2

Topic: B.11. Glial Mechanisms

Support: PROMEP Apoyo NPTC FE018/2012

Title: Expression of insulin-like growth factors I and II and their receptors in iron-deficient murine astrocytes

Authors: E. MORALES-GONZÁLEZ, *I. CONTRERAS, J. A. ESTRADA
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Abstract: Iron deficiency is an important public health problem worldwide, affecting vulnerable populations, such as children and pregnant women, causing developmental and functional alterations of the central nervous system. Growth factors, including the insulin-like growth factors I and II, act as neurotrophins, enhancing the survival and differentiation of neurons, thus having neuroprotective functions. However, the effect of iron deficiency on the expression of these growth factors has not been properly assessed. The objective of this study was to determine the expression of IGF-I, IGF-II, IGF-IR and IGF-IIR on murine astrocytes under iron-deficient conditions. To this end, we prepared primary mixed glial cultures from cerebral tissue from newborn mice (<24 hours old) and cultured them for 14 days. Astrocytes were dissociated from these cultures by treatment with trypsin/EDTA and cultured until 80% confluence was achieved. Two groups of cells were cultured: the first group was treated with deferoxamine (100 μM/mL) for 24 hours prior to analysis, to simulate iron-deficient conditions; the second group was maintained under standard culture conditions. After treatment, cells from both groups were lysed and their protein content was quantified using the Bradford method. Proteins were loaded in polyacrilamyde gels and IGF expression was analyzed by western blot. Our results showed decreased expression of IGF-II in iron-deficient astrocytes, compared to controls. IGF-I expression did not show differences in expression. IGF-IR and IGF-IIR expression was not altered either in iron-deficient conditions. Previous work has shown decreased expression of IGF-II in iron-deficient mixed glial cell cultures, accompanied by enhanced expression of IGF-IIR, with no discernible differences in the expression of these molecules in either isolated microglial or neuronal primary cultures. Together, our data suggest a differential regulation of IGF expression on neuronal and glial cells under iron deficient conditions, which may be important in the induction of neurological deficits in iron-deficient patients.

Disclosures: E. Morales-González: None. **I. Contreras:** None. **J.A. Estrada:** None.

Poster

508. Astroglia Function and Reaction to Pathological Processes

Location: Halls A-C

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Topic: B.11. Glial Mechanisms

Support: UC Berkeley Bakar Fellows Program

NSF Graduate Research Fellowship

Title: Albumin-induced reactive astrocytosis following blood-brain barrier disruption: A model for age-related seizure susceptibility

Authors: *V. SENATOROV, JR, G. CHIN, N. JAHAN, D. KAUFER
UC Berkeley, Berkeley, CA

Abstract: Post-traumatic epilepsy (PTE), occurring after brain insult, is one of the most common epilepsies, affecting millions of people worldwide. The progression of PTE is marked by a period of neuronal network reorganization in which post-injury inflammatory responses are thought to contribute to a hyperexcitable neural environment, ultimately leading to chronic and spontaneous seizures. Since post-injury epilepsy is often resistant to current anti-seizure medications, elucidating the mechanisms of the "silent period" of epileptogenesis may reveal novel treatments to prevent seizures before onset. Our previous research developed a novel hypothesis for how vascular injury leads to epilepsy. We found that breakdown of the blood-brain barrier (BBB), as occurs during injury, allows the serum protein albumin from the blood to enter the brain. Once in the neuropil, albumin binds selectively to transforming growth factor beta receptors (TGF-βR) on astroglia, causing inflammatory TGF-β signaling and initiating a variety of effects including reactive astrocytosis, increased neuronal excitability and epileptogenesis. Furthermore, pharmacologically blocking TGF-β signaling prevented the onset of seizure activity in rodents exposed to albumin. Interestingly, an increase in reactive astrocytosis, pro-inflammatory TGF-β signaling and BBB permeability is found in the aging brain, suggesting that the elderly will be more susceptible to epileptogenesis mediated by BBB injury. Indeed, the incidence and prevalence of seizures significantly increases in the elderly, but the causes and mechanisms of age-related vulnerability are poorly studied and remain elusive. Therefore, investigating the identity of potentially epileptogenic astrocytes, along with probing their possible roles in causing susceptibility to seizure onset, may elucidate the cellular and molecular factors underlying epileptogenesis. We hypothesize that the aged brain will show heightened astrocytic reactivity to albumin signaling, resulting in increased vulnerability to albumin-induced epilepsy. To test this, we characterized the astrocyte response to albumin in both the young adult and aged mouse brain. We also quantified albumin signal transduction in both age groups, used specific transgenic approaches to analyze the spatio-temporal patterns of

reactive astrocytosis and block astrocytic TGF-β signaling, and assessed the effects of astrocyte signaling and age on seizure induction threshold. These experiments explore the potential for preventative treatments targeted at astrocyte reactivity before the onset of epilepsy.

Disclosures: **V. Senatorov:** None. **G. Chin:** None. **N. Jahan:** None. **D. Kaufer:** None.

Poster

508. Astroglia Function and Reaction to Pathological Processes

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 508.04/J4

Topic: B.11. Glial Mechanisms

Support: MinJiang Scholar Fund

NSFC 31271153

Title: Glioma-induced astrocyte apoptosis: A new mechanism promoting glioma growth

Authors: ***Z. YE**[1,2], B. R. RANSOM[1], Y. ZHOU[2], R.-Y. LIN[2], W. WANG[2]
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Abstract: Glioma cells release glutamate and this behavior is believed to be an important mechanism underlying brain tumor-induced epilepsy and neuronal death. Excess glutamate is presumed to over-stimulate neurons promoting epileptiform discharge and/or killing them in a manner termed excitotoxicity. The end point of these glutamate effects is loss of neuronal tissue in and around the glioma mass, thus facilitating glioma expansion. The fate of normal astrocytes in and around gliomas, however, remains largely unknown. As shown in a previous report (sfn 2013, 131.03), astrocyte glutamate uptake is the chief mechanism counteracting glioma glutamate release, and can protect neurons from glioma glutamate excitotoxicity. Because the extracellular glutamate levels are determined by the equilibrium between glutamate release and glutamate uptake, the ratio between the numbers of astrocytes and glioma cells determines extracellular glutamate concentration. In glioma-astrocyte co-culture studies, however, we also found that when astrocytes are greatly outnumbered by glioma cells for periods greater than 24 hours, they can lose their ability to counteract glioma glutamate release, which is accompanied by astrocyte morphological changes, apoptosis and reduction in numbers. These results suggest that gliomas make room for tumor expansion by killing off both neurons and astrocytes. In fact,

the lethal effect on astrocytes might actually be permissive for neuronal destruction because it removes the main mechanism protecting neurons from excitotoxicity.

Disclosures: Z. Ye: None. **B.R. Ransom:** None. **Y. Zhou:** None. **R. Lin:** None. **W. Wang:** None.

Poster

508. Astroglia Function and Reaction to Pathological Processes

Location: Halls A-C

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Program#/Poster#: 508.05/J5

Topic: C.05. Aging

Support: NIH-NINDS 2U54NS041069-06AI

NIH IMBRE NCRR (5P20RR016466-12)

Title: Insulin signaling influences morphology and proteostasis in the aging of healthy and diseased neurons

Authors: H. N. CURREY[1], S. C. HUNTER[1], M. DRISCOLL[2], J. PARKER[3], C. NERI[4], *B. E. TAYLOR[1]
[1]Biol. & Wildlife, Univ. of Alaska Fairbanks, Fairbanks, AK; [2]Dept. of Mol. Biol. and Biochem., Rutgers, Piscataway, NJ; [3]Dept. of Neurosci., Univ. of Montreal, Montreal, QC, Canada; [4]Lab. of Neuronal Cell Biol., Sorbonnes Universites, Paris, France

Abstract: Aging of healthy brains is characterized by neuronal sprouting, synaptic deterioration and restructuring with little to no neuronal cell loss (Yankner, 2008). However, the physiological factors that affect the decline of neuronal morphology over time *in situ* remain poorly understood. This is true in particular at the single cell level. Insulin signaling has been shown to regulate the rate of aging in organisms ranging from yeast to mice, and there is evidence that in humans, single nucleotide polymorphisms (SNPs) of the FOXO transcription factor, an insulin signaling target, can predict whether an individual will reach the age of 100 (Suh et al, 2007). There are many aging related diseases, some of which are neurodegenerative, for example Huntington's disease. Huntington's is a dominantly inherited disorder caused by polyglutamine expansion in the N-terminus region of the huntingtin protein. This protein interacts with over 200 others and plays a major role in trafficking vesicles along axon microtubules (Schulte and Littleton, 2011). Here, we present an RNAi screen of insulin genes one through forty, in the wild

type and Huntington's neurodegeneration *C. elegans* models. Both the wild type control and the neurodegeneration model have six fluorescently labeled mechanosensory neurons, as well as huntingtin aggregates labeled with CFP in the neurodegeneration Huntington's model (Parker et al. 2001). Significant changes in neuronal morphology and aggregation were observed between insulin knockdowns and empty vector controls. In addition, differences in neuronal morphology were observed when comparing wild type and neurodegeneration models. Together, these RNAi screen findings shed light on the role of insulin signaling in the aging of healthy versus diseased neurons.

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Poster

508. Astroglia Function and Reaction to Pathological Processes

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 508.06/J6

Topic: B.11. Glial Mechanisms

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Associazione Le\1-Montalcini (fellowship to GC)

Title: Purinergic modulation of spinal neuroglial maladaptive plasticity following peripheral nerve injury

Authors: *M. PAPA[1], G. CIRILLO[2], C. DE LUCA[2], L. SAVARESE[2], L. ALBERGHINA[3], A. COLANGELO[4]
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Network Morphology, Naples, Italy; [3]SYSBIO Ctr. of Systems Biol., Milan, Italy; [4]Univ. of Milano Bicocca, Milan, Italy

Abstract: Modulation of spinal reactive gliosis following peripheral nerve injury (PNI) is a promising strategy to restore synaptic homeostasis. Oxidized ATP a non-selective antagonist of P2X receptors has been reported to recover neuropathic behavior following PNI. We investigated the effect of intraperitoneal OxATP treatment in restoring the expression of neuronal and glial markers in the mouse spinal cord after sciatic spared nerve injury. Using *in vivo* two photon microscopy, we imaged Ca2+-transients in neurons and astrocytes of the dorsal horn spinal cord at rest and upon right hind-paw electrical stimulation in different groups: Control, SNI and OxATP-treated mice. Spinal cord was processed for analysis of glial markers (GFAP, Iba1), glial (GLT-1 and GLAST) and neuronal aminoacid transporters (EAAC1, vGLUT1), and GABAergic markers (vGAT and GAD65/67). Analysis revealed that SNI was associated with i) increased glial response (Iba1 and GFAP), paralleled by ii) decreased glial aminoacid transporters (GLAST and GLT1) and iii) increased levels of neuronal EAAC1 and vGAT. In SNI animals, *in vivo* analysis of spinal neurons and astrocytes showed a persistent increase of Ca2+ levels. Administration of OxATP reduced glial activation, modulated the expression of glial and neuronal transporters, restored neuronal and astrocytic Ca2+ levels. Reduced astrocytes proliferation and levels of ROS in neurons and astrocytes, in presence of OxATP, as vGLUT levels increase, comes out from *in vitro* studies. Data support the correlation between reactive gliosis, the mechanisms underlying the perturbation of the synaptic circuitry and the role played by the purinergic system in modulating spinal plasticity following PNI

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Poster

508. Astroglia Function and Reaction to Pathological Processes

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 508.07/J7

Topic: B.11. Glial Mechanisms

Title: Effects of cortical freeze injury on primary cilia of glial cell populations

Authors: M. CORONEL, S. R. BHATTARAI, S. LEWIS, *H. D. SCHWARK, J. L. FUCHS Biol. Sci., Univ. of North Texas, Denton, TX

Abstract: Glial cells maintain homeostasis in the nervous system that is essential to neuronal function. Injury to the nervous system leads to the activation and proliferation of glial cells, which may contain damage and help restore homeostatic conditions. Sonic hedgehog signaling has been implicated in astrogliosis following injury. Sonic hedgehog signaling requires primary cilia, but there are few reports of primary cilia in glial cells, and glial cilia are currently thought to be rare. Primary cilia are microtubule-based organelles that arise from the centrosome, and are retracted when the centrosome nucleates the mitotic spindle prior to cell division. Among proteins concentrated in cilia are components of several proliferative, sensory and mechanosensory pathways. We investigated the effects of cortical freeze injury on the incidence of primary cilia in the wound region exhibiting reactive gliosis. Astrocytes, polydendrocytes and pericytes were classified by immunohistochemistry based on cell-type markers GFAP, NG2 and Olig2, and cilia were identified mainly by immunoreactivity for Arl13b. In normal adult mice, primary cilia were present in a majority of each cell type examined: astrocytes, 98±2%; polydendrocytes, 87±6%; and pericytes, 79±13% (mean ± SD). Three days post-injury, cilium incidence decreased by 24% in astrocytes ($p<0.008$) and 41% in polydendrocytes ($p<0.002$), but there was no significant effect in pericytes. Considering post-injury rates of proliferation for astrocytes and polydendrocytes, it appears that resorption of cilia during the cell cycle may account for much of the loss of cilia in polydendrocytes but is not sufficient to account for the loss of cilia in astrocytes. Losses in glial cilia might influence the capacity for recovery from injury in the CNS.

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Poster

508. Astroglia Function and Reaction to Pathological Processes

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Topic: C.05. Aging

Support: The Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (NRF-2012R1A1A2039477), South Korea

Title: A plasma membrane redox enzyme, cytochrome b5 reductase, can protect cells from insults through maintaining redox homeostasis

Authors: *D.-H. HYUN[1], H.-K. KIM[1], S.-K. KIM[1], M. P. MATTSON[2]
[1]Dept. of Life Sci., Ewha Womans Univ., Seoul, Korea, Republic of; [2]Lab. of Neurosciences, Natl. Inst. on Aging, Baltimore, MD

Abstract: The plasma membrane redox system (PMRS) contains several NADH-dependent enzymes and plays a key role in maintaining levels of NAD+/NADH and reduced coenzyme Q. Brain aging and neurodegenerative disorders involve impaired energy metabolism and oxidative damage, but the involvement of the PMRS in these processes is still unknown. In this study, neuronal cells overexpressing cytochrome b5 reductase (b5R) were used to investigate how the PMRS regulates cellular stress responses. Overexpression of b5R made cells more resistant to death induced by 2-deoxyglucose, KCN, H2O2 and MG132. The NAD+/NADH ratio was significantly increased in the b5R transfectants compared to the control cells, consistent with enhanced levels of b5R activity. Increased levels of b5R induced lower production of reactive oxygen species. Our data suggest that a higher NAD+/NADH ratio and lower ROS levels are closely related to ability of neural cells to maintain redox homeostasis under conditions of energetic stress. These results illustrate that an up-regulated PMRS can protect cells from insults due to an improved antioxidant capacity, and suggest important roles for the PMRS in protecting brain cells against age-related increases in oxidative and metabolic stress.

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Poster

508. Astroglia Function and Reaction to Pathological Processes

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 508.09/J9

Topic: B.11. Glial Mechanisms

Title: The role of Sonic hedgehog signaling in reactive gliosis

Authors: *R. ALLAHYARI, A. D. R. GARCIA
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Abstract: Understanding the process of neural repair is critical to developing therapeutic treatments and improving functional recovery following injury to the central nervous system (CNS). Astrocytes play an instrumental role in neural repair mechanisms. They undergo reactive gliosis, characterized by an increase in glial fibrillary acidic protein (GFAP) expression, and in severe injuries, undergo cell proliferation and glial scar formation. Reactive astrocytes segregate

the damaged tissue, in order to restrict secondary damage to surrounding tissue. The molecular mechanisms regulating these processes are poorly understood. In the adult forebrain, distinct astrocyte populations express the transcription factor, Gli1, indicating active and high level Sonic hedgehog (Shh) signaling. Disruption of Shh signaling in astrocytes leads to mild astrogliosis, suggesting that Shh is important in regulating normal astrocyte function. The functional significance of Shh signaling in astrocyte function is unknown. Here, we use a conditional knock out (CKO) approach in which the obligatory Shh receptor, Smoothened (Smo) is specifically ablated in GFAP-expressing cells. We performed a forebrain stab injury in *GFAPCre;Smo*$^{fl/fl}$ (GFAP Smo CKO) mice and subsequently investigated the effect on various measures of astrogliosis. Our data show that disrupting Shh signaling in astrocytes leads to reduced proliferation and smaller lesion volumes following an acute injury. In wild type mice, reactive astrocytes immediately adjacent to the lesion extend a single process directed towards the necrotic lesion core. Reactive astrocytes from GFAP Smo CKO mice fail to exhibit this morphological phenotype, suggesting that Shh signaling plays a role in regulating the morphology of these cells. Taken together, these data point to a critical role for astrocytic Shh signaling in mediating pathological processes following injury.

Disclosures: R. Allahyari: None. **A.D.R. Garcia:** None.

Poster

508. Astroglia Function and Reaction to Pathological Processes

Location: Halls A-C

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Topic: B.11. Glial Mechanisms

Support: Ben B. and Iris M. Margolis Foundation Award

NIH R01 NS078331

Title: Upregulation of astrocytic metabotropic glutamate receptor (mGluR) expression in a murine model of virus-induced epilepsy

Authors: *M. TAHERI, J. M. GEE, R. S. FUJINAMI, K. S. WILCOX, J. A. WHITE
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Abstract: Increasing evidence suggests that astrocytes communicate bidirectionally with neurons, are central to information processing, and are involved in the initial stage or progression

of neurological disorders such as epilepsy. Astrocytes express metabotropic glutamate receptor (mGluR) types 3 (a group II mGluR) and 5 (a group I mGluR) that regulate a variety of intracellular signaling systems through G-protein activation and are thought to play important roles in maintaining synaptic homeostasis and synaptic activity. Studies on temporal lobe epilepsy (TLE) patients report an upregulation of mGluRs in hippocampal astrocytes and neurons. In particular, mGluR3 and mGluR5 are upregulated in these patients. These studies also report astrogliosis, in which astrocytes undergo changes in morphology and exhibit a dramatic increase in the expression of intermediate filament protein glial fibrillary acidic protein (GFAP). Similar astrogliosis and mGluR upregulation has also been observed in some animal models of epilepsy. From these studies, it is hypothesized that reactive astrocytes, through their upregulation of mGluR 3 and 5 and downstream release of bioactive molecules, may contribute to the hyperexcitability of neural networks and the development of behavioral seizures in epilepsy. In this study, we assessed levels of astrogliosis and astrocyte mGluR 3 and 5 expression during the development of epilepsy in the Theiler's Murine Encephalomyelitis Virus (TMEV)-induced seizure model in C57Bl/6 mice. This newly developed epilepsy model closely resembles infection-induced TLE in humans. TMEV-injected mice progress through three phases: acute symptomatic seizures; a latent period, in which no seizures are observed and epileptogenesis occurs; and chronic spontaneous and recurrent seizures. We are specifically interested in studying cortical and hippocampal astrocytes in TMEV-injected mice during the latent period. Using immunohistochemical techniques, we have confirmed the appearance of astrogliosis in the cortex and hippocampus of TMEV-injected mice, as well as increased expression of mGluR 3 and 5 in hippocampal GFAP-positive astrocytes. Furthermore, we found that mGluR3 expression is highly colocalized with GFAP expression during the latent period. Our present results suggest that changes in astrocyte phenotypic expression, in particular of mGluR expression, may be an important factor in the development of behavioral seizures in epilepsy and may be a potential pharmaceutical target for the treatment of this disorder.

Disclosures: **M. Taheri:** None. **J.M. Gee:** None. **R.S. Fujinami:** None. **K.S. Wilcox:** None. **J.A. White:** None.

Poster

508. Astroglia Function and Reaction to Pathological Processes

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 508.11/J11

Topic: B.11. Glial Mechanisms

Support: NS058674

NS070824

UL1 RR024139

Title: Glutamine synthetase inhibition and epileptic seizures

Authors: E. PEREZ[1], H. ZAVERI[1], H. WANG[1], E. DAMISAH[1], R. DHAHER[1], *T. EID[2]
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Abstract: We have previously demonstrated that glutamine synthetase (GS) is deficient in astrocytes in the epileptogenic hippocampal formation in patients with MTLE (Eid et al. Lancet 2004; 363: 28-37). We have also shown that recreating a state of chronic GS deficiency in rats by continuously infusing methionine-sulfoximine (MSO), an inhibitor of GS, into the entorhinal-hippocampal area leads to recurrent seizures. However, the exact role of GS deficiency in the development of MTLE (epileptogenesis) and the initiation of seizures remains unclear. Here we test the working hypothesis that chronic (28 days) but not acute (72 hrs) inhibition of GS in the entorhinal-hippocampal area is necessary to induce epileptogenesis and cause spontaneous recurrent (epileptic) seizures. A total of twenty-five rats were infused with MSO into the entorhinal-hippocampal area. Seven rats had the infusion continue for 28 days. Eighteen rats had the infusion stopped after 72 hours. All of the rats had seizures during the infusion. However, rats with infusion stopped after 72 hours typically had several weeks of no seizures followed by reappearance of spontaneous recurrent seizures. These results suggest that brief inhibition of GS in the entorhinal-hippocampal area is sufficient to start epileptogenesis. We expect that these studies can provide important insights into the role of astrocytes and GS inhibition in epileptogenesis in MTLE.

Disclosures: E. Perez: None. **T. Eid:** None. **H. Wang:** None. **H. Zaveri:** None. **E. Damisah:** None. **R. Dhaher:** None.

Poster

508. Astroglia Function and Reaction to Pathological Processes

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 508.12/J12

Topic: B.11. Glial Mechanisms

Support: NSC 100-2320-B-345-001

Title: Aryl hydrocarbon receptor differentially regulates curcumin-induces proinflammatory and neuroprotective factor expressions in astrocytes

Authors: *C.-H. LIN[1], M.-S. LIN[2], P.-C. HSU[3], Y.-J. HUANG[3], Y.-H. LEE[3]
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Abstract: Curcumin, a natural bioactive polyphenol, has been proposed to provide prevention of neurodegeneration via the regulation of the immune response in the central nervous system. Aryl hydrocarbon receptor (AhR) has also been found to mediate immune cell differentiation and maturation, but its role in the brain disorders remains unclear. Our previous study has shown that curcumin can provide neuroprotection by increasing neuroprotective chemokine RANTES secretion from astrocytes. In this study, we demonstrate that curcumin can activate astrocytic AhR.. Astrocytes treated with curcumin alone (1 μM) caused increase in luciferase activity driven by the dioxin response element (DRE), the DNA binding sequence of the ligand-activated AhR. Curcumin by itself not only increased RANTES but also the proinflammatory iNOS expression, but did not affect the expression of IL6, which has both proinflammatory and neuroprotective effects. Intriguingly, knockdown AhR expression by siRNA abolished the cucumin-induced iNOS, but great enhanced the curcumin-induced RANTES and IL-6 expressions. TET1, a member of Dioxygenases of the Ten-Eleven Translocation (TET) that is involved in the process of DNA demethylation. We found that curcumin can profoundly increase TET1 expression in astrocytes, and this effect was abolished by AhR knockdown. In summary, our preliminary results suggest that AhR is differentially involved in the expression of proinflammatory mediators in astrocytes that may also provide neuroprotection, and one of the possibilities might involve its upregulation of DNA demethylation machinery for epigenetic regulation.

Disclosures: **C. Lin:** A. Employment/Salary (full or part-time):; bernice428@gmail.com. **Y. Lee:** None. **M. Lin:** None. **Y. Huang:** None. **P. Hsu:** None.

Poster

508. Astroglia Function and Reaction to Pathological Processes

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 508.13/K1

Topic: B.11. Glial Mechanisms

Title: Effects of 6-hydroxydopamine on striatal and cortical mouse astrocyte cultures

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Abstract: Intraventricular injection of 6-OHDA results in increased proliferation and de-differentiation of a subpopulation of $GFAP^+$ cortical astrocytes (Wachter *et al.*, 2010) towards a molecular phenotype resembling radial glia cells (expressing Pax6, Nestin Vimentin). Moreover, we observed a 15-fold increase in the number of tyrosine hydroxylase (TH)-positive somata in the temporo-parietal cortex of 6-OHDA lesioned animals. Most of these cells immunhistochemically co-stained for GAD or Calretinin, but the expression of glial and neuronal progenitor markers (Sox2, S100b, PSA-NCAM) in some TH^+ cells was also demonstrated. However, the design of the above mentioned study did not allow to discriminate if the observed effects were brought about directly by 6-OHDA or if they were due to the 6-OHDA-induced dopaminergic de-afferentiation. To investigate direct effects of 6-OHDA on glial and neuronal cells in dopaminergic target areas, we treated striatal and cortical mouse astrocytes with 15µM and 30µM of 6-OHDA, concentrations that have been shown not to affect the viability of the cells. Since after the intraventricular injection of the neurotoxin we observed *in vivo* an increase in proliferation of $GFAP^+$ cells, we investigated the effects of the 6-OHDA on the proliferation capability of striatal and cortical mouse astrocytes. Surprisingly treatment of the cultures with 6-OHDA inhibited proliferation in a dose-dependent manner at all timepoints investigated. Furthermore, immuncytochemistry at 2^{nd}-4^{th} and 6^{th} day after the treatment with 15µM of 6-OHDA revealed a significant lower expression of GFAP in cortical astrocyte cultures after 4 and 6 days *in vitro*. No effect of the toxin was to be seen on mRNA level. In contrast to the emergence of TH^+ cells in the temporo-parietal cortex following intraventricular injection of 6-OHDA, we could not detect any TH^+ cell neither in cortical nor in striatal astrocytes cultures at any time point investigated. Taken together, these results provide evidence that the observed de-differentiation of $GFAP^+$ cells as well as the occurrence of TH^+ cells *in vivo* following 6-OHDA lesion seem to be induced by the dopaminergic de-afferentiation rather than by direct effects of 6-OHDA. Further studies have to be performed in order to confirm this and to characterize the *in vitro* phenotype of the investigated subpopulation of $GFAP^+$ cortical astrocytes.

Disclosures: **S. Caradonna:** None. **B. Wachter:** None. **J. Etschmann:** None. **E. Küppers:** None.

Poster

508. Astroglia Function and Reaction to Pathological Processes

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 508.14/K2

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Alzheimer's Drug Discovery Foundation

Alzheimer's Art Quilt Initiative

Title: Stalled capillary flow is a novel mechanism for hypoperfusion in Alzheimer's disease

Authors: *N. NISHIMURA[1], C. KERSBERGEN[1], J. CRUZ HERNANDEZ[1], I. IVASYK[1], Y. KANG[1], S. GHERKING[1], V. MUSE[1], J. ZHOU[1], J. D. BEVERLY[1], G. OTTE[1], T. P. SANTISAKULTARM[1], E. SLACK[1], C. IADECOLA[2], C. B. SCHAFFER[1]
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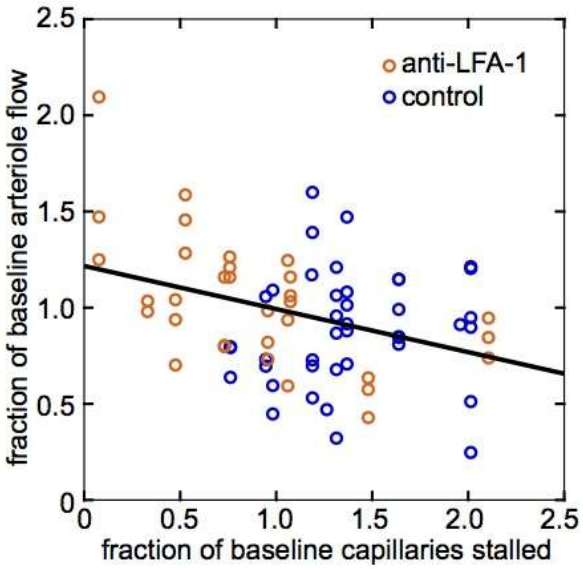
Abstract: Cerebral blood flow deficits of ~20-30% relative to healthy controls are observed in both patients and animal models of Alzheimer's disease (AD). We recently found that white blood cells often plug capillaries in the brains of AD mouse models. In AD mice, ~2% of brain capillaries are stalled while control animals have only 0.4% of capillaries stalled. We hypothesized that these plugged capillaries may be a cause of the drop in cortical perfusion. Using *in vivo* two-photon excited fluorescence imaging, we evaluate perfusion of capillaries in the cortex of AD mouse models (B6.Cg-Tg(APPswe,PSEN1dE9)85Dbo/J) and wildtype littermate controls. As a measure of overall blood flow to the cortex we quantified volume blood flux in individual penetrating arterioles. The majority of occlusions were caused by leukocytes, so to reduce the number of stalls, we injected antibodies against lymphocyte functional antigen-1 (LFA- 1, clone M17/4, 4 mg/kg, BD Biosciences) into AD mice. Using flow cytometry, we found that this treatment depletes circulating neutrophils (CX3CR1 macrophages) to 68% (57%) of the baseline value at 24 hours. We imaged these animals before and 24 hours after injection and quantified capillary stalls and measured blood flow speed in penetrating arterioles. In antibody-injected animals (n =6), the incidence of capillary stalling dropped to 47% +/- 18% of baseline ($p = 0.008$ from baseline) while saline injected controls remained nearly unchanged (average 119% +/- 42 of baseline, n=6, $p = 0.007$ from antibody treatment). Between the natural variability of the incidence of stalling and the decrease due to leukocyte depletion we were able to relate penetrating arteriole blood flow to the local incidence of capillary stalling. Volumetric blood flow increased to 120% of baseline when stalls dropped to near zero and decreased when stall rates increased. The brain blood flow increase we measured when capillary stalls were eliminated coincides with the observed deficiencies in blood flow measured in AD mice and patients, suggesting capillary plugging may contribute to brain hypoperfusion in AD.



Disclosures: **N. Nishimura:** None. **C. Kersbergen:** None. **J. Cruz Hernandez:** None. **I. Ivasyk:** None. **Y. Kang:** None. **S. Gherking:** None. **V. Muse:** None. **J. Zhou:** None. **J.D. Beverly:** None. **G. Otte:** None. **T.P. Santisakultarm:** None. **E. Slack:** None. **C. Iadecola:** None. **C.B. Schaffer:** None.

Poster

508. Astroglia Function and Reaction to Pathological Processes

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 508.15/K3

Topic: B.11. Glial Mechanisms

Title: Early life exposure to noise permanently reduces mpfc astrocyte numbers and t-maze alternation/discrimination task performance in male rats

Authors: *Y. RUVALCABA DELGADILLO[1], T. MORALES SALCEDO[2], G. YAÑEZ DELGADILLO[3], P. HERNANDEZ CARRILLO[3], G. CHIPRES TINAJERO[3], R. RAMOS ZUÑIGA[3], A. FERIA VELASCO[3], J. GARCIA ESTRADA[4], S. LUQUIN[3], F. JAUREGUI-HUERTA[3]
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Abstract: Environmental noise (EN) has become an important issue for modern societies. Its presence affects both, auditory and non-auditory functions. Non-auditory effects of noise are mostly due to annoyance and the subsequent activation of the stress response. Brain structure and function has been proposed as one of the main targets for the deleterious effects of noise outside the hearing organ. In this experiment, we evaluated the long-term effects of noise by assessing both, astrocyte changes in medial prefrontal cortex (mPFC) and mPFC related alternation/discrimination tasks. Methods: 21-day-old male Wistar rats were exposed to environmental noise in a 24-h fashion. We used for this purpose a standardized rats' audiogram-fitted adaptation of a human noisy environment. We measured corticosterone (CORT) serum levels at the end of the exposure and registered body weight gain during the first 7 weeks of the experiment. In order to assess non-auditory long-term effects of the early EN exposure, we assessed the rats' performance on T-Maze related PFC tasks 3 months after the end of the exposure. Astrocyte numbers and proliferative changes in mPFC were also evaluated 3 months later. We found that a 24-h EN exposure significantly increased serum CORT levels and negatively affected the body weight gain curve. Accordingly, enduring effects of noise were also demonstrated on mPFC structure and function. The ability to solve alternation/discrimination tasks were reduced as well as the numbers of astroglial cells. We also found a reduction on the cellular proliferation rate not related with astroglial lineage. Our results support the idea that early exposure to the widespread environmental stressor namely noise may have long-lasting consequences affecting complex cognitive processes. These results also suggest that glial changes may become an important element behind the cognitive and morphological alterations accompanying the PFC changes seen in some stress-related pathologies.

Disclosures: **Y. Ruvalcaba Delgadillo:** None. **T. Morales Salcedo:** None. **G. Yañez Delgadillo:** None. **P. Hernandez Carrillo:** None. **G. Chipres Tinajero:** None. **R. Ramos Zuñiga:** None. **A. Feria Velasco:** None. **J. Garcia Estrada:** None. **S. Luquin:** None. **F. Jauregui-Huerta:** None.

Poster

508. Astroglia Function and Reaction to Pathological Processes

Location: Halls A-C

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Program#/Poster#: 508.16/K4

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: NIGMS R01 GM066018

Title: Tolerance of hippocampal CA1 and CA3 interneurons to oxygen glucose deprivation

Authors: *G. BARRIONUEVO[1], N. V. POVYSHEVA[1], S. G. WEBER[2]
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Abstract: Neuronal populations as similar as hippocampal CA1 and CA3 pyramidal cells demonstrate different tolerance to hypoxic-ischemic insult, with the latter being substantially more resilient (Gee et al., 2006). In this study, we address the differential sensitivity of somatostatin (SST)-positive inhibitory neurons in hippocampal areas CA1 and CA3 to better understand the pathophysiological changes in the excitation/inhibition balance during ischemia. Whole-cell recordings were made from SST interneurons in coronal hippocampal slices of 4-7 months old transgenic mice with interneuron-specific GFP labeling FVBTg(GadGFP)45704Swn/J). SST interneurons were located in *strata pyramidale* and *oriens* of CA1 and CA3. SST interneurons from CA1 and CA3 had similar intrinsic membrane properties: spike duration (0.48±0.09 ms vs. 0.49±0.06 ms), firing without adaptation, time constant (19.4±5.5 ms vs. 21.1±9.2 ms), and input resistance (278±28 MΩ vs. 283±119 MΩ, respectively. During the oxygen-glucose deprivation (OGD) experiments, neurons were held at -70 mV at 32^{o}C. After recording of baseline responses in control conditions, the extracellular solution was changed to a solution containing sucrose (10 mM) substituted for glucose, and perfused with a mixture of 95% N_2/5% CO_2. The sucrose solution was applied for 10 minutes, and then switched to control solution for 15 minutes to explore the tolerance of cells to reperfusion. The neurons developed a strong inward current in OGD conditions or during reperfusion. We found that CA1 and CA3 SST interneurons had comparable latencies to onset of the OGD current (6.8±2.0 min, n=7 vs. 6.2±1.2 min, n=5), and comparable amplitudes of the OGD current (743±230 pA; n=7 vs. 521±460 pA; n=5). Four out of 6 CA1 and 4 out of 5 CA3 SST interneurons survived 10 min exposure to OGD. Two out of 4 CA1 and 2 out of 4 CA3 SST interneurons survived 15 min reperfusion. These preliminary data indicate that in contrast to pyramidal cells, SST-positive interneurons in CA1 and CA3 exhibit similar resilience to the hypoxic-ischemic insult. Further studies will address the involvement of SST-positive interneurons in the pathophysiology of hippocampal ischemia.

Disclosures: G. Barrionuevo: None. **N.V. Povysheva:** None. **S.G. Weber:** None.

Poster

508. Astroglia Function and Reaction to Pathological Processes

Location: Halls A-C

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Program#/Poster#: 508.17/K5

Topic: B.11. Glial Mechanisms

Support: NHRI PH-102-PP-40

Title: The multiple roles of lysophosphatidic acid receptor-1 in the regulation of lipopolysaccharide-induced immune responses in reactive astrocytes

Authors: *F.-S. SHIE, J.-J. LIANG, C.-S. LU
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Abstract: Increasing line of evidence suggest that reactivate astrocytes play an important role in the glia-mediated neuroinflammation in Alzheimer's disease (AD). Understanding the regulatory machinery of astrocyte reactivation may confer the molecular basis for the development of therapeutic approach for the disease. In this study, we demonstrate that immunoreactivity of lysophosphatidic acid receptor-1 (LPAR1) is present in reactive astrocytes in mice genetically engineered to develop AD and is increasing in parallel with the progression of the disease, whereas immunereactivity of LPAR1 is absent in the age-matched wild-type littermates. These findings lead us to speculate that LPAR1 may be involved in the regulation of immune responses in reactive astrocytes and alteration of LPAR1 expression may affect the pathogenesis of AD. Indeed, our data show that significant increases of immune responses are found in lipopolysaccharide (LPS)-activated primary astrocytes with siRNA against LPAR1 as compared to the controls. These immune responses include expressions of inducible nitric oxide synthase (iNOS), cyclooxygenase 2 (COX-2), IL-6, and TNFa. In contrast, overexpression of LPAR1 in primary astrocytes suppresses the LPS-induced immune responses. Intriguingly, the LPS-induced immune responses in primary astrocytes are partially inhibited by an LPAR1 antagonist, Ki16425. Taken together, our data suggest that multiple mechanisms may underlie the regulatory roles of LPAR1 in astrocytic immune responses.

Disclosures: F. Shie: None. **J. Liang:** None. **C. Lu:** None.

Poster

508. Astroglia Function and Reaction to Pathological Processes

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Topic: B.11. Glial Mechanisms

Support: The scientific and technological research council of Turkey Grant 113S266

Title: Glia limitans alterations in lipopolysaccharide induced parenchymal neuroinflammation

Authors: *I. TATAR[1,2], S. LULE[2], M. YEMISCI[2], M. HAYRAN[1], E. ERDEMLI[3], Y. OZDEMIR-GURSOY[2,4], T. DALKARA[2,4]
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Abstract: Glia limitans (GL) is the outermost layer of the cerebral cortex and is formed by astrocytic end-feet and basal membrane surrounding vascular structures, thereby functioning like a modified blood-brain barrier for the regulation of the movement of molecules and cells to in and out of the brain parenchyma. Neuroinflammation (NI) in brain parenchyma leads to activation of microglia and astrocytes resulting in increased gene expression of many potentially neurotoxic chemoattractant molecules. Although the effects of systemic inflammation on GL are known, there are no data on how the parenchymal inflammation affects GL and the associated micro-vasculature. The aim of the study is to investigate the effects of parenchymal NI induced by intracerebroventricular (icv) injection of lipopolysaccharide (LPS) on GL by using immunohistochemical, confocal and electron microscopic methods. 2.5µg/2.5µl LPS (Sigma, E.coli (0127:B8)) or saline was applied icv to Swiss albino male mice (28-30g). After 2 hours, mice were perfused with 4% paraformaldehyde and 20 µm thick sagittal sections were taken from frozen brains, stained with antibodies against Iba-1, ALDH1L1, IL-1beta, TNF-alfa and visualised with a confocal microscope. Another set of sections were evaluated and imaged with transmission electron microscopy. Activation of microglia and astrocytes were observed in LPS treated mice, especially in the superficial GL. Surface associated astrocytic end-feet were hypertrophic and microglia were in reactive state. Increased expression of inflammatory cytokines, TNF-alfa and IL-1beta, were detected in GL and the underlying cortex with immunofluorescent staining. In electron microscopic studies; edematous changes were seen especially at astrocytic end-feet of vascular (internal) GL but interestingly endothelial cells were intact and micro-vessels were not compressed. There are studies showing the cellular migration from blood to the brain parenchyma via GL during NI, but there is no study showing how NI affects GL and the micro-vasculature. In this preliminary study, we demonstrated that inflammation triggered within the parenchyma could affect both the superficial GL and the associated micro-vasculature, leading increased inflammatory cytokine formation as well as structural changes.

Disclosures: I. Tatar: None. **S. Lule:** None. **M. Yemisci:** None. **M. Hayran:** None. **E. Erdemli:** None. **Y. Ozdemir-Gursoy:** None. **T. Dalkara:** None.

Poster

508. Astroglia Function and Reaction to Pathological Processes

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Topic: B.11. Glial Mechanisms

Support: NIH Grant P01NS062686

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Wellcome Trust 086185/Z/08/Z

MRC MR/J002976/1

UCL Charlotte and Yule Bogue Research Fellowship

Title: Local GABAergic regulation of cerebellar NG2 cell development is altered in perinatal diffuse white matter injury

Authors: *M. ZONOUZI[1], J. SCAFIDI[3], P. LI[3], L. HARVEY[4], D. SUN[5], S. CULL-CANDY[2], M. FARRANT[2], V. GALLO[6]
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Abstract: Diffuse white matter injury (DWMI) is a common finding in children born very prematurely (<32 weeks of gestation) and is a leading contributor to neurodevelopmental disabilities. DWMI is characterized by significantly reduced myelination of the cerebral white matter (WM) by oligodendrocytes. Myelinating oligodendrocytes are formed from oligodendrocyte precursor cells (OPCs). The molecular mechanisms that control the proliferation and migration of OPCs, and thus the formation of myelinating oligodendrocytes, following WM damage remain unknown. Whilst the majority of studies on DWMI in neonates have focused on abnormalities occurring in the sub-cortical WM, there is evidence of significant injury to the cerebellum. In this study, we used an established rodent model of DWMI that mimics the disrupted pattern of brain development in very preterm neonates (chronic perinatal hypoxia; P3-P11). We examined the effects of chronic neonatal hypoxia on the number and distribution of WM GABAergic interneurons and NG2+-OPCs in the cerebellum at P7, P11, P15 and P30. We found that hypoxia resulted in 1) a significant increase in NG2+/Ki67+ OPCs and 2) a reduction in the number of Pax2+ immature interneurons. In a variety of brain regions NG2+-OPCs have

been shown to receive synaptic input from neurons during development. Using patch-clamp recording in acute brain slices we found a marked decrease in the prevalence and frequency of GABAergic synaptic input to NG2+-OPCs. As GABA signaling is known to regulate both neuronal and glia development, this loss may contribute to the NG2+-OPC disruption seen following neonatal hypoxia. There are currently no treatments for DWMI associated with very preterm birth. Thus understanding the mechanisms that control the properties of OPCs under pathological conditions is imperative.

Disclosures: **M. Zonouzi:** None. **J. Scafidi:** None. **P. Li:** None. **L. Harvey:** None. **D. Sun:** None. **S. Cull-Candy:** None. **M. Farrant:** None. **V. Gallo:** None.

Poster

508. Astroglia Function and Reaction to Pathological Processes

Location: Halls A-C

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Program#/Poster#: 508.20/K8

Topic: B.11. Glial Mechanisms

Support: Grant-in-Aid for Scientific Research(B)25860493

Title: The role of sodium channel on ethanol-induced aquaporin-4 expression

Authors: ***R. KATADA**, K. SUGIMOTO, M. YOSHIDA, H. MATSUMOTO
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Abstract: Ethanol is the bad player of traumatic brain injury (TBI). We previously reported that ethanol decreased survival rate to around half by brain edema augmentation after TBI. Aquaporin-4 (AQP4), an water channel protein, is involved in brain edema augmentation after TBI under ethanol consumption. We also reported that blood sodium ion concentration was decreased after TBI. AQP4 is regulated by Na-K ATPase, Na(+)-K(+)-2Cl(-) co-transporter. Ethanol is also involved in those channels. From these findings, sodium ion channel may affect AQP4 expression under ethanol condition. In this study, rat primary astrocyte was incubated in iso-sodium MEM (NaCl: 680 mg/dl) and hypo-sodium (NaCl: 410 mg/dl) or hyper-sodium MEM medium (NaCl: 950 mg/dl) with 10% calf serum for 30 min to 24 hr. Ethanol (25, 50, 100 mM) was added to each medium simultaneously. And it was exposed in ethanol (25, 50 mM) with tetrodotoxin (400 nM, 800 nM), voltage-gated sodium channel blocker, for 1 or 3 hr. After exposure, AQP4 protein expression was analyzed by western blotting and mRNA by real-time RT-PCR. Three hour hypo-sodium medium increased AQP4 expression. Hypo-sodium and

ethanol did not affect AQP4 expression, however, AQP4 expression was decreased by hyper-sodium and ethanol for 3 hr exposure, and was increased for 6 hr. Tetrodotoxin affected AQP4 expression under ethanol exposure. These findings indicate that AQP4 expression is involved in sodium ion channel with ethanol exposure.

Disclosures: R. Katada: None. **K. Sugimoto:** None. **M. Yoshida:** None. **H. Matsumoto:** None.

Poster

508. Astroglia Function and Reaction to Pathological Processes

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Program#/Poster#: 508.21/K9

Topic: B.11. Glial Mechanisms

Support: CIHR MOP 123298

NSERC PGS D2 391871

Title: Systemic administration of ultra-low dose alpha2-adrenergic antagonist atipamezole attenuates morphine-induced gliosis associated with the development of tolerance

Authors: *P. GRENIER[1], B. MILNE[2], M. C. OLMSTEAD[3], C. M. CAHILL[1,4]
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Abstract: Background: Prolonged use of opioids is limited by the development of analgesic tolerance as increasing the dose exacerbates side effects such as constipation and opioid-induced hyperalgesia. Chronic opioid administration leads to spinal glial activation, causing a shift to a pro-inflammatory state. Spinal administration of ultra-low dose (ULD) α2 adrenergic receptor (AR) antagonists paradoxically enhances morphine effectiveness and attenuates analgesic tolerance in pain-naïve conditions. The mechanism through which ULD α2 antagonists attenuate opioid tolerance is unknown, but could involve changes in the activation states of spinal glia. Methods: Rats were treated once daily with morphine (5mg/kg, s.c.), morphine plus ULD atipamezole (5ng, s.c.), atipamezole alone or vehicle (saline) for seven days. Thermal tail flick responses were assessed daily before injection and thirty minutes post-injection (at peak drug effect), and a full two hour time course was performed on day 1 and day 7. Following the last

day of behavioural testing, all animals were perfused and spinal cords were collected for immunohistochemical analysis of gliosis. The L4-L5 lumbar region of the spinal cord was isolated and sectioned on a freezing microtome. Sections were incubated with primary antibodies for microglia (CD11b) and astrocytes (glial fibrillary acidic protein/GFAP). Images were captured within the deep and superficial dorsal horn on a confocal microscope, layered stacks were collapsed, converted to greyscale and mean grey values were quantified. Results: Animals administered morphine alone experienced rapid development of analgesic tolerance. Atipamezole administered on its own had no effect on thermal tail flick latencies, but when co-administered daily with morphine it attenuated the development of morphine tolerance. Morphine treatment induced astrogliosis in the spinal dorsal horn, which was prevented by concomitant ULD atipamezole administration. Astrocyte labeling in the animals that received ULD atipamezole alone was not different from saline. No differences in microglial labeling were observed across any of the groups. Conclusion: ULD α2 AR antagonists attenuate opioid tolerance through the attenuation of morphine-induced astrogliosis in the spinal dorsal horn.

Disclosures: **P. Grenier:** None. **B. Milne:** None. **M.C. Olmstead:** None. **C.M. Cahill:** None.

Poster

508. Astroglia Function and Reaction to Pathological Processes

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 508.22/L1

Topic: B.11. Glial Mechanisms

Support: This study was supported by the research fund of the IRP, NIMH, NIH

Title: Valproic acid and other HDAC inhibitors regulate fibroblast growth factor 21 gene expression in astroglia

Authors: J. WANG, *Y. LENG, Z. WANG, H.-M. LIAO, P. LEEDS, D.-M. CHUANG
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Abstract: Fibroblast growth factor 21 (FGF-21) is a newly identified regulator of glucose and lipid metabolism, and has been proposed as a potential target for treating diabetes, obesity, and hyperlipidemia. Valproic acid (VPA), a mood stabilizer and an anticonvulsant drug, is a histone deacetylase (HDAC) inhibitor, and regulates gene expression via chromatin remodeling through epigenetic mechanisms. Recently, our lab reported that FGF-21 mRNA and protein can be markedly induced in rat brain-derived neurons by combined treatment with the mood stabilizers

lithium and VPA (Leng et al., Mol Psychiatry 2014, advance online). We also documented that FGF-21 has robust neuroprotective properties against insults such as glutamate excitotoxicity, at least in part, by inhibiting glycogen synthase kinase-3 (GSK-3) and activating the cell survival factor Akt. Notably, VPA alone failed to induce FGF-21 in multiple types of primary neurons. The present study was undertaken to investigate whether VPA and related HDAC inhibitors regulate the expression of FGF-21 in non-neuronal cells such as rat C6 glioma cells and primary cortical astrocyte cultures. Incubation of C6 cells with VPA caused a dose and time-dependent increase in FGF-21mRNA levels with an approximately 30-fold increase at 3 mM after 7-day treatment. A comparable extent of FGF-21 mRNA elevation was also observed by treating C6 cells with two other HDAC inhibitors, phenylbutylate butyrate (PB) and sodium butyrate (SB), which are structurally and functionally similar to VPA. The effects of VPA on FGF-21 mRNA in C6 cells were associated with dose-dependent increases in the length of neurite-like processes and levels of histone acetylation, a marker of HDAC inhibition. In primary rat cortical astrocytes, VPA also induced a seven-fold increase in FGF-21 mRNA levels following incubation with 5 mM of the drug for 7 days. Together, our results suggest that HDAC inhibition by VPA and related compounds up-regulates FGF-21 mRNA in astroglial cells. The neurobiological significance of this up-regulation in glia requires further investigation.

Disclosures: J. Wang: None. **Y. Leng:** None. **Z. Wang:** None. **H. Liao:** None. **P. Leeds:** None. **D. Chuang:** None.

Poster

508. Astroglia Function and Reaction to Pathological Processes

Location: Halls A-C

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Program#/Poster#: 508.23/L2

Topic: B.11. Glial Mechanisms

Title: PKC activation is necessary and sufficient for astrocytic group I mGluR potentiation of astrocyte glutamate uptake

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Abstract: Uptake of synaptically released glutamate by astrocytes is essential for maintaining a healthy level of excitatory activity in the brain and for shaping neuronal synaptic currents. Yet the mechanisms by which astrocytic glutamate uptake is acutely modulated remain to be

clarified. It is known that during neuronal activity, astrocytic metabotropic glutamate receptors (mGluRs) and other G protein-coupled receptors (GPCRs) are activated. We have previously demonstrated that activating astrocytic mGluRs via a tetanic high-frequency stimulus (HFS) applied to Schaffer collaterals (SCs) in acute hippocampal slices leads to short-term potentiation of the synaptically evoked glutamate transporter currents (STCs). Furthermore, there is no similar potentiation of STCs in the presence of group I mGluR antagonists or during selective inhibition of astrocytic PKC, suggesting that HFS-induced potentiation of astrocyte glutamate uptake is dependent upon astrocytic group I mGluRs and PKC. New data indicate that dialysis of astrocytes with the PKC activator (-)Indolactam-V, but not the inactive enantiomer (+) Indolactam-V, results in potentiation of astrocyte glutamate transporter currents over a similar timecourse as observed following HFS of SCs. Astrocytic transporter current amplitude gradually increased over the course of the recording, peaking at 30 min. after cell break-in. Collectively, the findings suggest that astrocytic PKC is both necessary and sufficient for rapid potentiation of astrocyte glutamate uptake. Experiments are underway to determine if potentiation of astrocyte glutamate uptake following stimulation of a transgenic GPCR (MrgA1R) expressed exclusively in astrocytes, is also PKC-dependent. Additional studies will determine whether modulation of astrocyte glutamate uptake is bidirectional, as well as the effects of rapid modulation of astrocyte glutamate uptake on neuronal excitability, including extrasynaptic NMDA receptor currents and LTP.

Disclosures: I.M. Holman: None. **P. Devaraju:** None. **T. Fiacco:** None.

Poster

508. Astroglia Function and Reaction to Pathological Processes

Location: Halls A-C

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Topic: B.11. Glial Mechanisms

Support: PROMEP 103.5/10/7324

UASLP-FAI C10-FAI-05-55.84

Title: AMPA receptors modulate changes in Bergmann glia morphology through calpain activation

Authors: *Y. BASTIAN[1], R. ROSAS-HERNÁNDEZ[2], J. MENDEZ[2]
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Abstract: The cerebellum is important for fine and coordinated movement as well as motor learning and some cognitive functions. The cerebelar functions depends on the neural activity of Purkinje cells. The neural activity of Purkinje cells depends on the adaptive synaptic plasticity through the integration of excitatory synaptic inputs from the parallel and the climbing fibers. The lamellar processes of Bergmann glia tightly ensheath the Purkinje cells synapses. Bergmann glia AMPA receptors lack iGluR2 subunits, therefore their stimulation induces calcium entry. Remarkably, this calcium entry through AMPA receptors is essential for establishment and maintenance of Purkinje cells synapses. Furthermore, AMPAr inactivation in Bergmann glia induces an impairment in fine motor coordination triggered by the retraction of Bergmann glia processes. Given that calcium is important for this retraction and that processes like this involves changes in the cytoskelatal structure, we used coronal cerebelar acute slices treated with 200 μM glutamate to evaluate the involvement of the calcium-dependent cystein protease calpain, a well known modulator of cytoskeleton structure. A 10 min stimulation with glutamate induces the activation of calpain that is accompanied with a change in the morphology of Bergmann glia. This change in morphology is prevented by calpain blockade with calpeptin, a calpain inhibitor. Moreover, both the activation of calpain and the change in morfology were prevented when AMPAr were blocked with NBQX. We suggest that the effects of calpain in remodeling of cytoskeleton involves the cleavage of either spectrin, ankyrin or other proteins implicated in actin cytoskeleton organization, for instance the focal adhesion proteins.

Disclosures: Y. Bastian: None. **R. Rosas-Hernández:** None. **J. Mendez:** None.

Poster

508. Astroglia Function and Reaction to Pathological Processes

Location: Halls A-C

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Program#/Poster#: 508.25/L4

Topic: B.11. Glial Mechanisms

Support: NSF GRFP 2388357

Title: Ca2+ responses in astrocytes of unanesthetized mouse visual cortex

Authors: *R. GARCIA, M. GOARD, J. PETRAVICZ, M. SUR
Brain & Cognitive Sci., MIT, Cambridge, MA

Abstract: Astrocytic intracellular Ca^{2+} signaling has become a prominent feature in neuronal-glial interactions. The majority of data concerning astrocyte Ca^{2+} signaling come from either culture or *in situ* brain slices, approaches that rely on electrical stimulation or pharmacological methods to examine the spatial and temporal coding of astrocyte Ca^{2+} signals. Recently, several studies have utilized *in vivo* Ca^{2+} imaging in response to physiologically relevant stimuli or combined with electrical stimulation of brain nuclei to examine the role of astrocyte Ca^{2+} transients in intact circuits. Further, *in situ* data shows that localized Ca^{2+} elevations in distal processes of astrocytes occur at a higher frequency than somatic increases. This is difficult to examine *in vivo*; bulk loading of SR101 and OGB require anesthesia and allow for imaging primarily of somatic response, with limited detection of Ca^{2+} activity in processes. Additionally, anesthesia has been shown to influence both neuronal and astrocytic activity, which may alter the spatial and temporal coding of Ca^{2+} transients in response to stimuli. We are currently investigating visually evoked Ca^{2+} responses in visual cortex astrocytes of an awake, head-fixed animal using two-photon microscopy. To achieve this, we have generated a new astrocyte reporter line that expresses tdTomato in cortical astrocytes driven by the shortened human GFAP promoter, together with viral mediated delivery of a membrane-bound genetically encoded Ca^{2+} indicator, Lck-GCamp5G, to specifically target cortical astrocytes. We present evidence that Ca^{2+} transients in distal processes of cortical astrocytes are more frequent than has been observed for anesthetized preparation, with variable relationship to somatic responses. Furthermore, we are able to identify structurally identifiable regions of distal processes from single astrocytes that are responsive to visual stimuli and display orientation tuning. The combination of these technologies will allow us to further explore the functional role of astrocytes in the primary visual cortical circuit.

Disclosures: R. Garcia: None. **M. Goard:** None. **J. Petravicz:** None. **M. Sur:** None.

Poster

508. Astroglia Function and Reaction to Pathological Processes

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 508.26/L5

Topic: B.11. Glial Mechanisms

Title: A quantitative evaluation of optogenetically-induced calcium signaling in astrocytes

Authors: *L. BALACHANDAR, A. RAYMOND, M. NAIR, J. RIERA DIAZ
Florida Intl. Univ., Miami, FL

Abstract: Optogenetics is a modern technique used to understand functioning principles of targeted neurons and their circuits. Studies have shown opsin gene expression in neurons modulate neural activity by light stimulation, which has been expanded to non-neuronal cells (i.e. astrocytes, Figueiredo et al., 2010). However, the mechanisms by which light stimulation affects channelrhodopsins on astrocytes have not been quantified. The main purpose of this study is to provide a full protocol to evaluate light stimulation profiles and viral transduction parameters which are optimal to produce calcium signaling in rat astrocytes. A DNA plasmid with an optogenetic probe (from Addgene) having a Chr2 and GFAP promoter was used to specifically target astrocytes. Creation of the virus by transformation, packaging by transfection and expression studies by transduction after titration were done. Primary rat astrocytes (Sciencell) were transduced with different viral loads and varying durations. Transfected astrocytes were stained with Sulphorhodamine 101 and Fluo-4 to evaluate morphometric changes and light-induced calcium signaling measurement (Stimulation with 470-490 nm light flashes with different durations and intensities on a DV Elite microscope) Transductions of astrocytes *in vitro* at all loads are indicated in Fig. 1A. Transduction time and viral load played a crucial role in light-induced calcium signaling (Fig. 1B). Calcium signaling evoked optogenetically resembled patterns obtained with standard low-doses of exogenous glutamate. We used a model of calcium signaling in astrocytes to estimate the total flux of calcium ions entering the cytoplasm for each condition (Riera et al., 2011). We developed a method to quantify AAV/light-induced calcium signaling in astrocytes. We evaluated ideal stimulation parameters (e.g. intensity, duration, frequency) to evoke astrocytic calcium waves. This would thereby pave way in understanding one of our long-term goals of the role of changes in calcium activity in astrocytes, as a result of epileptic seizures.

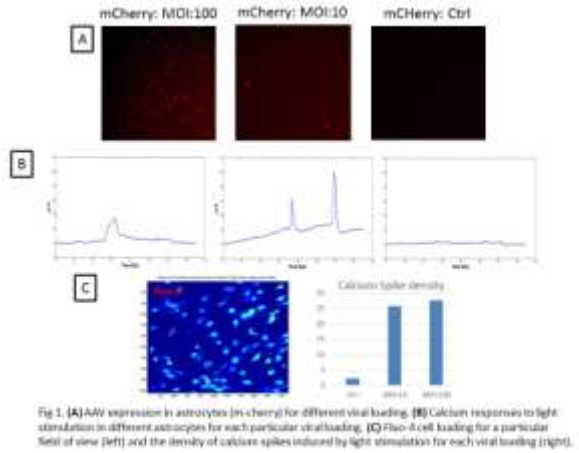

Fig 1. (A) AAV expression in astrocytes (m-cherry) for different viral loading. (B) Calcium responses to light stimulation in different astrocytes for each particular viral loading. (C) Fluo-4 cell loading for a particular field of view (left) and the density of calcium spikes induced by light stimulation for each viral loading (right).

Disclosures: L. Balachandar: None. **A. Raymond:** None. **M. Nair:** None. **J. Riera Diaz:** None.

Poster

508. Astroglia Function and Reaction to Pathological Processes

Location: Halls A-C

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Topic: B.11. Glial Mechanisms

Support: Migraine Research Foundation (SMB)

The Skirball Foundation (AC)

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Title: Cortical spreading depression induced optogenetically in awake mice

Authors: *S. M. BACA, A. BARTH, R. T. JONES, I. MODY, A. C. CHARLES
Neurol., UCLA, Los Angeles, CA

Abstract: Cortical spreading depression (CSD) is a wave of increased brain activity followed by a profound and lasting inhibition. CSD is a likely mechanism for migraine aura, and CSD is consistently observed when the brain is injured. Photostimulation of channelrhodopsin-2 receptor in astrocytes in awake mice triggered CSD with local field potential changes similar to those observed previously in anesthetized mice and brain slices. CSDs could be reliably elicited from the same location although the time needed to elicit subsequent CSDs varied. The minimally invasive approach of optogenetically triggering CSD in awake animals is a novel platform for studying basic mechanisms of CSD and may provide a translational platform to screen migraine therapeutics.

Disclosures: S.M. Baca: None. **A. Barth:** None. **R.T. Jones:** None. **I. Mody:** None. **A.C. Charles:** None.

Poster

508. Astroglia Function and Reaction to Pathological Processes

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Topic: B.11. Glial Mechanisms

Support: NICHD R01HD061946

Swiss National Science Foundation 31003A-122166

Title: Off targeted effects of aquaporin 4 RNA interference on connexin 43 expression via changes of microRNA expression

Authors: A. JULLIENNE[1], A. M. FUKUDA[2], N. NISHIYAMA[1], *J. BADAUT[4,3]
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Abstract: Background: Water movements in the brain are critical for cellular functions by regulating cell volume, and homeostasis in extracellular and intracellular compartments. We previously showed that with a 27% decrease of the water channel aquaporin 4 (AQP4) after intracortical injection of siRNA targeting AQP4 (siAQP4) in rat, water mobility was decreased by 50% as interpreted from apparent diffusion coefficient (ADC) value changes. In fact, it has been previously reported that siAQP4 also resulted in a decreased expression of the gap junction protein connexin 43 (Cx43) in primary astrocyte cultures. Interestingly, the absence of Cx43 and Cx30 has consequences on the distribution of AQP4 in astrocyte end feet. MicroRNA (miRNA) are small (21-22 nucleotides) noncoding RNA, which have a regulatory activity in animals. Several miRNA target both AQP4 and Cx43 mRNA and could therefore be good candidate to regulate simultaneously the level of expression of AQP4 and CX43. We hypothesized that the off target effects of siAQP4 on Cx43 expression occur via modifications of expression of miRNA targeting both proteins. Methods: P17 rats were intracortically injected with siAQP4, siCx43 or siGLO (control), on day 0 and day 2. On day 3, animals were euthanized after T2WI and DWI scans. One part of the animals was used for protein assessment (western blot and immunohistochemistry) and the other one was used for miRNA assessment (PCR). To determine miRNA targeting both AQP4 and Cx43 we used literature and databases (targetscan.org and microrna.org). We selected 6 miRNA among the most conserved between species. Results: Western blot and immunohistochemistry showed that siAQP4 injection indeed resulted in decreased levels of Cx43 *in vivo* compared to siGLO-injected rats and a decrease of ADC values. In contrast, injection of siCx43 did not change the levels of AQP4 and no changes in T2WI and ADC. The lower expression of Cx43 in siAQP4 treated animals compared with control animals is associated with an up-regulation of miR224. Discussion: The changes of miRNA expression could be one of the molecular mechanisms explaining in part the effect of siAQP4 on Cx43 expression. Decrease of CX43 is not enough to induce changes in ADC signal; AQP4 decrease is mandatory to observe ADC decrease.

Disclosures: A. Jullienne: None. **A.M. Fukuda:** None. **N. Nishiyama:** None. **J. Badaut:** None.

Poster

508. Astroglia Function and Reaction to Pathological Processes

Location: Halls A-C

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Berry postdoctoral fellowship

NIH T32GM007365

NHMRC (Australia)

CJ Martin Fellowship

NIH R00 GM95713

Title: An RNA-Seq transcriptome and splicing database of glia, neurons, and vascular cells of mouse and human cerebral cortex

Authors: *Y. ZHANG[1], K. CHEN[2], S. SLOAN[1], M. BENNETT[1], A. SCHOLZE[1], S. O'KEEFFE[3], H. PHATNANI[3], P. GUARNIERI[3], C. CANEDA[1], N. RUDERISCH[4], S. DENG[2], S. LIDDELOW[1], C. ZHANG[3], R. DANEMAN[4], T. MANIATIS[3], J. WU[2], B. BARRES[1]
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Abstract: The major cell classes of the brain differ in their developmental processes, metabolism, signaling, and function. To better understand the functions and interactions of the cell types that comprise these classes, we prospectively purified representative populations of neurons, astrocytes, oligodendrocyte precursor cells, newly formed oligodendrocytes, myelinating oligodendrocytes, microglia, endothelial cells, and pericytes from the mouse cerebral cortex. We generated a transcriptome database for these 8 cell types by RNA sequencing and used a highly sensitive algorithm to detect alternative splicing events. Bioinformatic analyses identified thousands of new cell type-enriched genes and splicing isoforms that will provide novel markers for cell identification, tools for genetic manipulation, and insights into the biology of the brain. For example, our data provides clues as to how astrocytes dynamically regulate glycolytic flux and lactate generation. This dataset will provide a

powerful new tool for understanding the development and function of the brain. We are currently expanding our RNA-seq based transcriptome analysis to include human neurons and glia. We have developed an immunopanning-based method to purify neurons, astrocytes, oligodendrocytes, and microglia from fetal and postnatal human surgical brain samples. Astrocytes promote synapse formation and function in both rodent and human neurons, but whether human astrocytes are more potent stimulators of synapse formation remains unknown. Our gene profiling of human and rodent astrocytes is revealing many genes that are expressed specifically by human astrocytes. Thus, we are testing the function of several candidate synaptogenic molecules that are specific to human astrocytes to unveil functional differences between human and rodent astrocytes.

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Poster

509. Microglia Activation Pathways

Location: Halls A-C

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Program#/Poster#: 509.01/L9

Topic: B.11. Glial Mechanisms

Title: SUMO-1 is an upstream regulator of NFκB-mediated inflammatory response of activated microglia

Authors: ***P. RANGARAJAN**[1], A. KARTHIKEYAN[2], B. TAN[2], E. A. LING[2], S. T. DHEEN[2]
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Abstract: Microglial cells are the resident immune cells of the Central Nervous System. Chronic activation of microglia resulting in excessive release of cytokines has been implicated in several neurodegenerative disorders. Sumoylation, a post translational modification whereby the small ubiquitin like modifier - 1 (SUMO-1) is covalently attached to the target protein, has been identified as a key regulatory mechanism of several cellular processes including inflammation. The role of sumoylation in microglia-mediated inflammation has not been well established. In this study, we aim to characterize the function of SUMO-1 in activated microglia. SUMO-1 expression has been localized in microglia from tissue sections of early postnatal rat brains. Its expression was found to be increased in activated BV-2 microglia *in vitro*. Further, in activated

BV-2 microglia, the nuclear fraction of SUMO-1 was found to be significantly higher than the cytosolic fraction. It has been previously shown that sumoylation of NFκB essential modulator (NEMO) by SUMO-1 causes the activation and nuclear translocation of NFkB. In conjunction with this, our study showed that knockdown of SUMO-1 in activated BV-2 microglia decreased protein levels of NFκB and the proinflammatory cytokine, tumour necrosis factor-alpha (TNF-α). Nuclear translocation of NFκB has also been found to be decreased upon SUMO-1 knockdown in activated BV-2. In silico screening has identified other potential SUMO-1 targets like TRIKA1, TRAF6, IKKα, IKKβ, IκBα and p65 which are all involved in the NFκB-mediated inflammatory pathway. Our study suggests that SUMO-1 expression in microglia upon LPS stimulation appears to regulate the nuclear translocation of NFκB which activates the transcription of pro-inflammatory cytokines like TNF-α. Further studies are required to elucidate the role of sumoylation in the inflammatory pathway mediated by activated microglia.

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Poster

509. Microglia Activation Pathways

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Support: PUMC scholarship to Dr. Jianmin Zhang (2012RC01)

PUMC predoctoral fellowship to Huaishan Wang (2012-1001-006)

Title: A potential role of TSPO in inflammasome activation

Authors: H. WANG[1,2], Q. YANG[3], J. YANG[3], W. WANG[4], S. CHAI[3], L. CUI[3], Y. HU[3], H. CHEN[3], W. HE[3], *J. ZHANG[5,3,2,1]
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Abstract: Neuronal damage induced by the activation of microglia through secreting inflammatory cytokines, such as IL-1β, TNF-α and IL-6, is a major causal contributor to

neurodegenerative diseases, such as Alzheimer's disease and Parkinson's disease. Inflammasome activation in microglia causes the neuroinflammation in the brain, which is also a critical step to initiate the innate immune responses. Previous studies shown that mitochondrial plays crucial roles in the inflammasome activation and regulation. Mitochondrial translocator protein (18kDa) (TSPO), an outer membrane mitochondrial protein, was originally described as a peripheral benzodiazepine receptor. TSPO participates in steroid biosynthesis by translocating cholesterol into mitochondria from cytoplasmic side, which is also involved in kinds of mitochondria functions and secondary signal transduction. Recently, many clinical studies demonstrate an association of TSPO with neuroinflammation. However, the roles of TSPO in inflammasome activation and neuroinflammation remain unclear. Here, we investigated the roles of TSPO in the activation of inflammasome. Our results showed that TSPO were mainly expressed in the monocytes and the platelets, which were sorted from human peripheral blood mononuclear cells by FACS. Low level of TSPO expression was observed in T cells, but not in granulocytes, B cells and NK cells. Lipopolysaccharide (LPS) treatment could significantly upregulate the expression of TSPO and two inflammasome proteins, Pro-caspase-1 and Pro-IL-1β, in a mouse microglia cell line BV2 cells. All inflammasome activators used in this study, including Nigericin, ATP, Alum, CPPD, MSU, Poly(I:C), Poly(dA:dT), Flagellin and MDP, could induce the release of IL-1β in murine peritoneal macrophages after primed 3h by 1μg/ml LPS, and all of which could be inhibited by a TSPO antagonist ligand Ro5-4864. However, another TSPO ligand PK11195 only displayed partial inhibitory effect, and neither of two ligands plays any roles in pyroptosis (inflammasome activation associated apoptosis) induced by Nigericin. Under confocal microscope, the colocolization of TSPO and NLRP3 could be decreased by TSPO ligands but significant increased by Nigericin in LPS-primed mouse microphages. All these results taken together indicate that TSPO could play a potential role in inflammasome activation producing IL-1β, but not pyroptosis. Furthermore, TSPO may be involved in the neuroinflammation in neurodegenerative diseases. Keywords: TSPO, Neuroinflammation, Inflammasome, PK11195, Ro5-4864.

Disclosures: **H. Wang:** None. **Q. Yang:** None. **J. Yang:** None. **W. Wang:** None. **S. Chai:** None. **L. Cui:** None. **Y. Hu:** None. **H. Chen:** None. **W. He:** None. **J. Zhang:** None.

Poster

509. Microglia Activation Pathways

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Program#/Poster#: 509.03/L11

Topic: B.11. Glial Mechanisms

Support: CNRM

DMRDP

Title: The Angiotensin II receptor blockers reduce LPS-mediated microglial activation via AT1 independent pathways

Authors: *K. O. AFFRAM, S. VILLAPOL, J. P. SHAH, K. MITCHELL, A. J. SYMES
Pharmacol., Uniformed Services Univ., Bethesda, MD

Abstract: Traumatic brain injury (TBI) can lead to chronic neuroinflammation. Drugs which reduce neuroinflammation after TBI should therefore be beneficial. Studies in our laboratory have shown that FDA-approved angiotensin II type I receptor (AT1R) blockers (ARBs) improve recovery from CCI in mice, suggesting they may be a potential treatment for patients with TBI. Mice treated with ARBs after TBI show reduced activation of microglia, pointing to anti-inflammatory actions of ARBs. However, it is not known whether angiotensin II acts directly through AT1Rs on microglia providing a target for ARB activity. ARBs not only block signaling through the AT1R but also act as agonists at PPAR-γ. Thus, ARBs may act to reduce inflammation in microglia either through blocking signaling by the AT1R or through directly stimulating PPAR-γ. We therefore investigated the expression of AT1Rs in primary microglia, and determined whether ARBs could reduce inflammation directly in cultured primary microglia from rats and wild type and AT1A knockout (KO) mice. Primary microglial cultures were pretreated with the ARBs, telmisartan (100μM) or candesartan (200μM) for two hours before addition of lipopolysaccharide (LPS) for 16 hours. RNA was isolated and gene expression determined by qPCR. AT1AR mRNA was expressed in primary microglia at low levels, and its expression was not altered by LPS treatment. As expected, LPS induced the expression of the pro-inflammatory genes, IL-1β and iNOS. Treatment with either telmisartan or candesartan or the PPAR-γ agonist rosiglitazone, reduced the LPS-stimulated induction of both IL-1β and iNOS in rat microglia showing that ARBs directly ameliorate LPS-induced microglial activation. Telmisartan and candesartan treatment of microglia derived from AT1A KO mice showed the same effect, reducing LPS stimulation of IL-1β and iNOS expression. These data show that this effect does not require the AT1 receptor and is therefore mediated by a non-angiotensin driven mechanism, possibly through stimulation of the PPAR-γ receptor. We continue to investigate the relative contribution of AT1R and PPAR-γ to the ARB-mediated reduction of LPS-stimulated gene expression. Our data show that multiple mechanisms may contribute to the ability of ARBs to ameliorate chronic neuroinflammation after TBI.

Disclosures: K.O. Affram: None. **S. Villapol:** None. **J.P. Shah:** None. **K. Mitchell:** None. **A.J. Symes:** None.

Poster

509. Microglia Activation Pathways

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 509.04/L12

Topic: B.11. Glial Mechanisms

Support: CIHR MOP82743

Title: Multiple separable mechanisms of statin treatment on primary cultured microglia

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Abstract: As the primary immune cells of the central nervous system, microglia contribute to neural development, homeostatic mechanisms, and cellular and synaptic plasticity, in addition to their well characterized roles in the foreign body response and inflammation. Increasingly, inappropriate activation of microglia is being reported as a component of inflammation in a wide range of neurodegenerative and neuropsychiatric disorders. The statin class of cholesterol-lowering drugs have been observed to have anti-inflammatory and neuroprotective effects in both neurodegenerative diseases and ischemic stroke, and are suggested to act by attenuating microglial activity. We sought to investigate the effects of statin treatment on the secretory profile and phagocytic activity of primary cultured microglia, and to dissect the mechanism of action of statins on microglial activity. Statin treatment altered the release of cytokines and trophic factors from microglia, including interleukin-1-β, tumour necrosis factor-α, and brain derived neurotrophic factor in a cholesterol-dependent manner. Conversely, statins inhibited phagocytosis in microglia in a cholesterol-independent manner, suggesting the two effects occur through two distinct and separable molecular mechanisms.

Disclosures: M.A. Churchward: None. **K.G. Todd:** None.

Poster

509. Microglia Activation Pathways

Location: Halls A-C

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Program#/Poster#: 509.05/M1

Topic: B.11. Glial Mechanisms

Support: NIA P01 AG022550

NIA P01 AG027956

Title: Soluble oligomeric Abeta42 affects microglia activation and mitochondrial respiration

Authors: *C. TAN, S. SARKAR, J. SIMPKINS
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Abstract: Microglia activation is associated with many neurodegenerative diseases such as Alzheimer's disease (AD). When activated, microglia cells change their morphology and become phagocytic. In the Alzheimer's brain, microglia play an important role in scavenging plaque-damaged cells and in clearing Aβ plagues. Soluble oligomeric Aβ42 is capable to stimulate microglia activation. In AD brain, microglia accumulate around senile plaques as a hallmark of the pathology and Aβ42 has been shown to rapidly activate microglia through the nuclear factor kappa-B (NFkB) pathway. We found that mitochondrial oxidative phosphorylation is affected in a dose-dependent manner in a microglia cell line (C8-B4). We demonstrated that basal, maximal and spare respiration capacities of mitochondria increase at low concentrations of oligomeric Aβ42 and decrease at higher concentrations. Flow cytometry for markers of microglial activation (Iba-1 upregulation) similarly shows a low dose increase and a high dose inhibition. In contrast, the Aβ42 stimulation does not alter mitochondrial oxidative phosphorylation in a mouse hippocampal neuronal cell line (HT22), suggesting a brain cell-specific response to Aβ42. Our experiment results suggest that at low concentrations of Aβ42, which would normally occur in healthy brains, microglia activation and phagocytosis are associated with increased mitochondrial function. In contract, at higher pathological levels of Aβ42, mitochondrial activation and its associated mitochondrial functions are inhibited, which may prevent phagocytosis and allow the accumulation of Aβ42. (Supported by NIH grants NIA P01 AG022550 and NIA P01 AG027956.)

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Poster

509. Microglia Activation Pathways

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Topic: B.11. Glial Mechanisms

Support: JSPS KAKENHI 10J00463

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Title: Fosb gene products contribute to excitotoxic microglial activation by regulating the expression of complement C5a receptors in microglia

Authors: *H. NOMARU[1], K. SAKUMI[1,2], A. KATOGI[1], Y. N. OHNISHI[3], D. TSUCHIMOTO[1,2], E. J. NESTLER[3], Y. NAKABEPPU[1,2]
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Abstract: The Fosb gene encodes subunits of the activator protein-1 transcription factor complex. Two mature mRNAs, Fosb and ΔFosb, encoding full-length FOSB and ΔFOSB proteins respectively, are formed by alternative splicing of Fosb mRNA. Fosb products are expressed in several brain regions. Moreover, Fosb-null mice exhibit depressive-like behaviors and adult-onset spontaneous epilepsy, demonstrating important roles in neurological and psychiatric disorders. Study of Fosb products has focused almost exclusively on neurons; their function in glial cells remains to be explored. In this study, we found that microglia express equivalent levels of Fosb and ΔFosb mRNAs to hippocampal neurons and, using microarray analysis, we identified six microglial genes whose expression is dependent on Fosb products. Of these genes, we focused on C5ar1 and C5ar2, which encode receptors for complement C5a. In isolated Fosb-null microglia, chemotactic responsiveness toward the truncated form of C5a was significantly lower than that in wild-type cells. Fosb-null mice were significantly resistant to kainate-induced seizures compared with wild-type mice. C5ar1 mRNA levels and C5aR1 immunoreactivity were increased in wild-type hippocampus 24 hours after kainate administration; however, such induction was significantly reduced in Fosb-null hippocampus. Furthermore, microglial activation after kainate administration was significantly diminished in Fosb-null hippocampus, as shown by significant reductions in CD68 immunoreactivity, morphological change and reduced levels of Il6 and Tnf mRNAs, although no increase in the number of Iba-1-positive cells was observed. These findings demonstrate that, under excitotoxicity, Fosb products contribute to a neuroinflammatory response in the hippocampus through regulation of microglial C5ar1 and C5ar2 expression.

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Poster

509. Microglia Activation Pathways

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Program#/Poster#: 509.07/M3

Topic: B.11. Glial Mechanisms

Support: BrightFocus Foundation grant (A2012115)

Title: NFATc2 regulates microglial proinflammatory phenotype both *in vitro* and *in vivo*

Authors: *A. GHATAK, K. L. PUIG, C. K. COMBS
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Abstract: Nuclear factor of activated T cells (NFAT) is known to play a critical role in initiating and regulating transcription of specific pro-inflammatory genes. Previous work from our laboratory described a role of NFAT in regulating stimulus-induced microglial activation. Based upon this, we hypothesized that a particular isoform of NFAT may represent an attractive target for microglial modulation strategies. Using primary murine microglial cultures, we observed that NFATc2 was the most abundantly expressed isoform. To evaluate the relative importance of NFATc2 activity in regulating microglial phenotype, we first compared primary microglial cultures from wild type and NFATc2 knockout mice. Although basal levels of cytokine secretion did not differ between the cultures, NFATc2 knockout cells had significantly attenuated TNFα and IL-6 secretion compared to wild type microglia in response to stimulation with either Aβ peptide or lipopolysaccharide. Consistent with the *in vitro* data, hippocampal lysate ELISAs from 8 month old NFATc2 knockout mice demonstrated significantly reduced cytokine levels compared to wild type controls. Surprisingly, immunohistochemistry demonstrated increased immunoreactivity for the microglial marker, Iba1, in the brains of NFATc2 knockout mice compared to controls. These findings were validated by western blotting results. Importantly, neither light/dark box testing nor Y maze spontaneous alternation behavior showed any significant behavioral differences between the two strains of mice indicating no dramatic behavioral compromise in NFATc2 knockout mice. Our findings demonstrate that NFATc2 has a positive role in regulating microglial cytokine secretion and phenotype *in vitro* and *in vivo*. These data support the idea that inhibiting microglial NFATc2 activity may represent a valid approach for limiting microgliosis.

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Poster

509. Microglia Activation Pathways

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Topic: B.11. Glial Mechanisms

Support: Japan Society for the Promotion of Science(JSPS) KAKENHI Grant Number 26460338

JSPS Research Fellow 26・5605

Title: EP4 receptor-associated protein (EPRAP) in microglia promotes inflammation in the brain

Authors: *R. FUJIKAWA[1,2], M. MINAMI[1], S. HIGUCHI[1], M. YASUI[1], T. IKEDO[1], M. NAGATA[1], M. YOKODE[1]
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Abstract: Background and aims: EP4 receptor-associated protein (EPRAP) was isolated as a novel cytoplasmic signaling partner of EP4 receptor. Recently, we have generated EPRAP-deficient (EPRAP-KO) mice, and demonstrated that EPRAP attenuated macrophage activation in the periphery using various animal models of chronic inflammation. EPRAP also abundantly exists in the brain; however, little is known about the function of EPRAP in the central nervous system. In this study, we investigated the role of EPRAP in brain inflammation. Methods: The localization of EPRAP was assessed by immunohistochemical analyses. To explore the role of EPRAP in microglial activation, primary microglial cells from WT and EPRAP-KO mice were isolated and cultured: cytokine levels in cell-conditioned media were measured after 24-hour incubation with 10 ng/mL of lipopolysaccharide (LPS). In *in vivo* experiments, LPS was administered intraperitoneally at a dose of 100 mg/kg both in WT and EPRAP-KO mice, and the mRNA levels of pro-inflammatory cytokines in the cerebral cortex were measured 6-hour after the administration. In addition, to clarify the effect of EPRAP against neuronal cell death, kainic acid (0.2 μg in 4.0 μl of PBS) was injected intraventricularly in these mice, and brain samples were harvested 72-hour later. Sections including the CA1 field of the dorsal hippocampus were examined for karyopyknotic cells. Results: Immunohistochemical analyses demonstrated that EPRAP was colocalized in Iba1-positive microglial cells, suggesting that EPRAP is associated with inflammatory responses in the brain. EPRAP-deficiency markedly decreased LPS-induced expressions of pro-inflammatory cytokines and chemokines including TNFα, MCP-1 and IL-6 in primary microglial cells. Accordingly, EPRAP-KO mice showed less microglial accumulation

and pro-inflammatory cytokine expression in the cortex induced by intraperitoneal administration of LPS. Furthermore, compared with WT, EPRAP-KO mice showed less microglial activation as well as neuronal cell death in the hippocampus induced by intraventricular injection of kainic acid. Conclusion: Our results indicated that, in contrast to peripheral macrophage, EPRAP in microglia promoted pro-inflammatory activation and may be a key to deteriorate neuronal damages by brain inflammation. EPRAP could be a novel therapeutic target for inflammatory disorders of the central nervous system including Alzheimer's disease or stroke.

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Poster

509. Microglia Activation Pathways

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Topic: B.11. Glial Mechanisms

Support: NIH Grant 1P50 AT006273

Title: Elderberry and its active polyphenol components on oxidative and anti-oxidative signaling pathways in microglial cells

Authors: *G. Y. SUN[1], Z. CHEN[2], D. AJIT[2], M. HANNINK[2], K. L. FRITSCHE[2], A. SIMONYI[2], A. L. THOMAS[1], Z. GU[3], D. L. LUBAHN[2]
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Abstract: Many edible berries are rich source of polyphenols with potent anti-oxidative properties, and have been shown to offer beneficial effects to promote human health. Elderberry (*Sambucus nigra*) is regarded as a medicinal plant for treatment of a number of maladies and for boosting the immune system. The action of elderberries and their active components on oxidative and anti-oxidative responses has not been investigated in microglial cells. In this study, we examined effects of freeze-dried ethanol extract of elderberry pomace and a few of its putative bioactive components (namely, quercetin, rutin, cyanidin, and cyanidin-3-glucoside) on liposaccharide (LPS)- induced oxidative and inflammatory pathways for induction of free radicals including NO and reactive oxygen species (ROS) in BV-2 microglial cells. While elderberry extract inhibited LPS-induced ROS production, its effect on induction of NO was

typically an inverted U shape curve. Among the active flavonol components, quercetin was the most potent in inhibiting LPS-induced NO and ROS. LPS also activated expression of heme oxygenase-1 (HO-1) via the anti-oxidant pathway involving Keap1/Nrf2. Interestingly, quercetin alone could induceHO-1, and quercetin exposure followed by stimulation with LPS further enhanced HO-1 protein expression. In addition, stimulation of HO-1 synthesis by quercetin and LPS was regulated by ERK1/2 and p38MAPK, indicating multiple modes of regulation of the Keap1/Nrf2 pathway. These results suggest that botanicals exhibiting electrophilic properties are capable of conferring resilience against the oxidative-inflammatory stress by upregulation of the anti-oxidative pathway.

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Poster

509. Microglia Activation Pathways

Location: Halls A-C

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Topic: B.11. Glial Mechanisms

Title: Investigating the effect of microglial α7 nicotinic receptor activation on lipopolysaccharide mediated tumor necrosis factor-α secretion

Authors: *H. PATEL[1], A. DUNAH[1], R. LORING[2]
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Abstract: Microglia, the resident immune cells of the brain, plays an important role in inflammation in neurodegenerative diseases such as Alzheimer's and Parkinson's disease. Activating α7 nicotinic receptors on macrophages has anti-inflammatory effects in the peripheral nervous system, and α7 nicotinic receptors are also found in brain microglia, which share properties with macrophages. We hypothesize that activating α7 nicotinic receptors on microglia has anti-inflammatory properties. We studied whether α7 receptor activation of cultured rat microglia blocks lipopolysaccharide (LPS)-mediated secretion of the inflammatory cytokine, tumor necrosis factor-α (TNF). A five hour treatment of cultured rat microglia with LPS (1-10 ng/ml) significantly increased TNF levels in cell medium, measured using an enzyme-linked immunosorbent assay. Pretreatment with nicotine as well as the α7-selective agonist PNU282987 significantly inhibited LPS mediated TNF secretion, with no evidence of cell death. Nicotinic activation also changed the morphology of LPS activated microglia more towards a resting state

phenotype. Thus, our results imply that α7 nicotinic receptors have anti-inflammatory effects by significantly blocking TNF secretion in activated microglia. Future research should focus on examining the signaling interactions between α7 nicotinic receptors and TNF secretion and characterizing the α7 receptors expressed in microglia.

Disclosures: **H. Patel:** A. Employment/Salary (full or part-time):; Biogen Idec. **A. Dunah:** A. Employment/Salary (full or part-time):; Biogen Idec. **R. Loring:** None.

Poster

509. Microglia Activation Pathways

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Topic: B.11. Glial Mechanisms

Support: Grant-in-Aid for Youg Scientists (B)

Title: Changes of microglial characters in response to extracellular stimulation

Authors: T. TANAKA, K. MURAKAMI, Y. BANDO, *S. YOSHIDA
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Abstract: Microglia, generally considered to be immune cells of the central nervous system (CNS), are involved in many types of inflammatory processes in the brain. They are critical in developmental processes and are imperative for the maintenance of neuronal homeostasis. Recent studies have demonstrated that under specific polarization conditions microglia develop into different phenotypes, termed M1 and M2. However, the characterization of M1- and M2-polarized microglia and the mechanisms for regulating polarization are largely unknown. Here, we examined the characterization of LPS-treated M1 and IL-4-treated M2 microglia *in vitro*. The addition of M2 microglial conditioned media (CM) resulted in elongated neurite length compared with M1 microglial CM due to promotion of expression of neurotrophic factors and inhibition of toxic factors from M2 microglia. M2 microglia exhibited greater phagocytic capacity than M1 microglia. Morphology of M1 microglia were characterized by larger soma and that of M2 microglia were characterized by long processes. These characterization were converted in response to polarization switches. These results suggest that endogenous molecule in microglia control a dramatic phenotypic change but not originally different population. We found that interferon regulatory factor (IRF) 7 and IRF9 were elevated during M2-to-M1

microglia *in vitro* and *in vivo*. Thus, our findings reveal that endogenous IRF signaling including IRF7 and IRF9 implicated in switching the microglial polarization.

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Poster

509. Microglia Activation Pathways

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Topic: B.11. Glial Mechanisms

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Title: A potential role of TSPO in inflammasome activation

Authors: J. ZHANG[1,2,3], Q. YANG[1,2], J. YANG[1,2], W. WANG[4], S. CHAI[1,2], L. CUI[1,2], Y. HU[1,2], H. CHEN[1,2], W. HE[1,2], *H. WANG[1,2]
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Abstract: Neuronal damage induced by the activation of microglia through secreting inflammatory cytokines, such as IL-1β, TNF-α and IL-6, is a major causal contributor to neurodegenerative diseases, such as Alzheimer's disease and Parkinson's disease. Inflammasome activation in microglia causes the neuroinflammation in the brain, which is also a critical step to initiate the innate immune responses. Previous studies shown that mitochondrial plays crucial roles in the inflammasome activation and regulation. Mitochondrial translocator protein (18kDa) (TSPO), an outer membrane mitochondrial protein, was originally described as a peripheral benzodiazepine receptor. TSPO participates in steroid biosynthesis by translocating cholesterol into mitochondria from cytoplasmic side, which is also involved in kinds of mitochondria functions and secondary signal transduction. Recently, many clinical studies demonstrate an association of TSPO with neuroinflammation. However, the roles of TSPO in inflammasome activation and neuroinflammation remain unclear. Here, we investigated the roles of TSPO in the activation of inflammasome. Our results showed that TSPO were mainly expressed in the monocytes and the platelets, which were sorted from human peripheral blood mononuclear cells

by FACS. Low level of TSPO expression was observed in T cells, but not in granulocytes, B cells and NK cells. Lipopolysaccharide (LPS) treatment could significantly upregulate the expression of TSPO and two inflammasome proteins, Pro-caspase-1 and Pro-IL-1β, in a mouse microglia cell line BV2 cells. All inflammasome activators used in this study, including Nigericin, ATP, Alum, CPPD, MSU, Poly(I:C), Poly(dA:dT), Flagellin and MDP, could induce the release of IL-1β in murine peritoneal macrophages after primed 3h by 1μg/ml LPS, and all of which could be inhibited by a TSPO antagonist ligand Ro5-4864. However, another TSPO ligand PK11195 only displayed partial inhibitory effect, and neither of two ligands plays any roles in pyroptosis (inflammasome activation associated apoptosis) induced by Nigericin. Under confocal microscope, the colocolization of TSPO and NLRP3 could be decreased by TSPO ligands but significant increased by Nigericin in LPS-primed mouse microphages. All these results taken together indicate that TSPO could play a potential role in inflammasome activation producing IL-1β, but not pyroptosis. Furthermore, TSPO may be involved in the neuroinflammation in neurodegenerative diseases. Keywords: TSPO, Neuroinflammation, Inflammasome, PK11195, Ro5-4864.

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Poster

509. Microglia Activation Pathways

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Program#/Poster#: 509.13/M9

Topic: B.11. Glial Mechanisms

Title: Analysis of MAP kinase cascade in M-CSF-dependent microglial proliferation

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Abstract: Transection of rat facial nerve leads to an increase of microglial cell number in the ipsilateral facial nucleus. In the previous study, we demonstrated that up-regulated macrophage-colony stimulating factor (M-CSF) in the transected facial nucleus triggers the induction of cFms (receptor for M-CSF), proliferating cell nuclear antigen (PCNA) and cell cycle-associated proteins, including cyclins, cyclin-dependent protein kinases (Cdks) and Cdk inhibitors (CdkIs) in microglia and causes the microglia to divide. The microglial proliferation was found to be

regulated by the interactions among cyclin A, cyclin D, Cdk2, Cdk4 and p21, and the induction of cyclins/PCNA and cFms, which are requisite for microglial proliferation, was found to be differentially regulated by c-Jun N-terminal kinase (JNK) and p38 MAP kinase (p38) in M-CSF-stimulated microglia. However, the knowledge about signaling mechanism for M-CSF-dependent microglial proliferation is largely limited. In the present study, we analyzed the MAP kinase cascade in M-CSF-triggered microglial proliferation. We first confirmed that cFms transmembrane receptor is phosphorylated just after M-CSF stimulation. Subsequently, both JNK and p38 were phosphorylated. These activations were significantly suppressed by pretreatment with cFms inhibitor, suggesting that JNK and p38 are downstream of cFms. The upstream kinases of JNK and p38, MKK4 and MKK3/6, were also recognized to be activated by stimulation with M-CSF. Furthermore, we demonstrated that mitogen activated protein kinase activated protein kinase-2 (MAPKAPK-2), cyclic AMP responsive element binding protein (CREB), mitogen-and stress-activated protein kinase-1 (MSK-1) and ETS family of transcription factor (ETS-1) are all activated in M-CSF stimulated microglia. Our results indicated that M-CSF/cFms signaling in microglia is linked to MKK4-JNK and MKK3/6-p38 cascades, and at downstream of these MAP kinases MAPKAPK-2, CREB, MSK-1 and ETS-1 are serving for the induction of cyclins and cFms.

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Poster

509. Microglia Activation Pathways

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Topic: B.11. Glial Mechanisms

Support: KAKENHI 24890083

Title: A new tracer [11C]DPA713 tops a conventional tracer [11C]PK11195 in visualizing activated microglia in the living human brain

Authors: *M. YOKOKURA[1], Y. OUCHI[2], K. TAKEBAYASHI[1], E. YOSHIKAWA[3], M. FUTATSUBASHI[3], Y. IWATA[1], T. TERADA[2], K. NAKAIZUMI[1], N. MORI[1]
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Abstract: Introduction; Translocator protein (TSPO) or a peripheral benzodiazepine receptor is established as a marker of microglial activation in the process of neuroinflammation in the brain. Activated microglia have been reported to increase in number with age in many studies with the most common TSPO PET tracer [11C]PK11195. This tracer, however, is reputed to suffer from high nonspecific binding. In contrast, a new TSPO PET tracer [11C]DPA713 has been recently reported to bind to TSPO more specifically than [11C]PK11195. To examine the power of [11C]DPA713 in their depiction, we estimated binding potentials (BPs) of [11C]DPA713 for the multiple brain regions in young and old healthy adults and compared them with [11C]PK11195 BPs. Methods; Thirteen healthy young (mean age 21.5±1.8 years old) and 12 healthy elderly (71.6±2.6) adults underwent [11C]DPA713 PET and MRI measurements. In a different group, 13 healthy young (mean age 21.4±2.0 years old) and 10 healthy elderly (72.2±8.1) adults also underwent [11C]PK11195 PET and MRI measurements. BP was estimated by a simplified reference tissue model analysis in which a normalized normal input was used as a reference tissue time activity curve as reported previously. We manually set regions of interest (ROIs) bilaterally on multiple brain regions in individual MRI images and obtained the values of BP by transferring ROIs onto corresponding PET BP images. Then, we compared [11C]DPA713 and [11C]PK11195 BP levels between young and old subjects in all ROIs and also examined the differences in magnitude between [11C]DPA713 and [11C]PK11195 BP. Results; In all ROIs, the levels of [11C]DPA713 BP were significant higher in old subjects than in young subjects. There are no significant differences in the levels of [11C]PK11195 BP between young and old subjects. Comparison between two tracers showed that levels of [11C]DPA713 BP in the healthy old subjects were significantly higher than those of [11C]PK11195 BP in a different set of healthy old subjects. Discussion; A new tracer [11C]DPA713 can depict a greater activation of microglia in the brains of old subjects than in young counterparts. Because [11C]DPA713 has a higher binding capacity to activated microglia than [11C]PK11195 in old subjects, [11C]DPA713 would suit for delineation of activated microglia occurring in the elderly brains irrespective of normal or disease states.

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Poster

509. Microglia Activation Pathways

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Topic: B.11. Glial Mechanisms

Support: Grant-in-Aid for Scientific Research from the Ministry of Education, Sports and Culture

Title: Mechanism of TLR4-mediated survival of microglia: Roles of GM-CSF self-production and the activation of JAK2/STAT5 signaling pathways

Authors: *M. KAMIGAKI[1], I. HIDE[2], Y. YANASE[3], S. TANAKA[2], T. SHIRAFUJI[2], M. HIDE[3], N. SAKAI[2]
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Abstract: The activation of Toll-like receptor 4 (TLR4) of rat primary cultured microglia by lipopolysaccharide (LPS) induced rapid death in a concentration dependent manner. Subpopulation of microglia escaped from death and survived for much longer than control cells, which gradually underwent death within two day. Survival of microglia is generally dependent on astrocyte-derived survival factors, such as macrophage colony-stimulating factor (M-CSF) and granulocyte macrophage colony-stimulating factor (GM-CSF). It is not clear, however, how LPS-stimulated microglia could keep surviving after the isolation from astrocytes. To clarify the mechanism underlying TLR4-mediated survival, we examined the possibility that microglia can produce their survival factors by themselves in response to LPS. The mRNA expression of M-CSF and GM-CSF in rat primary microglia after TLR4 activation were measured by using real-time PCR. The mRNA levels of M-CSF were not affected by LPS stimulation. On the other hand, LPS induced a marked increase in GM-CSF mRNA expression from the basal levels which was nearly undetectable in microglia. Moreover, the M-CSF receptor α subunit (GM-CSFRα) mRNA expression was also elevated in LPS-stimulated microglia. To explore the role of JAK2/STAT5 signaling pathway downstream GM-CSFR activation in LPS-stimulated microglial survival, the activation of STAT5 was tested by Western blot analysis. The TLR4 activation of microglia by LPS resulted in the phosphorylation of STAT5. Furthermore, pre-treatment with a specific JAK2 inhibitor, NVP-BSK805, suppressed both STAT5 phosphorylation and survival in microglia after LPS stimulation. These results suggest that TLR4 activation may enhance survival of a subpopulation of microglia at least through self-production of GM-CSF and up-regulation of GM-CSFR. This GM-CSF autocrine pathway may promote the activation of downstream signals including JAK2/STAT5 which controls transcription of survival-related genes.

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Poster

509. Microglia Activation Pathways

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 509.16/M12

Topic: B.11. Glial Mechanisms

Support: NIH PO1 MH64570

NIH F30 MH095664

NIH F32 MH099913

Title: LRRK2 and the MAPK pathway: Friend or foe during neuroinflammation?

Authors: *J. M. PUCCINI[1], D. F. MARKER[2], J. BARBIERI[2], V. S. GOODFELLOW[3], S.-M. LU[2], H. A. GELBARD[2]
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Abstract: Leucine rich repeat kinase 2 (LRRK2) is a known regulator of microglial activation during neuroinflammation in the central nervous system (CNS). However, the signaling mechanisms used by LRRK2 remain largely unknown. Given the homology of the LRRK2 kinase domain with mitogen activated protein kinases (MAPKs), we became interested in possible interactions between LRRK2 and the MAPK signaling pathway. In order to further elucidate these mechanisms, we employed a panel of small molecule kinase inhibitors which target LRRK2 and/or mixed lineage kinase 3 (MLK3), a MAPK known to be involved in neurodegenerative disease, such as Human Immunodeficiency Virus 1 (HIV-1) associated neurocognitive disorders (HAND) and Parkinson's disease (PD). We hypothesize that LRRK2 and MAPK signaling are intertwined and as a result can alter neuroinflammation within the CNS. We can induce inflammation in the BV-2 murine microglia cell line using either lipopolysaccharide (LPS) or the HIV-1 Trans activator of transcription (Tat) protein, both of which induce phosphorylation of LRRK2 at serine 935 (pS935-LRRK2), which is directly linked to LRRK2 kinase activity. Upon co-treatment with our inhibitors we can attenuate pS935-LRRK2 to varying degrees based on the targets of the given inhibitor. Specifically, LRRK2-IN-1, which inhibits LRRK2 but not MLK3, causes a significant decrease in pS935-LRRK2, as does URMC-099, which inhibits both LRRK2 and MLK3. Interestingly, our most recent data show that Califia Compound A, which inhibits MLK3 but not LRRK2, causes an increase in pS935-LRRK2. We have also found that LRRK2-IN-1 decreases phosphorylated p38 and c-Jun N-terminal kinase (JNK), which are downstream from MLK3. These results demonstrate that while

MLK3 inhibition increases LRRK2 kinase activity, LRRK2 inhibition decreases MAPK signaling. Further research will involve determining how these interactions effect neuroinflammation by analyzing microglial activation via cytokine expression and phagocytosis. Thus, we conclude that the interactions between LRRK2 and the MAPK pathway may be of high importance in the treatment of neurodegenerative disease, including both HAND and PD.

Disclosures: **J.M. Puccini:** None. **D.F. Marker:** None. **J. Barbieri:** None. **V.S. Goodfellow:** None. **S. Lu:** None. **H.A. Gelbard:** None.

Poster

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Topic: B.11. Glial Mechanisms

Support: DMRDP

Title: NFκb pathway mediates down regulation of Transforming Growth Factor β receptor 1 expression and inhibition of canonical TGF-β signaling in activated microglia

Authors: K. O. AFFRAM, *K. MITCHELL, J. P. SHAH, A. J. SYMES
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Abstract: Chronic neuroinflammation mediated by persistent microglial activation has been associated with neurodegeneration. TGF-β1 is an anti-inflammatory cytokine that plays a major role in maintaining microglial quiescence. Our previous studies demonstrated that TGF-β1 signaling is suppressed in LPS-activated microglia thereby allowing chronic activation of microglia. We also showed that the TGF-β receptor, TβR1 is significantly downregulated by LPS treatment. We therefore sought to understand the pathways and molecular mechanisms by which TGF-β1 signaling is suppressed by LPS signaling in activated microglia. We showed that the rapid LPS-mediated downregulation of TβR1 mRNA was prevented by inhibition of the NFκb pathway with Bay 11-7082, but not by inhibition of the MAP Kinase pathway using PD 98059. The reduction of TβR1 mRNA, which required de novo transcription within the first 3 h as determined by actinomycin D treatment, was further suppressed via transcriptional-independent mechanisms at later time points. We also observed induction of negative regulators of TGF-β1 signaling in LPS-activated microglia including BAMBI and SnoN. Suprisingly, Smad7, which is commonly induced in other cell types in an NFκb-dependent mechanism to negatively regulate

TGF-β signaling was not upregulated in LPS-activated microglia. Finally, we assessed the effect of other pathologically relevant microglial activators like Amyloid β oligomer (Aβ) on primary microglia. We demonstrated that, in Aβ-activated primary microglia, TβR1 is downregulated, and TGF-β1 is less able to suppress expression of pro-inflammatory cytokines. Aβ treatment also reduced the amount of microglial cell death induced by TGF-β1. In conclusion, we have demonstrated that in microglia LPS suppresses TβR1 expression through an NFκb-dependent pathway and also induces the expression of negative regulators of TGF-β signaling, Bambi and SnoN. Aβ also inhibit TGF-β signaling and downregulate TβR1. Thus, we have identified a common mechanism for microglia activators and their associated pathways to prolong activation of microglia. Identifying ways to restore TGF-β1 signaling in activated microglia may yield novel strategies for reducing chronic neuroinflammation and ameliorating the associated neurodegeneration.

Disclosures: K.O. Affram: None. **K. Mitchell:** None. **J.P. Shah:** None. **A.J. Symes:** None.

Poster

509. Microglia Activation Pathways

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Topic: B.11. Glial Mechanisms

Support: NIH R01NS073848

Title: c-Maf is a p53-dependent anti-inflammatory factor that regulates adult microglia behavior in response to neuroinflammation

Authors: *W. SU[1], B. SOPHER[1], D. J. KANG[2], M. S. ALOI[3], S. HOPKINS[1], G. A. GARDEN[1]
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Abstract: Neuroinflammation is observed in acute and chronic CNS injury, including stroke, traumatic brain injury and neurodegenerative diseases. Microglia are a specialized population of resident myeloid cells that mediate CNS innate immune responses to disease relevant stimuli, such as reactive oxygen species (ROS). Previously we described a novel mechanism by which p53, a ROS responsive transcription factor, modulates microglia behaviors *in vitro* and *in vivo*, promoting pro-inflammatory functions and suppressing down-regulation of the inflammatory response and tissue repair. We demonstrated that p53 negatively regulates the anti-inflammatory transcription factor c-Maf through two micro RNA (miRNA)-mediated pathways in cultured

neonatal microglia and a macrophage cell line. We observed that p53 is required for pro-inflammatory induction of miR-155, a suppressor of c-Maf mRNA and p53 promotes transcription of miR-34a and miR-145, which both suppress expression of Twist-2, a transcriptional activator of c-Maf. We observed that microglia extracted from adult p53 deficient mice expressed increased mRNA for c-Maf, supporting the hypothesis that this regulatory pathway was not unique to cultured neonatal microglia. To determine if c-Maf is a critical target for p53 dependent modulation of microglia function in the adult brain, we successfully developed Adeno-Associated Virus (AAV) serotype 2 as a gene transfer vector to express a c-Maf transcriptional reporter construct and modulate expression of c-Maf. Compared to lentiviral gene transfer, AAV infection yields reduced inflammatory responses and decreased toxicity in both neonatal and adult microglia. Using AAV for gene transfer, we were able to successfully study the effect of modulating c-Maf expression in adult microglia. We observed the effects of c-Maf overexpression and c-Maf shRNA on markers of activation phenotype, phagocytosis of apoptotic cells and cytokine release in both adult and neonatal microglia. Taken together our findings support the hypothesis that in the adult brain, p53 activation by local ROS or accumulated DNA damage influences microglia function, at least in part, through miRNA mediated modulation of c-Maf expression.

Disclosures: W. Su: None. **B. Sopher:** None. **D.J. Kang:** None. **M.S. Aloi:** None. **S. Hopkins:** None. **G.A. Garden:** None.

Poster

509. Microglia Activation Pathways

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 509.19/N3

Topic: B.11. Glial Mechanisms

Title: Microglia expression of activation markers in iron-deficient conditions

Authors: *J. A. ESTRADA, M. A. LEÓN-DÁVILA, I. CONTRERAS
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Abstract: Iron deficiency is the most common nutritional deficiency in the world. It is estimated that, in developed countries, 20-60% of children under 4 years old and up to 50% of pregnant and fertile age women suffer from this pathology. Iron is involved in the immune system as a co-factor in the formation of oxygen radicals and the proliferation and effector functions of leukocytes in general. Microglia represent the resident arm of the immune system in the central

nervous system (CNS). These cells are essential for immune surveillance in the CNS and the activation of innate and adaptive immune responses within this tissue. The effect of iron deficiency on the phenotype and functions of these cells has not been determined. The objective of this study was to determine the expression of the activation markers MHC class I and II, CD80 and CD86, by microglia from iron deficient mice. BALB/c mice were separated in two groups before mating: Control (standard diet, 100ppm iron) and Iron deficient (iron deficient diet, 10ppm iron). Mice born from mothers in each group were further subdivided in four more groups: two Basal groups (21 days old, control and iron-deficient) and two Adult groups (60 days old, control and iron deficient). Each group was maintained with the corresponding diet. Central nervous systems were dissected from mice sacrificed by anesthetic overdose for mononuclear cell purification. Purified cells were stained with fluorochrome-conjugated antibodies for the surface markers CD11b and CD45, to identify the microglial population, as well as for MHC-I, MHC-II, CD80 and CD86. The analysis was performed by flow cytometry. Our results show an overall decrease in the percentage and number of cells expressing activation markers in microglia from iron-deficient mice, compared to controls. However, the mean fluorescence intensity was increased in the same population. Our results suggest that iron deficiency increases the expression of cell surface markers of activation in microglia, although the number of cells expressing these markers is lower. These results may have important implications for the capacity of microglia to induce an adaptive immune response in the CNS of iron deficient individuals; however, the functional phenotype of these cells must be explored further.

Disclosures: J.A. Estrada: None. **M.A. León-Dávila:** None. **I. Contreras:** None.

Poster

510. Microglia Functions

Location: Halls A-C

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Program#/Poster#: 510.01/N4

Topic: B.11. Glial Mechanisms

Title: Global PBR/TSPO-knockout reduces energy efficiency but not steroidogenesis

Authors: *R. BANATI[1,2,3], R. J. MIDDLETON[2], R. CHAN[1,3], C. R. HATTY[1,3], W.-Y. KAM[1,2], C. QUIN[1,3], M. B. GRAEBER[1,3], A. PARMAR[2], S. FOK[1], N. R. HOWELL[2], M. GREGOIRE[2], A. SZABO[2,4], T. PHAM[2], E. DAVIS[2], G.-J. LIU[2,1,3]
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Abstract: The evolutionarily conserved mitochondrial peripheral benzodiazepine receptor (PBR), or 18 kDa translocator protein (TSPO), is widely accepted as indispensable for life. Evidence accrued over 30 years ascribes to the PBR/TSPO an essential role in the translocation of cholesterol across the outer mitochondrial membrane, the vital rate-limiting step of steroid biogenesis. This regulatory function of the PBR/TSPO in steroid biogenesis is the common explanation for its involvement in disease and the mechanism of action of PBR/TSPO-binding drugs. The PBR/TSPO is extensively investigated as a biomarker of "neuroinflammation" due to its disease-dependent expression in activated microglia and a therapeutic drug target in neoplastic, inflammatory, metabolic, neurodegenerative, and behavioural disease. Here we show that global *Tspo* gene knockout mice (C57BL/6-*Tspo*$^{tm1GuWu(GuwiyangWurra)}$) are viable with normal cholesterol transport, blood pregnenolone concentration, fertility, protoporphyrin IX metabolism and under healthy conditions without overt clinical impairment. However, on high fat diet, homozygous *Tspo* knockouts, unlike wild-type mice, showed less than the expected weight gain. At the cellular level, the absence of the PBR/TSPO resulted in a substantially increased proton leak thereby reducing mitochondrial ATP synthesis. The PBR/TSPO appears to contribute to the efficient coupling of mitochondrial respiration and ATP synthesis and thus co-regulate the balance between energy intake and expenditure. Further, we demonstrate the high selectivity of both the isoquinoline PK11195, originally used to pharmacologically describe the PBR/TSPO, and the imidazopyridines CLINDE and PBR111 for the PBR/TSPO *in vitro* and *in vivo*. The global *Tspo* gene knockout animal model provides a crucial tool to evaluate, under naturalistic conditions, the diagnostic and therapeutic selectivity of existing and new chemical compounds with affinity to the PBR/TSPO. Reducing the reliance on conjecture, it enables a broad range of fundamental experiments into the controversially discussed pathways of steroid biogenesis, steroid-dependent systemic effects, including behavior, as well as mitochondrial energy production and the mechanisms of diet-induced obesity.

Disclosures: **R. Banati:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); •Patent or license related to the work being reported is held by the author and/or a university without direct corporate involvement at the time. **R.J. Middleton:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); •Patent or license related to the work being reported is held by the author and/or a university without direct corporate involvement at the time. **R. Chan:** None. **C.R. Hatty:** None. **W. Kam:** None. **C. Quin:** None. **M.B. Graeber:** None. **A. Parmar:** None. **S. Fok:** None. **N.R. Howell:** None. **M. Gregoire:** None. **A. Szabo:** None. **T. Pham:** None. **E. Davis:** None. **G. Liu:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent

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Poster

510. Microglia Functions

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Topic: B.11. Glial Mechanisms

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Title: Microglia: Synaptic scavengers in the healthy and diseased juvenile brain

Authors: *D. P. SCHAFER, C. T. HELLER, C. GORDON, L. LITVINA, C. CHEN, B. STEVENS
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Abstract: Abnormal glial cells and synaptic circuits have emerged as hallmarks of several neurodevelopmental disorders. However, it has remained a mystery whether these two abnormalities may be linked. Recently, we demonstrated in the healthy, developing brain that microglia, a highly specialized innate immune cell, play a key role in activity-dependent synaptic pruning in the early, postnatal (P5) mouse thalamus. Specifically, we showed that microglia engulf a subset of synapses in a manner dependent upon neural activity and the classical complement cascade. Here, we provide new data that, in the absence of any inflammation, microglia-mediated synaptic engulfment can be re-activated much later postnatally (P40). This engulfment occurs precisely at time points and locations where late-stage, fine-scale structural synapse elimination occurs in the healthy, juvenile brain. In addition, we provide evidence that late-stage synaptic engulfment is aberrantly upregulated in a neurodevelopmental disorder with late-onset and widespread synaptic abnormalities, Rett Syndrome (RTT). In mice deficient in the gene underlying RTT, Mecp2-/-, microglia-synapse interactions appear normal until late-stage

synaptic pruning (P40). During this later period, engulfment of synapses is aberrantly upregulated and is coincident with a weakening of synaptic connectivity and symptom onset. Taken together, we provide evidence that microglia-mediated engulfment of synapses in the healthy brain can be reactivated during multiple developmental windows. Furthermore, during a specific developmental window in RTT syndrome, microglia appear to be playing key roles in synaptic circuit abnormalities associated with the disease.

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Poster

510. Microglia Functions

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Topic: B.11. Glial Mechanisms

Support: UTHSC College of Pharmacy

Title: Polarization of microglia through functionally distinct cannabinoid compounds

Authors: *C. PRESLEY, B. M. MOORE, II
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Abstract: Microglia, as the brain's primary immunomodulators, can throw the brain's homeostasis into turmoil following classical activation (M1), a state associated with inflammatory neurodegenerative disorders. However, microglia may move along an activation continuum and polarize toward an anti-inflammatory or alternative activation state (M2) following the influence of anti-inflammatory cytokines such as interleukin 4 (IL-4) or cannabinoid compounds. We have established a new electrochemiluminescent platform to evaluate murine microglial polarization markers such as CD16/32, Fc γ receptor II/III, CD206, the mannose receptor, as well as cannabinoid receptors 1 and 2 (CB1, CB2) after a polarizing stimuli such as lipopolysaccharide (LPS) for an M1 state or IL-4 for an M2 state. Additionally, we have established the effect of cannabinoid compounds such as CP55,940, SR141716A, SR144528, as well as novel cannabinergic compounds on these same markers following polarization to either an M1 or an M2 state.

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Poster

510. Microglia Functions

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Topic: B.11. Glial Mechanisms

Support: All authors are employees of Lundbeck Research USA

Title: Characterization of kynurenine pathway metabolite production in rat primary microglia

Authors: *M. E. HAMBY[1], D. P. BUDAC[2], E. CHARYCH[1], T. MÖLLER[1], B. M. CAMPBELL[1]
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Abstract: Levels of metabolites in the kynurenine (KYN) pathway, the major route of tryptophan catabolism, are altered in several central nervous system (CNS) disorders. Alterations in metabolite levels by neuroinflammation or changes in central substrate (i.e., L-KYN) availability due to peripheral inflammation may be protective in or contribute to disease, depending on the context and disease/disorder under study. Evidence suggests that the pathway is partially segregated by cell type. KYN aminotransferase II (KATII) produces kynurenic acid (KYNA) primarily in astrocytes, whereas KYN 3-monooxygenase (KMO) produces 3-hydroxykynurenine (3-HK) predominantly in microglia. While 3-HK, through 3-hydroxyanthranilic acid (3-HAA), can be converted to the NMDAR agonist quinolinic acid, 3-HK and 3-HAA can also be oxidized to produce free radicals that contribute to oxidative stress. The present study examined KYN metabolite production in primary rat microglia, as measured via LC-MS, under basal, substrate supplemented (L-KYN) or toll-like receptor 4 (TLR4) activated (control standard endotoxin, (CSE)) conditions. Under basal conditions (48 hr), rat primary microglia produced 3-HK and small amounts of anthranilic acid (AA) and KYNA. Initial studies using a high concentration of L-KYN (500 μM; 48 hr) demonstrated a heightened production of 3-HK, KYNA and 3-HAA relative to non-L-KYN-treated microglia. The increases in KYNA, 3-HK and 3-HAA occurred in an L-KYN concentration-dependent manner (10-1000 μM; 48 hr). The production of 3-HK and 3-HAA, but not KYNA, elicited by exposure to 10 μM L-KYN was inhibited in a concentration-dependent manner by the KMO inhibitors UPF 648 and RO 61-8048 (0.01-30 μM; 48 hr), suggesting that 3-HAA is derived from 3-HK and not AA under non-inflammatory conditions in rat microglia. To test whether activation of the canonical pro-inflammatory signaling pathway TLR4 might alter KYN metabolite production in microglia, primary cultures were exposed to CSE. CSE (300 EU/ml; 48 hr) triggered an increase in KYN and 3-HK production; however, AA, KYNA and 3-HAA levels were largely unaffected. Similar

to L-KYN-treated cultures, the CSE-induced increase in 3-HK was attenuated by the KMO inhibitors RO 61-8048 and UPF 648 (10 μM; 48 hr). In sum, these studies demonstrate that the capacity of microglia to metabolize KYN and its metabolites varies based on the experimental conditions or environment. Current studies are underway to evaluate the functional significance of these changes in KYN metabolite levels in relation to oxidative stress in microglia and neurons.

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Poster

510. Microglia Functions

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Topic: B.11. Glial Mechanisms

Title: Opposite effects of BDNF and proBDNF on NO release from activated rodent microglial cells

Authors: *Y. MIZOGUCHI, H. NABETA, Y. IMAMURA, Y. HARAGUCHI, A. MONJI
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Abstract: Microglia are immune cells which release many factors, including proinflammatory cytokines, nitric oxide (NO) and neurotrophins, when they are activated after the disturbance in the brain. There is increasing evidence suggesting that pathophysiology of neuropsychiatric disorders is related to the inflammatory responses mediated by microglia. Brain-derived neurotrophic factor (BDNF) is a neurotrophin well known for its roles in the activation of microglia as well as in the pathophysiology and/or the treatment of neuropsychiatric disorders. We have previously reported that pretreatment of BDNF significantly suppressed the release of NO from activated microglial cells, possibly mediated by sustained store-operated calcium entry (SOCE) induced by BDNF. Although BDNF is initially translated as the precursor proBDNF, which binds p75 neurotrophin receptor (NTR), it remains obscure whether proBDNF affects the release of NO from activated microglial cells. In this study, we observed that pretreatment of proBDNF significantly potentiated the release of NO from activated microglial cells. RT-PCR and immunocytochemical techniques revealed that p75NTR were highly expressed in rodent microglial cells. Opposite effects of BDNF and proBDNF on microglial function might

contribute to the dichotomy of BDNF actions on neuroinflammation and also be involved in the pathophysiology and/or the treatment of neuropsychiatric disorders.

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Poster

510. Microglia Functions

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Topic: B.11. Glial Mechanisms

Title: Role of microglia in the hippocampus of chronic stressed mice

Authors: *A. M. CARNEIRO, B. BASSETT, S. VARNEY, C. CHUNG
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Abstract: Chronic stress elicits biological changes that may have deleterious consequences upon higher brain functioning, thus influencing risk for several psychiatric disorders, including depression and anxiety. The adverse consequences of chronic stress, likely arise from alterations in modulatory neurotransmitter systems such as serotonin and noradrenaline. Cognitive symptoms of depression are associated with decreased neurogenesis and synaptogenesis in the hippocampus, which can be prevented by the concomitant administration of drugs targeting these systems, such as selective serotonin reuptake inhibitors (SSRIs). One of the early events triggered by chronic stress exposure is the activation of the immune system, in the brain represented by microglia. Recent studies indicate that chronic stress increases microglial activation, effects that can be normalized by SSRI administration. Here, we examine how chronic stress influences hippocampal microglial activation. We exposed mice to the chronic unpredictable stress model for three weeks to elicit behavioral responses associated with depression. After three weeks, mice were tested behaviorally in the forced swim and novelty-suppressed feeding tests, and tissue collected for neuroanatomical staining and quantification of tissue levels of monoamines by HPLC. Three weeks of chronic stress resulted in significant decreases in monoamine levels in cortices, higher immobility time in the forced swim test, and a larger latency to feed in the novelty-suppressed feeding test. Immunofluorescence analysis of hippocampal slices revealed that stress modifies specific microglial populations in the hippocampus. While stress elicited no changes in the number of microglia, it induced significant increases in microglial activation in the hippocampus. To identify hippocampal regions sensitive

to microglial response, we performed neuroanatomical analysis of microglia in the dentate gyrus, CA1 and CA3 regions of the hippocampus. Here we identified differential responses to chronic stress in dorsal versus ventral hippocampus, with increased microglial activation in the dentate gyrus. We further explored the role of microglia in the hippocampus by testing whether activated microglia contained neuronal fragments. We observed a significantly higher percentage of microglia containing neuronal fragments in the ventral hippocampi of stressed mice, compared to control animals. These studies provide evidence of a region-specific increase of neuronal phagocytosis by activated microglia in the hippocampus.

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Poster

510. Microglia Functions

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Title: Hv1 proton channels in spinal microglia contribute to astrocyte activation and neuropathic pain in mice

Authors: *J. PENG[1], H.-J. JEONG[4], P. SWIATKOWSKI[1], U. B. EYO[1], N. GU[1], S. GANATRA[2], Y. HUANG[3], C. F. DREYFUS[3], Y. REN[2], S. OH[4], L.-J. WU[1]
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Abstract: Activation of spinal cord microglia contributes to peripheral nerve damage-induced neuropathic pain development. However, molecular mechanisms underlying microglia in neuropathic pain are not fully understood. Using a single cell gene array screen technique, we identified that the expression of the HVCN1 gene encoding the voltage-gated proton channel Hv1 was significantly increased in spinal cord microglia after spinal nerve transection (SNT). Hv1 channels mediated functional voltage-gated proton currents in spinal microglial and mice

lacking Hv1 (Hv1 KO) displayed attenuated pain hypersensitivities after SNT, especially in the later maintenance phase compared with wildtype (WT) mice. Consistently, microglial production of reactive oxygen species (ROS) and subsequent astrocyte activation in the spinal cord was reduced in Hv1 KO mice after SNT. Cytokine screening and immunostaining further revealed that IFN-γ expression was comprised in spinal astrocytes of Hv1 KO compared with WT mice. Intrathecal neutralization of IFN-γ reversed mechanical allodynia in WT but not Hv1 KO mice, while exogenous IFN-γ transiently rescued SNT-induced allodynia in Hv1 KO mice. These results demonstrate that the Hv1 proton channel contributes to microglial ROS production, astrocyte activation, IFN-γ upregulation, and subsequent pain hypersensitivities after SNT. Therefore, this study provides a rational for microglial Hv1 proton channel as a novel therapeutic target for alleviating neuropathic pain.

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Poster

510. Microglia Functions

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Program#/Poster#: 510.08/N11

Topic: B.11. Glial Mechanisms

Support: MSIP No. 2012R1A3A2048834

Title: Single-cell analysis reveals neuropathic pain-specific gene-expression signature in spinal microglia following peripheral nerve injury

Authors: *H. JEONG[1], Y. KIM[1], Y.-J. NA[2], G. CHUNG[1], M. KANG[3], J. KIM[2], S. OH[1]
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Abstract: Microglia become activated in response to peripheral nerve injury, and microglia activation, accompanied by global changes in gene expression, contributes to develop neuropathic pain. Here we employed microarray assay of individually collected pools of 10 cells to identify changes in microglia-specific gene expression that may be associated with microglial activation after peripheral nerve injury. With microglia located on superficial dorsal horn from

spinal cord slices of CX3CR1$^{+/GFP}$ mice after L4 spinal nerve transection, post-operative day (POD)1 and POD7 microglia were compared to sham-operated microglia, respectively. We found that microglia were highly heterogeneous at single-cell level, and while the variability of gene profile generally tended to increase over time after nerve injury, there existed 64 microglial genes showing lower variation, potentially linked to neuropathic pain. Our results also indicate that early microglia and late microglia exhibit significant differences in gene expression profile and recruit distinctive signaling pathways following peripheral nerve injury, possibly leading to phenotype changes during development and maintenance of neuropathic pain.

Disclosures: H. Jeong: None. **Y. Kim:** None. **Y. Na:** None. **M. Kang:** None. **J. Kim:** None. **G. Chung:** None. **S. Oh:** None.

Poster

510. Microglia Functions

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Topic: B.11. Glial Mechanisms

Support: NINR Division of Intramural Research, NR000020-03

Title: Radiation-induced fatigue linked with microglial activation not with skeletal muscle alterations

Authors: G. HOLDER[1], Z. YU[2], L. N. SALIGAN[1], *K. FUKUHARA[1]
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Abstract: Background: Fatigue related to cancer and its therapy is a persistent sense of tiredness that negatively affects the overall quality of life of patients. We developed a mouse model to mechanistically investigate the relationship of radiation-induced fatigue with skeletal muscle alternations and microglial activation. **Methods:** 24 male Hsd:ICR (CD1), 10-week old mice were assigned to 3 different groups: non-irradiated control group (N=12), where 6/12 mice were sacrificed on day 7 for tissue collection, one day before start of irradiation; and two groups of irradiated mice sacrificed on day 13 (D13, N=6) and day 25 (D25, N=6). The irradiated mice received a total of 18 Gy to their lower abdomen administered at 6 Gy/day on days 8, 9, and 10. Voluntary wheel running was measured from days 7-25. Histological integrity of skeletal muscles were examined using hematoxylin and eosin stainings, and Iba-1 staining of CA1 and CA3 hippocampal regions were used to detect microglial activation. **Results:** Irradiated mice

(D13 and D25) had lower wheel running activity than non-irradiated (D7) mice, (D13, F=25.5, $p<0.001$; D25, F=13.4, $p<0.001$). There was no evidence of inflammation, damage or histological alterations in all skeletal muscle samples from each group. D13 mice reached nadir in wheel running activity 3 days after incremental irradiation and had reduced lba-1 expression (mean count per high power field (HPF)=9) compared with D7 (mean/hpf)=20) and D25 mice (mean/hpf=19). **Conclusion:** Histological skeletal muscle alterations are not observed in irradiation-related fatigue. However, acute but not chronic irradiation-related fatigue is influence microglial activation.

Disclosures: G. Holder: None. **Z. Yu:** None. **L.N. Saligan:** None. **K. Fukuhara:** None.

Poster

510. Microglia Functions

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Topic: B.11. Glial Mechanisms

Support: CIHR Grant MOP259183

Title: Increases in microglial Iba1 immunoreactivity in the frontal cortex and hippocampus in response to chronic sleep restriction in rats

Authors: *S. HALL[1], S. DEURVEILHER[1], J. BURNS[1], K. SEMBA[1,2,3]
[1]Med. Neurosci., [2]Psychology & Neurosci., [3]Psychiatry, Dalhousie Univ., Halifax, NS, Canada

Abstract: Chronic sleep restriction (CSR) has negative consequences on cognitive performance, as well as cardiovascular, metabolic, and immune functions. Although the neurobiological mechanisms underlying these impairments are unclear, emerging evidence suggests a role of neuroinflammation, which is mediated by microglia, the resident immune cells in the brain. In this study, we examined changes in immunohistochemically-identified microglia in the rat brain during a '3/1' protocol of CSR, which features continuous cycles of 3 h of sleep deprivation using slowly rotating wheels and 1 h of sleep opportunity, for 4 days. We previously showed that this protocol induced both homeostatic and adaptive changes in sleep patterns, sustained attention performance, and the levels of brain-derived neurotrophic factor and FosB family transcription factor proteins. Adult male Wistar rats were randomly assigned to 4 groups (n = 2-5/group): two CSR groups were housed in motorized wheels and underwent the 3/1 protocol for 27 or 99 h, and two locked wheel control groups were kept in stationary wheels for corresponding intervals.

Following perfusion at the end of the experimental protocols, microglia in the brain were visualized using an antibody against ionized calcium-binding adaptor molecule 1 (Iba1) and a standard ABC/DAB-Ni method. Iba1-positive cell bodies were counted and the density of Iba1 immunoreactivity (percentage of immunoreactive area in a total area of analysis) was measured in selected brain regions known to be responsive to sleep loss, including the prelimbic cortex, hippocampus, and perifornical lateral hypothalamic area, using ImageJ software. The number of Iba1-immunoreactive cells and the density of Iba1 immunoreactivity in the prelimbic cortex and hippocampus tended to increase above control levels after 27 h, with a slight decline after 99 h, of CSR. The number of Iba1-immunoreactive cells and the density of immunoreactivity were positively correlated in each of the two regions (r=0.80 and 0.83). No changes were apparent in the perifornical lateral hypothalamic area. Analyses of the morphology of immunoreactive microglia are in progress. These preliminary results indicate an increase in the number of microglia in the cortex and hippocampus in response to CSR, suggesting that CSR initiates neuroinflammatory processes. These changes could contribute to cognitive and other impairments associated with CSR.

Disclosures: **S. Hall:** None. **S. Deurveilher:** None. **J. Burns:** None. **K. Semba:** None.

Poster

510. Microglia Functions

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Program#/Poster#: 510.11/O2

Topic: B.11. Glial Mechanisms

Support: NIH/NINDS R01NS082308 (Loane)

Title: NOX2 inhibition modulates microglial polarization, improves functional recovery and reduces chronic neurodegeneration following experimental TBI

Authors: *A. KUMAR, B. A. STOICA, F. TCHANTCHOU, A. I. FADEN
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Abstract: Signals from the microenvironment polarize microglia toward either a classical (M1) or alternative (M2) activation state, which mediates post-traumatic neuroinflammation or promotes tissue repair, respectively. Activation of NADPH oxidase (NOX2) is an important mechanism that initiates pro-inflammatory signaling in microglia. We have recently demonstrated that NOX2 is chronically expressed in M1-polarized microglia that surround the

lesion at 1 year following experimental traumatic brain injury (TBI) in mice. In the present study we used wild-type (gp91phox+/+) and NOX2-deficient (gp91phox-/-) mice to investigate the role of NOX2 in microglial polarization following TBI. Three-month old gp91phox+/+ and gp91phox-/- male mice were subjected to controlled cortical impact (6m/sec, 2mm depth) and cohorts of mice were followed for 1d, 3d, 7d and 21d post-injury. Changes in M1/M2 polarization were analyzed by qPCR, flow cytometry and immunohistochemical analyses. Long-term motor function recovery was assessed through 21d post-injury using a beam walk test, and lesion volume and cortical neurodegeneration was assessed by stereological methods. Flow cytometry analysis found no differences in the total numbers of activated microglia (CD11b+ cells) or infiltrating macrophages (CD11b+/CD45high) in gp91phox+/+ and gp91phox-/- mice following TBI. In contrast, genetic ablation of NOX2 modulated M1/M2 polarization: at 1d post-injury M1 gene expression (eg. TNFα & iNOS) was significantly reduced whereas M2 gene expression (eg. Ym1, Arg1, Fizz1, IL-4Rα) was significantly increased in the cortex of gp91phox-/- TBI mice compared to gp91phox+/+ TBI mice. Similar changes in protein expression of M1 (iNOS) and M2 (Ym1, Arg1, TGFβ) markers were confirmed by flow cytometry at 3d post-injury in MACS purified CD11b+ cells from the TBI cortex. Further, immunohistochemical analysis demonstrated that there was reduced expression of M1 microglia (CD16/32+ Iba1+) at 7d post-injury and increased expression of M2 microglia (Ym1+) at 21d post-injury in the cortex of gp91phox-/- TBI mice compared to gp91phox+/+ TBI mice. The dominant M2 polarization in gp91phox-/- TBI mice was accompanied by improved motor function recovery and significantly reduced lesion volume and cortical neurodegeneration at 21d post-injury. Our data indicate that NOX2 drives the M1 polarization of microglia following experimental TBI. The repolarization of microglia towards an M2 activation state in gp91phox-/- TBI mice may be in part responsible for reduced neurodegeneration and improved functional recovery.

Disclosures: **A. Kumar:** None. **B.A. Stoica:** None. **F. Tchantchou:** None. **A.I. Faden:** None.

Poster

510. Microglia Functions

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Topic: B.11. Glial Mechanisms

Support: NIH R21AA018823 (MED)

NIH 5 P30 DC010362 (SHG)

Title: Microglial responses to cortical cell death during a two-day alcohol exposure in neonatal mice

Authors: *K. AHLERS, F. OOI, K. MAH, J. KERSIGO, B. FRITZSCH, S. H. GREEN, M. E. DAILEY
Biol., Univ. of Iowa, Iowa City, IA

Abstract: Brief episodes of high alcohol exposure during critical periods of brain development can induce widespread neuroapoptosis and microglial activation in the neocortex and other brain regions. We previously reported (Ahlers et al., Soc. Neurosci. Abstr. 2013) that a single day of alcohol exposure (5 g/kg alcohol administered via 2 intraperitoneal injections spaced 2 h apart) in postnatal day (P) 7 or P8 mice leads to robust but transient microglial activation and changes in the expression of proinflammatory factors. Work in BAX-null mice demonstrated that microglial activation in this model was strongly linked to BAX-dependent neuroapoptosis, not the alcohol. Remarkably, most dead cells were cleared and microglia began to de-activate within 2 d of the initial insult. Given that alcohol exposure on either P7 or P8 induces comparable levels of neuroapoptosis and microglial activation, we hypothesized that alcohol exposure on two sequential days (P7 and P8) would exacerbate neuroapoptosis and extend the period of microglial activation. Instead, we found that the period of neuroapoptosis and microglial activation was similar after a 1 d alcohol exposure on P7 or after a 2 d exposure on both P7 and P8. This was also observed at lower alcohol levels (3 g/kg) that show reduced levels of neuroapoptosis. Potentially the low cell death produced by the second injection in a two-day alcohol exposure model may be due to neuroprotective mechanisms elicited by the first injection. In support of this idea, a preliminary microarray analysis of cortical gene expression 12 and 24 h after exposure to 5 g/kg alcohol showed a decrease in expression of several pro-apoptotic factors and concomitant increase in expression of pro-survival factors, including neurotrophins. Of particular interest, the microarray showed a 15 fold increase in BDNF expression at 24 h (confirmed by qPCR) and *in situ* hybridization showed strong BDNF expression in cortical regions showing high levels of cell death. Future studies will be needed to extend the analysis of microglial activation states in this two-day injection model and to further explore the possibility that increased levels of BDNF following an initial alcohol exposure enact neuroprotective mechanisms against a second insult.

Disclosures: K. Ahlers: None. **F. Ooi:** None. **K. Mah:** None. **J. Kersigo:** None. **B. Fritzsch:** None. **S.H. Green:** None. **M.E. Dailey:** None.

Poster

510. Microglia Functions

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Topic: B.11. Glial Mechanisms

Support: NNSF Grant 81100833

Shenzhen KQ Grant 2013237

Title: Microglia inhibition is a potential mechanism underlying neuropathic pain prevention by Botulinum Toxin A

Authors: *W. XIE[1,2], X. FENG[2], Z. ZHOU[2], Y. QIU[2], L. XIAO[3]
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Abstract: Botulinum toxin A (BoTN/A) has shown long-term symptomatic anti-nociceptive efficacy against neuropathic pain in clinical applications, such as postherpetic neuralgia and diabetic neuropathic pain. The BoTN/A analgesic mechanism currently focuses on release inhibitory of neurotransmitters in peripheral and/or central terminals of somatosensory neuronal system. To identify if neuroglia related mechanism involved in BoTN/A induced analgesia, primary cultured rat microglia were incubated with BoTN/A. The BoTN/A pretreated microglia displayed slight morphological changes after LPS activation compared with untreated control group. Moreover, the real-time RCR results indicated LPS induced up-regulation tendency of inflammatory cytokines mRNA expression level, significantly decreased in BoTN/A pretreated microglia. Microglia activation in pathological condition, is believed has pivotal role in chronic neuropathic pain origin and maintenance. Then, we confirmed BoTN/A pre-intrathecal injection were able to inhibit initiation of neuropathic pain like behaviors including mechanical allodynia and thermal hyperalgesia in rat partial sciatic nerve ligation model. The immunofluorescence and western blot results indicated peripheral injury causing spinal microglia activation was partially blocked by BoTN/A pre-intrathecal injection. These *in vitro* and *in vivo* experimental results suggest besides neuronal release inhibition, suppressing microglia activation may underlie the analgesic efficacy of BoTN/A, which shows a promising preventive possibility on neuropathic pain. Further investigation related this latent capacity of BoTN/A is needed to be completed.

Disclosures: W. Xie: None. **X. Feng:** None. **Z. Zhou:** None. **Y. Qiu:** None. **L. Xiao:** None.

Poster

510. Microglia Functions

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Topic: B.11. Glial Mechanisms

Support: AHFMR

Title: Modeling of the glial scar through 3D hydrogel cultures

Authors: A. F. JEFFERY[1], M. A. CHURCHWARD[2], A. L. ELIAS[1], *K. G. TODD[3]
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Abstract: Neural interfacing often requires electrode implantation into the central nervous system (CNS). These electrodes must be biocompatible (elicit a minimal foreign body response) to optimize long-term safety and functionality. Glial scarring, initiated by the native immune cells of the CNS (microglia and astrocytes) is believed to be a major contributing factor to the loss in electrode functionality. Due to variability, expense and time required for *in vivo* testing, a parallel *in vitro* model is desired. This model must not only support microglia and astrocytes, but also mimic the mechanical properties of the CNS. Our study investigates the use of a three-dimensional culture technique to analyze glial scarring in response to a microwire. The three-dimensional culture was based on methacrylated hyaluronic acid, a hydrogel capable of photocrosslinking. Gels were formed at four macromer concentrations (0.5, 0.75, 1.0, and 1.5 % (w/v)). Stiffness of the hydrogels was shown to increase with increasing macromer concentrations via compressive strain analysis. A macromer weight fraction of 1.0 % (w/v) had a modulus similar to adult rat brain tested under identical conditions. Hydrogel structure was analyzed using Cryo Scanning Electron Microscopy. Although no significant difference in pore size was observed, scaffold walls appeared thicker with increasing macromer weight fraction. Microglia and astrocytes isolated from whole brain were then encapsulated in the HA scaffolds to assess cell viability, density and morphology. Of the cell seeding densities tested, 1x10^7 cells/mL was the most consistent and stable and was therefore used for all further studies. Cultures were viable at all macromer weight fractions tested though the 1.5 % (w/v) sample was significantly lower than other gels tested (0.5, 0.75 and 1.0 % (w/v)). The temporal response of mixed glial cultures to a microelectrode was followed over a period of 2 weeks. Microglia responded first to the electrode, exhibiting an activated morphology at 3 days, while a layer of astrocytes was observed around the electrode at day 6. This cellular response is similar to results reported for *in vivo* trials and appeared to model acute glial scarring. A 3D HA hydrogel culture model will allow *in vitro* testing of the physical and chemical properties of neural interfacing electrodes in *in vivo*-like conditions. This model will enable robust and repeatable comparisons of glial scarring to electrodes as well as other foreign bodies in the CNS.

Disclosures: **A.F. Jeffery:** None. **M.A. Churchward:** None. **A.L. Elias:** None. **K.G. Todd:** None.

Poster

510. Microglia Functions

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Health and Labor Science Research Grant for Research on New Drug Development from MHLW, Japan

Title: Microglia accelerate the maturation of barrier function of blood brain barrier

Authors: ***K. SATO**, Y. SHIGEMOTO-MOGAMI, K. HOSHIKAWA, Y. SEKINO
Div. Pharmacol, Natl. Inst. Hlth. Sci., Tokyo, Japan

Abstract: The blood-brain barrier (BBB) restricts the transport of substances between vasculature and brain. It is not known whether microglia have roles in the BBB development. In this study, we investigated the role of microglia in the maturation of barrier function of BBB using in *in vitro* BBB model. Co-existence of non-stimulated microglia with astrocytes in the brain side during the maturation period of *in vitro* BBB model significantly increased the trans-endothelial electrical resistance (TEER) and the expression levels of tight junction proteins. On the contrary, co-existence of LPS-stimulated microglia significantly decreased the TEER and the expression levels of tight junction proteins. These results suggest that microglia accelerate the

maturation of barrier function of BBB but their effects are reversed in the pathological conditions. We also discovered that BBB acquired the capability of ejecting extracellular L-Glu in the brain. Further, addition of non-stimulated microglia significantly increased the capability of ejecting L-Glu via increasing the expression levels of GLAST and GLT1 in endothelial cells. However, addition of LPS-stimulated microglia reversed the effects. These results strongly suggest that microglia is important for the maturation of the barrier function of BBB and reveal negative effects in the pathological conditions. In this study, we have developed the novel *in vitro* BBB model including microglia. The investigation of the precise role of microglia in the maturation of BBB barrier function is currently being undertaken.

Disclosures: K. Sato: None. **Y. Shigemoto-Mogami:** None. **K. Hoshikawa:** None. **Y. Sekino:** None.

Poster

510. Microglia Functions

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Topic: B.11. Glial Mechanisms

Support: CIHR grants to ESR

Title: Inflammation increases axonal structural remodeling in a developing neural circuit

Authors: *N. FAROOQI[1], E. S. RUTHAZER[2]
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Abstract: Multiple lines of evidence implicate immune genes and immune/inflammatory activation in the development of neuropsychiatric disease. We investigated the effects of inflammation on a developing neural circuit *in vivo* by exposing larval zebrafish to bacterial lipopolysaccharide (LPS) to induce an innate inflammatory response. We demonstrate that exposure to LPS upregulates transcription of pro-inflammatory cytokines and induces morphological activation of microglia, confirming an active immune response. We subsequently expressed EGFP under a retinal ganglion cell (RGC) promoter to sparsely label axons arborising in the tectum and performed *in vivo* two-photon microscopy at high spatio-temporal resolution. Morphometric analysis of RGC axon arborization before and after exposure to LPS or a control solution demonstrates that an inflammatory insult increases structural remodelling as measured by rates of RGC axonal branch addition and retraction. Axons in larvae exposed to LPS also

demonstrate greater increases in overall axon arbor length than controls. These findings may reflect a failure to stabilize synaptic contacts and may underlie aberrant circuit formation. Microglia are key regulators of inflammatory responses in the CNS and have been implicated as having roles in normal neural development. The SpI1/Pu.1 transcription factor is necessary for normal differentiation of the myeloid lineage, including microglia and macrophages. One-cell stage injection of a morpholino oligonucleotide against SpI1/Pu.1 produces morphant animals lacking microglia and macrophages. We confirmed this effect and demonstrated that the increased remodelling of axonal arbors in response to LPS does not occur in SpI1/Pu.1 morphants. Myeloid-cell mediated responses are therefore necessary for the effect of an inflammatory stimulus on neuronal development. Funding sources: CIHR Vanier (NF), CIHR (ESR)

Disclosures: **N. Farooqi:** None. **E.S. Ruthazer:** None.

Poster

510. Microglia Functions

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Topic: B.11. Glial Mechanisms

Support: Heart and Stroke Foundation

FRQS

CIHR

Title: Microglia rapidly change their morphological and dynamic properties during metabolic stress

Authors: ***L.-P. BERNIER**, L. DISSING-OLESEN, B. MACVICAR
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Abstract: Microglia are highly motile cells that play a pivotal role in monitoring brain homeostasis by constantly probing the environment and responding to extracellular cues. They are involved in stroke-related pathologies, mediating a nonspecific neuroinflammatory reaction that could lead to long-term deleterious effects following transient ischemia. However, the acute functional response of microglia during ischemic periods remains unclear. Here, we used real-time two-photon imaging in acute brain slices to monitor the initial effect of anoxic and

aglycemic insults on the morphological phenotype and dynamic properties of microglia. Microglia in resting conditions display a highly branched morphology with motile processes, however oxygen depletion induces a rapid, actin-dependent retraction of processes that is fully reversible upon reoxygenation. This finding indicates that microglia sense decreased oxygen levels in brain tissue within minutes. The rapid loss of major processes, normally needed by microglia to probe the environment, translates into a significant change in microglia function. Under normal conditions, microglia quickly extend their major processes towards focal injury, however this mechanism is inhibited during anoxia. We show that this phenotype is mimicked by multiple signaling molecules hypothesized to be released during brain anoxia, indicating a common intracellular pathway is activated to induce microglial process retraction. Characterizing the molecular cues responsible for this functional switch of microglial behaviour will likely provide valid targets for stroke treatment.

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Poster

510. Microglia Functions

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Support: OTKA (NK 81983)

Hungarian Academy of Sciences MTA TKI 02001

Hungarian Brain Research Program - Grant No. KTIA_13_NAP-A-III/6

Title: The metabolic profile of primary microglia and BV-2 microglial cell line

Authors: *L. TRETTER[1,2], A. M. NAGY[1,2], R. FEKETE[3], Z. KORNYEI[3], V. ADAM-VIZI[1,2]
[1]Med. Biochem., Semmelweis Univ., Budapest, Hungary; [2]MTA-SE Lab. for Neurobiochemistry, Budapest, Hungary; [3]Inst. of Exptl. Med. of the Hungarian Acad. of Sci., Budapest, Hungary

Abstract: Microglial cells play a key role in the patomechanism of neurodegenerative disorders. They can enter into metabolically different compartments in the CNS. We investigated, which compounds can serve as metabolic fuels for these cells. Cells were incubated in Artificial Cerebrospinal Fluid (ACSF) supplemented with those substrates, which are available for the

cells in the CSF: glutamine, glucose, lactate, pyruvate or ketone bodies. The oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) was measured on primary microglial cells and on the BV-2 microglial cell-line with Seahorse Extracellular Flux Analyzer (Seahorse). ECAR was considered as a parameter of glycolytic activity. Cell viability was determined using MTT, Annexin/Calcein and Tunel essays. All of the substrates applied supported the metabolism of the cells and none of them influenced their viability negatively. In the presence of glutamine the basal rate of respiration was increased. However in the presence of glucose the OCR was decreased, the ECAR raised and the addition of a lactate dehydrogenase inhibitor after glucose was able to reverse this effect. Adding a mitochondrial fatty acid transporter inhibitor further increased the ECAR in the presence of glucose. We conclude that microglial cells show high metabolic plasticity, using wide range of substrates. From the ECAR results we claim, that these cells show high glycolytic capacity. Furthermore it was found that besides glucose glutamine is the most preferred substrate for microglial cells.

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Poster

510. Microglia Functions

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Topic: B.11. Glial Mechanisms

Support: Alzheimer's Association NIRG-12-242598

ONO Pharmaceuticals

Title: Inhibition of microglial tyrosine kinases, Hck and Syk, impairs phagocytic activation and exacerbates Alzheimer’s disease-like neuropathology

Authors: *S.-L. LIM, C. J. RODRIGUEZ-ORTIZ, M. KITAZAWA
Univ. of California, Merced, Merced, CA

Abstract: Growing evidence has shown that microglia - the resident macrophage in the brain - plays a key role in regulating neuroinflammation and removing amyloid-beta (Aβ) species by phagocytosis. These beneficial activities of microglia are thought to protect one from developing Alzheimer’s disease (AD). However, functional inactivation and improper senescence of

microglia significantly impair its ability to clear Aβ, leading to a pathological buildup of Aβ and the onset of AD. Hematopoietic cell kinase (Hck) is a member of the Src family tyrosine kinases which mediate immunoreceptor-induced phagocytic activation in microglia upon Aβ stimulation, while spleen tyrosine kinase (Syk) is a non-receptor tyrosine kinase acting downstream of the Src family tyrosine kinases. We hypothesize that inactivation of Hck and Syk impairs microglial phagocytosis and Aβ clearance, leading to the development of AD neuropathology and cognitive decline. Murine microglial/macrophage cells (BV-2) were stimulated by naturally secreted oligomers (AβO) from 7PA2 cells, opsonized zymosan (mOZ) and lipopolysaccharide (LPS) in the presence or absence of Src-pan inhibitor (PP2) or Syk-specific inhibitor (BAY 61-3606). AβO markedly activated phagocytosis in BV-2 cells, and its activation was significantly inhibited by the Src and Syk inhibitors. This signified that the activation of BV-2 cells phagocytic activity by AβO stimulation was mediated by the Src family tyrosine kinases and Syk pathways, and inactivation of either kinase significantly attenuated the phagocytic capacity of BV-2 cells. We then examined whether a deficiency of Hck impacted cognition and Aβ pathology in Tg2576 mice. Behavioral studies on the Tg2576/Hck-knockout (KO) mice showed exacerbated cognitive decline in hippocampal-dependent spatial memory tests as compared with age-matched Tg2576, Hck-KO, and wild-type (WT) mice. The *in vivo* studies provided evidence that the inactivation of Hck and subsequent functional impairment of microglia may precede the buildup of Aβ and downstream AD neuropathology. Our data demonstrate that Hck and Syk are neuroprotective and important for AβO-stimulated microglial phagocytosis and clearance. These findings are imperative in delineating the contribution of microglial dysfunctionality in AD progression.

Disclosures: S. Lim: None. **C.J. Rodriguez-Ortiz:** None. **M. Kitazawa:** None.

Poster

510. Microglia Functions

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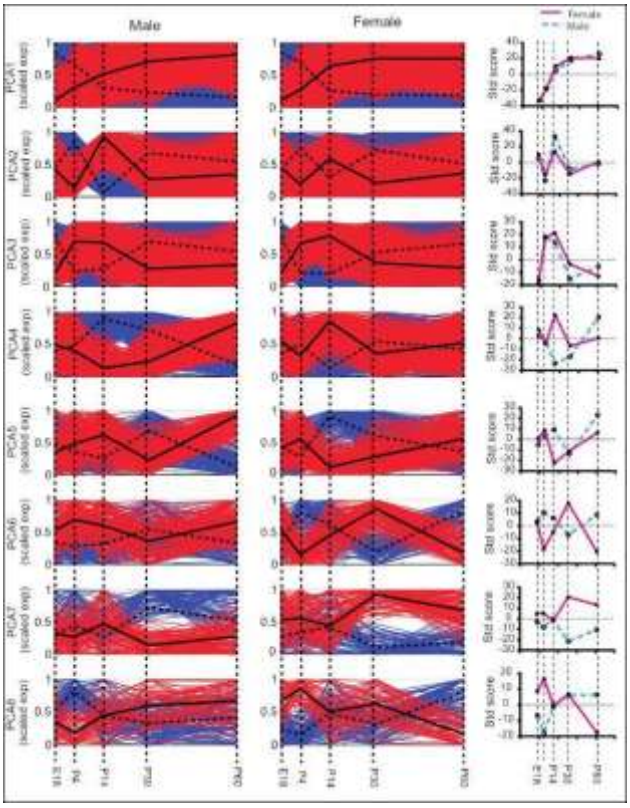
Topic: B.11. Glial Mechanisms

Support: MH101183

Title: Sex differences in developmental gene expression patterns in hippocampal microglia of mice: Relevance for neurodevelopmental disorders

Authors: *R. HANAMSAGAR[1], J. BOLTON[1], M. ALTER[2], S. BILBO[1]
[1]Duke Univ. Med. Ctr., Durham, NC; [2]Univ. of Pennsylvania, Philadelphia, PA

Abstract: Several neuropsychiatric disorders have been associated with immune abnormalities that likely originate during development. Many such disorders exhibit a marked sex difference in their prevalence and age of onset. Males are more likely to have disorders that present early in childhood, including autism and learning disabilities. Females are more often diagnosed with disorders that arise around or after the onset of puberty, including anxiety and depression. We have shown that microglial activation induced by early-life infection can adversely impact cognition in adult males, but not in females corelating to increased number of amoeboid microglia observed in male hippocampus (HP) during early post-natal developmental stages. However, the functional differences between male and female microglia at a basal level have not been studied. We performed whole transcriptome profiling using microarray and measured gene expression levels in microglia purified from HP of male and female at embryonic day 18 (E18), postnatal day 4 (P4), P14, P30 and P60. Data was examined with principal components analysis (PCA). The first 8 principal components accounted for 94% of the variance in gene expression levels (Fig). With respect to males and females, the first 3 principal component scores accounted for 53% of the variance and behaved similarly in males and females. The 4th through 8th principal component scores accounted for 41% of the variance and behaved differently in males and females. We hypothesize that sex-related functional differences are related to sex-based differential gene expression that follow PCA4-8. On-going studies on a bigger cohort of male and female mice will also involve treating mice at P60 with either lipopolysaccharide or saline (i.p.) to assess how a systemic inflammatory response can induce differential changes in microglial gene expression in males and females. These studies will help us understand how male and female microglia are intrinsically different, and will help explain sex-based differences in behavioral and cognitive outcomes.



Disclosures: **R. Hanamsagar:** None. **J. Bolton:** None. **M. Alter:** None. **S. Bilbo:** None.

Poster

510. Microglia Functions

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 510.21/O12

Topic: B.11. Glial Mechanisms

Support: unrestricted grant from Merck-Serono

Title: ATP stimulation induces plasma membrane budding and shedding of the human microglia cell line CHME-5: A good model for studying microvesicles biology

Authors: ***F. COLOMBO**, A. FINARDI, G. RACCHETTI, J. MELDOLESI*, R. FURLAN
Inst. Scientifico San Raffaele, Milano, Italy

Abstract: Cell-to-cell communication is probably one of the most studied and promising field of the current cell biology since the recent demonstration that molecular information (protein and nucleic acids) can be stored and transferred among cells by lipid particles of different size known as microvesicles. Their first descriptions date back decades ago nevertheless they were considered artifacts until recently thanks to some observations reporting the specificity of their genesis and their crucial roles in physiological and pathological conditions, which rely on the messages they carry. Exosomes and shedding microvesicles (SVs) are the two main types of microvesicles isolated up to now thanks to their diffent size (10-100 nm the former, 150-1500 nm the latter) and density. Exosomes are particles of endosomal origin released upon the fusion of multivesicular bodies with the plasma membrane whereas SVs derive by the budding of the plasma membrane, a process also known as ectocytosis. The biology of SVs is still very mysterious starting from the molecular actors governing their formation and release to the processes involved in the sorting of the content inside the nascent vesicle. Recently our group has observed a strong enrichment of myeloid-derived SVs in the cerebrospinal fluid of multiple schlerosis patients, with a strong correlation between the microvesicles number and the disease severity; this opened the possibility to use SVs as a reliable marker for monitoring neuroinflammatory disorders. In order to study some aspects of the biology of SVs in the contest of human brain pathology we chose as model a microglia cell line derived from human embryonal brain, namely CHME-5. Here I report the panel of methods I used for describing in detail the ability of CHME-5 cells to release SVs upon ATP or cytokine treatment, conditions that properly mimick the inflammed tissues. The methods presented here include flow cytometry (FCS) and western blotting for microvesicles quantitation, transmission and scanning electron microscopy (SEM, TEM) along with dynamic light scattering (DLS) for the measurements of the particles diameter. Therefore the crucial advantage of using this cell line would be the possibility to isolate enough amounts of SVs to perform OMICS analysis in order to reveal their content and perhaps their functions.

Disclosures: **F. Colombo:** None. **A. Finardi:** None. **G. Racchetti:** None. **J. Meldolesi*:** None. **R. Furlan:** None.

Poster

511. Glial Physiology and Glia-Neuronal Physiology

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 511.01/P1

Topic: B.11. Glial Mechanisms

Support: Telethon Italy GGP12265

Telethon Italy GGP10138

Cariparo Foundation

CNR Aging Project

FIRB RBAP11X42L

Title: GABAergic signaling evokes calcium oscillations and glutamate release in cortical astrocytes

Authors: L. MARIOTTI[1], *G. LOSI[1], M. SESSOLO[1], I. MARCON[1], S. BOVETTI[2], T. FELLIN[2], G. CARMIGNOTO[1]
[1]Neurosci. Institute-Cnr and Univ. of Padova, Padova, Italy; [2]Dept. of Neurosci. and Brain Technologies, Inst. Italiano di Tecnologia, Genova, Italy

Abstract: During the last decade, there has been an increasing interest in the reciprocal signalling between principal excitatory neurons and astrocytes. Conversely, the signalling between inhibitory interneurons and astrocytes remains largely unexplored. The main goal of our study was to identify and characterize the astrocytic response to GABAergic signals. We used slice preparations from the somatosensory cortex of P14-P22 mice loaded with the Ca2+ indicator Fluo-4 AM and the astrocytic marker SR101-AM. We found that i) about 60% of layer V astrocytes showed large amplitude somatic Ca2+ increases in response to GABA or baclofen (Bac), a GABAB receptor agonist; ii) blocking Gi/o proteins by pertussis toxin prevented Bac-mediated Ca2+ transients in astrocytes; iii) both GABA and Bac failed to induce Ca2+ events in mice lacking the inositol-1,4,5-trisphosphate (IP3) receptor type 2 in astrocytes. These results reveal an involvement of the Gq/IP3 cascade and suggest possible Gi/o-Gq protein interactions in the astrocyte response to GABA signals. Optogenetic stimulation of ChR2-expressing parvalbumin fast-spiking (Pv-FS) interneurons also evoked astrocytic Ca2+ events, and current pulse depolarization of single Pv-FS or a somatostatin (Som) interneurons increased Ca2+ peaks in nearby astrocytes from 0.41 ± 0.04 to 0.65 ± 0.08 (events/minute; $p<0.05$) and from 0.10 ± 0.31 to 1.09 ± 0.16 ($p<0.001$), respectively. Patch-clamp recordings in the presence of TTX showed that GABAB activation triggered glutamate release in astrocytes and NMDAR-mediated slow inward currents (SICs) in nearby neurons. The frequency of SICs was strongly increased both in Pv-FS interneurons (from 0.15 ± 0.06 to 0.46 ± 0.04 event/min) and pyramidal neurons (from 0.30 ± 0.07 to 0.79 ± 0.17 event/min). The increase in SIC frequency lasted for about three minutes, outlasting the time of GABA agonist applications. Due to the intrinsic membrane properties, GABAB mediated SICs were more effective in inducing action potential firing in pyramidal neurons than in Pv-FS interneurons, suggesting that in local circuits astrocytes activated by GABAergic interneurons convert a transient inhibition into a delayed excitation. The activation of astrocytes by two of the major GABAergic interneurons in the brain (Pv and

Som) and the consequent gliotransmitter release represent a new form of homeostatic control of local network excitability.

Disclosures: **L. Mariotti:** None. **G. Losi:** None. **M. Sessolo:** None. **I. Marcon:** None. **S. Bovetti:** None. **T. Fellin:** None. **G. Carmignoto:** None.

Poster

511. Glial Physiology and Glia-Neuronal Physiology

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 511.02/P2

Topic: B.11. Glial Mechanisms

Title: Nerve Growth Factor regulates astrocyte and microglia physiology *in vitro* and *in vivo*

Authors: N. M. CARUCCI[1], C. RIZZI[1], L. GALLI-RESTA[2], S. CAPSONI[1], *A. CATTANEO[1]
[1]Scuola Normale Superiore, Pisa, Italy; [2]Inst. of Neuroscience, CNR, Pisa, Italy

Abstract: Cholinergic neurons of the basal forebrain are considered the main target cell population of NGF in the CNS. Selectively inhibiting the activity of mature NGF in the brain with a recombinant anti NGF antibody determines a more widespread, progressive neurodegeneration that recapitulates many hallmarks of AD. Also, intranasally delivery of NGF has been shown to rescue behavioural memory deficits and neuropathological alterations in APP based FAD mouse models, that show no cholinergic deficits. Here we show that neutralization of mature NGF in the AD11 mouse model induces, in an age-dependent manner, a marked astrocytic morphological change, which is reversed by intranasal NGF administration, showing a specific dependence on mature NGF deprivation. NGF neutralization determines also an early disregulated expression of mRNAs related to neuroinflammation, prior to the onset of any evident neurodegeneration. As both astrocytes and microglia cells are the principal mediators of neuroinflammatory states, we studied the effects of NGF neutralization in primary cell cultures of astrocyte or microglia. In these cells we confirmed the presence of NGF receptors TrkA and p75NTR. When incubated with NGF, we found an activation of both TrkA and p75NTR signaling pathways. *In vitro* neutralization of NGF with anti NGF mAb alfaD11 causes astrocyte morphological changes that are fully superimposable to those observed *in vivo* in AD11 mice. Moreover, in response to acute NGF deprivation, cultured astrocytes show changes in phosphorylation of TrkA, p75, Plcγ, Akt and modifications in their calcium dynamics. We demonstrated that the selective neutralization of mature NGF in the brain of AD11 mice, concomitantly to these astrocytes changes, also determines an early decrease in immune and

synaptic markers expression. Concomitantly, we found a decreased number of inhibitory interneurons in AD11 mice. To dissect the role of NGF receptors in microglia cells, these cells were incubated with NGF, in the presence/absence of p75NTR and TrkA immunoadhesins, or of the TrkA neutralizing antibody MNAC13. We observed a modulation of some inflammatory proteins and mRNA that are also modulated in the AD11 mouse. Microglia cells are known to phagocyte beta-amyloid. We found that NGF significantly increases the ability of microglial cell to phagocyte aggregated beta amyloid42. In conclusion we showed that NGF plays a significant role in the maintenance of astrocyte and microglia functions and homeostasis *in vivo* and *in vitro*. This physiological dependence could have a physiopathological consequence as well as a therapeutical implication, broadening the spectrum of NGF target cells in the brain.

Disclosures: N.M. Carucci: None. **A. Cattaneo:** None. **C. Rizzi:** None. **S. Capsoni:** None. **L. Galli-Resta:** None.

Poster

511. Glial Physiology and Glia-Neuronal Physiology

Location: Halls A-C

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Program#/Poster#: 511.03/P3

Topic: B.11. Glial Mechanisms

Support: Wellcom trust Grant

Title: Release of lactate via connexin hemichannels

Authors: *A. KARAGIANNIS[1], S. SYLANTYEV[2], S. KASPAROV[3], A. V. GOURINE[1]
[1]Neuroscience, Physiol. and Pharmacol., UCL, London, United Kingdom; [2]Clin. and Exptl. Epilepsy, UCL, Inst. of neurology, London, United Kingdom; [3]Physiol. and Pharmacol., Univ. of Bristol, Bristol, United Kingdom

Abstract: Neurons require constant and appropriate supply of energy to meet their metabolic demands. Astrocyte-to-neuron lactate shuttle hypothesis suggests that neuronal activity is fuelled by lactate provided by the neighbouring astrocytes. This mechanism requires an efficient transport system of lactate across plasma membrane. Monocarboxylate transporters (MCT) have been implicated by the majority of studies, but their existence does not exclude the involvement of other potential mechanisms. In this study we tested the hypothesis that lactate could be released via opening of the connexin hemichannels. Lactate biosensors were used to record tonic and hypoxia-induced release of lactate by the acute slices of the brainstem, cortex, and the

hippocampus. Connexin hemichannels open when extracellular [Ca^{2+}] decreases. In our experiments we found that reduction of extracellular [Ca^{2+}] (from 2 mM to 0 mM) increased tonic release of lactate (by ~50%) suggesting that connexin hemichannels contribute to this release. Compounds known to block connexins (carbenoxolone, 100 μM; NPPB 200 μM) reduced lactate tone (by ~40%) while MCTs blocker 4-CIN (250 μM) had no effect. Hypoxia-induced lactate release by brainstem slices was significantly reduced (by ~50%) in the presence of either connexin or MCT blockers. Stimulation of Schaffer collateral fibers in hippocampal slices triggered release of lactate in the vicinity of the patch-clamped CA1 neurons. Lactate release in CA1 in response to stimulation of Schaffer collaterals was increased in low [Ca^{2+}] (0.5 mM) conditions, reduced by carbenoxolone (200 μM) and abolished by lactate dehydrogenase inhibitor oxamate (20mM). These results suggest that release of lactate in the central nervous system may occur via the mechanisms other than MCTs. We show that connexin hemichannels may function as a conduit of lactate release and this mechanism is recruited during hypoxia or enhanced neuronal activity.

Disclosures: A. Karagiannis: None. **S. Sylantyev:** None. **S. Kasparov:** None. **A.V. Gourine:** None.

Poster

511. Glial Physiology and Glia-Neuronal Physiology

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Program#/Poster#: 511.04/P4

Topic: B.11. Glial Mechanisms

Support: University of Louisiana at Lafayette GSO grant

Title: Imaging intracellular Calcium Waves in astrocytes of FGFR1 knockout mice

Authors: *D. J. ROGERS, P. ACHI, J. COLLETTE, G. WATSON, K. M. SMITH
Univ. of Louisiana at Lafayette, Lafayette, LA

Abstract: Fibroblast Growth Factor Receptor 1 (FGFR1) is a tyrosine receptor kinase. Its primary ligand is Fibroblast Growth Factor 2 (FGF2), which binds to its extracellular domain along with Heparin. In addition to signaling from the lipid bilayer, the entire FGF2/FGFR1 complex may become internalized and transported to the nucleus to stimulate transcription of proliferation promoting genes. One of the main substrates of FGFR1 is Phospholipase C γ (PLCγ). PLCγ activates IP3, which causes the release of calcium from the intracellular stores,

primarily from the endoplasmic reticulum. An increase of cytoplasmic calcium facilitates the exocytosis of gliotransmitters and also other ionic signaling through gap junctions. Calcium released from the endoplasmic reticulum may also enter the nucleus to aid in transcription of calcium-dependent genes. We compared internal calcium waves in astrocyte tissue cultures prepared from P2-P4 $FGFR1^{Flox/Flox;NestinCre}$ knockout mice and Control littermates using Fluo2-AM. We observed prominent calcium waves throughout the cortical astrocyte cultures in both the control and $FGFR1^{Flox/Flox;NestinCre}$ groups. The mean number of intracellular waves observed in 60 seconds was not significantly different between the control and $FGFR1^{Flox/Flox;NestinCre}$ groups. Cell nuclei fluoresced the brightest during a calcium wave, and returned to a basal fluorescence level at the end of the wave. There was a nonsignificant trend that the $FGFR1^{Flox/Flox;NestinCre}$ astrocytes demonstrated less intensity at the peak of the calcium wave than the littermate controls. $FGFR1^{Flox/Flox;NestinCre}$ mean intensity difference from baseline = 39 grey values ± 35.8. ; control mean intensity difference from baseline = 63 grey values ± 44.6 p=0.06. The calcium wave duration at the nucleus was compared. The $FGFR1^{Flox/Flox;NestinCre}$ astrocytes exhibited about a 4 times slower wave than the control. $FGFR1^{Flox/Flox;NestinCre}$ wave duration = 14.1 sec ± 5.6. Control wave duration = 3.7 sec ± 2.34 $p=1.98x10^{-11}$. This highly-significant difference in wave duration diminished by the second and third calcium waves; showing no significant differences between the two groups. These results indicate that FGFR1 has a significant role in intracellular calcium signaling throughout the astrocyte and possibly, the movement of calcium into the nuclear space.

Disclosures: D.J. Rogers: None. **G. Watson:** None. **P. Achi:** None. **J. Collette:** None. **K.M. Smith:** None.

Poster

511. Glial Physiology and Glia-Neuronal Physiology

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Program#/Poster#: 511.05/P5

Topic: B.11. Glial Mechanisms

Support: Swiss National Science Fondation Grant # 31003A_135720

Title: Extracellular potassium tunes astrocytic glutamate clearance efficiency

Authors: T. S. RIMMELE, A.-B. ROCHER, *J.-Y. CHATTON
Univ. Lausanne, Lausanne, Switzerland

Abstract: Glutamate is released in the synaptic cleft during neuronal activity. Glutamate clearance is mainly performed by Na^+-dependent glutamate transporters of astrocytes. Astrocytes are also involved in the regulation of extracellular potassium concentration ($[K^+]_o$) which significantly increases during the repolarization phase of neurons firing action potentials. In this study, we evaluated how these two fundamental functions of astrocytes, directly associated with neuronal activity, coexist and if they interact. We investigated the impact of altering $[K^+]_o$ on glutamate transporter activity measuring the intracellular sodium concentration ($[Na^+]_i$) by microspectrofluorimetry in primary astrocytes and glutamate transporter currents in cortical slices. Glutamate uptake caused a reversible rise in $[Na^+]_i$, which was tightly modulated both in amplitude and rate of rise by $[K^+]_o$. Astrocyte glutamate transporter currents evoked in acute slices also showed amplitude modulation by $[K^+]_o$. Because the Na^+/K^+ ATPase constantly attempts to regulate intracellular Na^+, we inhibited it using ouabain in order to single out the impact of $[K^+]_o$ fluctuations on the kinetics of the glutamate transporter. Under these conditions, low $[K^+]_o$ enhanced transport activity, whereas high $[K^+]_o$ markedly slowed down glutamate capture, indicating that $[K^+]_o$ directly modulates the kinetics of glutamate transporters. We then found that these $[K^+]_o$ alterations had bioenergetic consequences directly proportional with the degree of glutamate transporter activation. Overall, these results indicate that high $[K^+]_o$ exerts a negative feedback on glutamate uptake in astrocytes, while lowered $[K^+]_o$ potentiates glutamate transporter activity. The physiological consequences of these findings have to be considered in the context of K^+ buffering, maintenance of neurotransmitter, and energy homeostasis.

Disclosures: **T.S. Rimmele:** None. **A. Rocher:** None. **J. Chatton:** None.

Poster

511. Glial Physiology and Glia-Neuronal Physiology

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 511.06/P6

Topic: B.11. Glial Mechanisms

Support: Spanish Government Grant BFU2012-38844

Title: CREB regulates calcium excitability in astrocytes via mitochondria

Authors: A. ERASO[1], E. VICARIO[1,2], R. VILLALONGA[1], L. PARDO[1], *E. GALEA[1], R. MASGRAU[1]
[1]Inst. of Neurosciences, and Dept. of Biochem. and Mol. Biol., Univ. Autònoma de Barcelona, Bellaterra, Barcelona, Spain; [2]Univ. of California, San Diego, CA

Abstract: HYPOTHESIS: Astrocytes are calcium-based excitable cells that modulate neurotransmission, learning and memory, although how they do it is not completely understood. Our working hypothesis is that experience induces CREB-mediated long-lasting changes in astrocytes that, in turn, modulate the activity of astrocyte-neuronal circuitries. In this study we have aimed at finding support for this idea by asking the straight question of whether CREB modulates the gliotransmitter-elicited increases in cytosolic calcium in rat cortical astrocyte cultures. METHODS: CREB-dependent transcription was triggered either by 1 hour-pulses with norepinephrine (NE 10 μM) or ATP (100 μM), which we have previously shown to CREB-dependent transcription in astrocytes within 6 hours, or by virally transducing a constitutively active form of CREB (VP16-CREB). Cytosolic calcium was assessed by calcium imaging in Fluo-4-loaded astrocytes (4 μM) challenged by gliotransmitters (100 μM ATP , 10 μM NE and 100 nM ET-1). RESULTS: Pre-stimulation with gliotransmitters reduced the calcium responses to the second stimulation with gliotransmitters by 15-38 %. Likewise, VP16-CREB caused a 27 % decrease in gliotransmitter-induced calcium response, as compared to astrocytes infected with an empty virus (Null). There were no alterations in resting cytosolic calcium levels. The CREB-induced decrease in calcium transients was still observed in the presence of EGTA but not if mitochondrial calcium uptake was blocked by the mitochondrial proton gradient uncoupler FCCP (5 μM). These results suggested that the CREB-induced decrease in cytosolic calcium transients was due to increased uptake by mitochondria rather than by reduced entry of external calcium. In agreement, ATP-induced mitochondrial calcium rises, monitored with 10 μM Rhod-2, were around 3 times higher in VP16-CREB-overexpressing than in Null astrocytes. CONCLUSION: CREB changes the calcium excitability of astrocytes by altering calcium compartimentalization between cytosol and mitochondria.

Disclosures: **A. Eraso:** None. **E. Vicario:** None. **R. Villalonga:** None. **L. Pardo:** None. **E. Galea:** None. **R. Masgrau:** None.

Poster

511. Glial Physiology and Glia-Neuronal Physiology

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Support: Owensenske Fond

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Novo Scholarship Programme

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Biomedicine Travel Stipend

Title: Purines released from astrocytes inhibit excitatory synaptic transmission in the ventral horn of the spinal cord

Authors: E. M. M. CARLSEN[1], *J.-F. PERRIER[2]
[1]Neurosci. and Pharmacol., Univ. of Copenhagen, Copenhagen, Denmark; [2]INF, Univ. Copenhagen, Copenhagen, Denmark

Abstract: Spinal neuronal networks are essential for motor function. They are involved in the integration of sensory inputs and the generation of rhythmic motor outputs. They continuously adapt their activity to the internal state of the organism and to the environment. This plasticity can be provided by different neuromodulators. These substances are usually thought of being released by dedicated neurons. However, in other networks from the central nervous system synaptic transmission is also modulated by transmitters released from astrocytes. The star-shaped glial cell responds to neurotransmitters by releasing gliotransmitters, which in turn modulate synaptic transmission. Here we investigated if astrocytes present in the ventral horn of the spinal cord modulate synaptic transmission. We evoked synaptic inputs in ventral horn neurons recorded in a slice preparation from the spinal cord of neonatal mice. Neurons responded to electrical stimulation by monosynaptic EPSCs. We used mice expressing the enhanced green fluorescent protein under the promoter of the glial fibrillary acidic protein to identify astrocytes. Chelating calcium with BAPTA in a single neighboring astrocyte increased the amplitude of synaptic currents. In contrast, when we selectively stimulated astrocytes by activating PAR-1 receptors with the peptide TFLLR, the amplitude of EPSCs evoked by a paired stimulation protocol was reduced. The paired-pulse ratio was increased, suggesting an inhibition occurring at the presynaptic side of synapses. In the presence of blockers for extracellular ectonucleotidases, TFLLR did not induce presynaptic inhibition. Puffing adenosine reproduced the effect of TFLLR and blocking adenosine A1 receptors with DPCPX prevented it. Altogether our results show that ventral horn astrocytes are responsible for a tonic and a phasic inhibition of excitatory synaptic transmission by releasing ATP, which gets converted into adenosine that binds to inhibitory presynaptic A1 receptors.

Disclosures: E.M.M. Carlsen: None. **J. Perrier:** None.

Poster

511. Glial Physiology and Glia-Neuronal Physiology

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 511.08/P8

Topic: B.11. Glial Mechanisms

Support: University of Minnesota, Department of Neuroscience

Ministerio de Ciencia e Innovación, Spain (CSD2010-00045)

Title: Astrocyte-neuron communication properties during aging

Authors: *M. MARTIN-FERNADEZ[1], M. GOMEZ-GONZALO[2], G. PEREA[2], A. ARAQUE[1]
[1]Univ. of Minnesota, Minneapolis, MN; [2]Inst. Cajal, Madrid, Spain

Abstract: The tripartite synapse is synaptic physiology concept, where astrocytes sense and control neuronal and synaptic activity. This concept is supported by studies from young animals. However the possible alteration of this functional unit during adulthood and aging is still unknown. Using electrophysiological and calcium imaging techniques we have investigated the bidirectional signaling between neurons and astrocytes in hippocampal and cortical slices of 0.5, 5, 12, and 20 months age mice. Astrocytes displayed spontaneous calcium elevations that were independent of neuronal activity. The local application of transmitter receptor agonist (ATP, ACh and DHPG), PAR1 agonist (TFLLR) or nerve electrical stimulation could induce calcium elevations. Electrophysiological recordings in neurons of adult and aged mice reveal the presence of slow inward currents (SICs) mediated by the activation of NMDA receptors by the released gliotransmitter glutamate. Therefore, during aging astrocytes are still able to affect neuronal excitability releasing the gliotransmitter glutamate. Furthermore, theses SICs can be induced after local application of agonists that activate astrocytes. These results reveal the existence of reciprocal signaling between neurons and astrocytes in adult and aged mice.

Disclosures: M. Martin-Fernadez: None. **M. Gomez-Gonzalo:** None. **G. Perea:** None. **A. Araque:** None.

Poster

511. Glial Physiology and Glia-Neuronal Physiology

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 511.09/P9

Topic: B.11. Glial Mechanisms

Title: Potential role of NF-κB p50 in astrocyte-neural progenitor cell communication within the neurogenic niche

Authors: *V. BORTOLOTTO, S. CVIJETIC, S. LOVECCHIO, P. L. CANONICO, M. GRILLI
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Abstract: One of the areas in which adult neurogenesis occurs is the SubGranular Zone (SGZ) in the dentate gyrus of the hippocampus. This region is characterized by the presence of the neurogenic niche, a highly specialized microenvironment which profoundly influences the neurogenic process. Previous work in our laboratory showed that members of the NF-κB family of transcription factors are expressed in zone of active neurogenesis and that NF-κB p50 knockout (KO) mice display impaired adult hippocampal neurogenesis, likely occurring at the transition stage between neuroblasts and mature neurons. However, when adult hippocampal neural progenitor cells (NPC) derived from wild type (WT) and p50 KO mice are cultured *in vitro*, no significant differences can be observed in their neuronal differentiation rate. These data suggest a potential contribution of other cell subpopulations present in the neurogenic niche, like astrocytes, to the defective neurogenesis in p50 KO mice. Recently, we have set up primary astrocyte cultures from cortex and hippocampi of p50 KO and WT neonatal mice and studied their interaction with WT and p50 KO NPC by using astrocyte-conditioned medium (ACM). When WT NPC where exposed to WT ACM, an increased differentiation towards both neuronal and glial lineages was observed, compared to standard medium. Conversely, p50 KO ACM was able to significantly increase the percentage of newly generated astrocytes, but devoid of proneurogenic effects. Additionally, WT ACM had no proneurogenic effect on p50 KO NPC. In all experimental settings both WT and p50 KO ACM had no significant effect on the survival rate of NPC and their progeny in both genotypes. These preliminary data revealed a complex role for the NF-κB p50 subunit in the NPC-astrocyte cross-talk. In addition, they suggested that neurogenic defects in p50 KO mice cannot be merely ascribed to neural progenitor cell autonomous effects, but that they may also involve glial cells. At present, we are actively pursuing the identification of the astrocyte-derived soluble factor(s) which may be responsible of the different proneurogenic potential of WT and p50 KO ACM.

Disclosures: V. Bortolotto: None. **S. Cvijetic:** None. **S. Lovecchio:** None. **P.L. Canonico:** None. **M. Grilli:** None.

Poster

511. Glial Physiology and Glia-Neuronal Physiology

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Topic: B.11. Glial Mechanisms

Support: DFG SFB 1089

DFG SPP 1757

Title: Excitatory synaptic depolarizations induce Ca2+ elevations in NG2 cells

Authors: *W. SUN, D. DIETRICH
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Abstract: As the fourth major type of glia cells in central nervous system (CNS), NG2 expressing oligodendrocyte precursor cells attracted much attention in the last decade because they receive direct synaptic input from neurons. NG2 cells themselves also express prominent levels of voltage gated ion channels (VGCs), including A-type and delay rectifier type potassium channels, and fast voltage-activated sodium channels. So far it has not been elucidated how presynaptic activity may trigger signaling pathways in postsynaptic NG2 cells potentially important for their proliferation and differentiation. In particular, it is unclear whether direct synaptic transmission can induce post-synaptic Ca^{2+} elevations in NG2 cells. In this study, we are investigating whether excitatory synaptic depolarizations are capable of triggering Ca^{2+} signaling in NG2 cells, and how VGCs modulate the Ca^{2+} elevation. We patched NG2 cells in the hippocampal CA1 region of NG2-DsRed mice (8-15 days old) in current clamp mode and held the membrane potential at -85mV. Intracellular calcium was monitored with 2-photon laser scanning microscopy. Excitatory post-synaptic potentials (EPSPs) were evoked by injecting mock excitatory post-synaptic current (moEPSCs) waveforms derived from miniature synaptic currents recorded in NG2 cells. moEPSC amplitudes were matched to the individual cell's passive properties. We did not detect any Ca^{2+} elevation in NG2 cells when single EPSCs were injected (N = 7). However, when we injected a burst of 10 moEPSCs at 100Hz, clear Ca^{2+} signals were observed which rose during the stimulation, peaked at 12.4 ± 2% $\Delta F/F$ and showed a 50%-decay-time of 2.3 ± 0.5 s in the proximal dendrite (N = 7). Combined application of 300 µM $CdCl_2$ and 200 µM $NiCl_2$ completely blocked the Ca^{2+} signal induced by burst moEPSCs injection (N =5). In striking contrast, in the presence of the A-type potassium channel blocker, 4-Aminopyridine (4-AP), 50% of NG2 cells showed Ca^{2+} elevation with only a single moEPSC injection (4 out of 8 cells), and the Ca^{2+} elevation in response to burst moEPSCs injection

significantly increased to 24.5 ± 6% in proximal dendrites. Our data suggest that excitatory synaptic depolarizations in NG2 cells are able to trigger Ca^{2+} elevation through the activation of voltage gated calcium channels. In addition, A-type potassium channels seem to be important for gating Ca2+ entry into NG2 cells.

Disclosures: W. Sun: None. **D. Dietrich:** None.

Poster

511. Glial Physiology and Glia-Neuronal Physiology

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 511.11/P11

Topic: B.11. Glial Mechanisms

Support: FNRS (31003A-130821/1)

NCCR Synapsy

Biaggi Foundation

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Title: Lactate stimulates NMDA receptor-mediated Erk signaling cascade to regulate plasticity-related gene expression

Authors: *I. ALLAMAN[1], E. RUCHTI[1,2], P. JOURDAIN[1,3], J. YANG[1], G. GRENNINGLOH[1], J.-M. PETIT[1,3], P. J. MAGISTRETTI[1,2,3]
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Abstract: Glycogen-derived lactate release by astrocytes is required for long-term memory formation (Suzuki et al, 2011). Here we report that in primary cultures of mouse cortical neurons L-lactate significantly stimulates mRNA expression of key immediate early genes (IEGs) involved in plasticity-related processes, in a time- and concentration-dependent manner. Following one hour of treatment with 20 mM L-lactate, Arc, Zif268 and c-Fos mRNA levels were increased by 5.2-, 3.7- and 8.2-fold, respectively. In addition, the increased IEGs mRNA expression levels induced by L-lactate are correlated at the protein level with respectively 5.5, 4.0 and 3.2 fold increase compared to control values. These effects were specific for L-lactate,

since D-lactate, L-pyruvate and D-glucose had no effect on gene expression. The effects of L-lactate involved potentiation of NMDA receptor activity and its downstream signaling cascade Erk1/2, as they were prevented in the presence of specific inhibitors of NMDA receptors (MK801, APV and the glycine site blocker L-689.560) and of Erk1/2 kinases (U0126). We also observed that L-lactate potentiated NMDA receptor-mediated currents (induced by co-application of glutamate and glycine) and the ensuing increase in intracellular calcium. Consistent with a direct modulation of NMDA receptor activity by L-lactate, NMDA application results in an increased IEGs expression that is potentiated by L-lactate. All effects of L-lactate are mimicked by NADH, suggesting that changes in redox state of neurons following conversion of L-lactate to L-pyruvate are involved in the effect of L-lactate. Taken together these findings demonstrate that L-lactate acts as a direct signaling molecule which regulates neuronal plasticity-related IEGs expression. Interestingly, the underlying mechanism of action of L-lactate involves NMDA receptors and changes in the redox state. This set of observations therefore reveals a novel signaling role of lactate which may represent a mechanism underlying the role of astrocytic-derived L-lactate on long term memory formation.

Disclosures: I. Allaman: None. **E. Ruchti:** None. **P. Jourdain:** None. **J. Yang:** None. **G. Grenningloh:** None. **J. Petit:** None. **P.J. Magistretti:** None.

Poster

511. Glial Physiology and Glia-Neuronal Physiology

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Topic: B.11. Glial Mechanisms

Support: Natural Sciences and Engineering Council of Canada

Title: Brain extracellular glucose and lactate levels in the mouse motor cortex: Effects of running and peripheral injections of glucose and insulin

Authors: *C. MESSIER, J. LARCHER
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Abstract: There are some uncertainties about the relative contribution of lactate and glucose to brain metabolism. A long-standing theory suggests that the brain uses primarily glucose that transits from the blood to the brain extracellular space and then enters brain cells. Another theory posits that glucose enters the brain extracellular space and is taken up immediately by astrocytes

that eventually release lactate. Lactate is taken up by neurons and used as metabolic fuel. In the present experiment, we aimed bilateral electrochemical electrodes measuring glucose (right side) and lactate (left side) at the primary motor cortex of CD-1 mice. Brain extracellular glucose and lactate were recorded in freely-moving animals that could use a running wheel. Sustained running wheel activity and locomotion were associated with up to 50% increases in lactate and glucose levels in the primary motor cortex. Lactate rose from 0.65 mmol to 0.9 mmol when mice initiated running and returned to baseline 4 min after the end of running. When mice were injected with 2g/kg ip glucose, lactate increases during locomotion were blunted and there was a monotonic brain extracellular glucose increase (190% from baseline) proportional to blood levels. When mice received a 0.4U/kg ip insulin injection, brain extracellular glucose levels decreased (3%) and lactate levels increased (10%), 1-2 min after the injection. When the mice ran in the running wheel after the peripheral glucose injection, brain lactate extracellular levels rises were blunted. Conversely, when mice ran after the peripheral insulin injection, glucose extracellular rises were blunted and exercise-related extracellular lactate levels rises were more prominent. The results show that glucose and lactate extracellular levels closely follow each other during neuronal activation in the motor cortex. Rises in blood and brain extracellular glucose levels appear to reduce the amount of lactate present in the extracellular space and blunt the lactate increase following neuronal activation. Decreases in blood and brain extracellular glucose levels appear to increase the amount of lactate present in the extracellular space and potentiate the lactate increase following neuronal activation. The results suggest that both glucose and lactate may contribute to neuronal metabolism but their relative role may depend on blood and brain glucose availability.

Disclosures: **C. Messier:** None. **J. Larcher:** None.

Poster

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Support: NEI Grant EY013319

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Title: Proteolytic regulation of synaptic plasticity in the mouse primary visual cortex- analysis of mmp-9 deficient mice

Authors: *E. A. KELLY[1], A. MAJEWSKA[2]
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Abstract: Matrix metalloproteinases (MMPs) constitute a large family of proteases which contribute to extracellular matrix degradation. MMP-9 is one of the most prevalent MMPs in the mammalian cerebral cortex and is known to participate in dendritic and synaptic remodeling. We have shown previously that MMP-9 is involved in the regulation of the intercellular adhesion molecule, ICAM-5 (telencephalin), and is responsible for its cleavage and subsequent removal, resulting in dendritic spine maturation. To investigate further the role of MMP-9 and extracellular matrix remodeling in activity-driven plasticity, we explored cortical plasticity in adolescent MMP9 null mice. We induced ocular dominance plasticity at P28 by monocularly depriving the contralateral eye of vision for a period 4-7 days. We then used intrinsic signal optical imaging to monitor the ocular dominance shift induced by monocular deprivation. We found that this shift was attenuated in MMP-9 KO mice following deprivation, suggesting that MMP-9 may play an important role in activity-dependent remodeling in the cortex. To evaluate the possible mechanisms of MMP-9 action during plasticity we evaluated the involvement of MMP-9 in several pathways that contribute to ocular dominance plasticity. Since MMP-9 has been shown to regulate dendritic structure and plastic changes involve dendritic spine remodeling, we next investigated anatomical changes at the level of the dendritic spine in MMP9 KO/GFP-M mice using a combination of two-photon and confocal microscopy. We also examined changes in extracellular matrix composition in MMP-9 KO mice using immunohistochemical techniques. Lastly, because MMP-9 is expressed in microglia and microglia have recently been shown to play an important role in ocular dominance plasticity, we quantified microglial morphology using immunohistochemistry and electron microscopy. Our preliminary findings suggest that loss of MMP-9 does not induce widespread changes in dendritic spine morphology or microglial morphology. Hence we conclude that MMP-9 loss affects activity-dependent plasticity through pathways that are distinct from the regulation of synapse and microglial morphology.

Disclosures: **E.A. Kelly:** None. **A. Majewska:** None.

Poster

511. Glial Physiology and Glia-Neuronal Physiology

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Topic: B.11. Glial Mechanisms

Support: Medical Research Council MR/J013110/1

Title: ATP-mediated signalling plays a key role in generation of BOLD fMRI responses

Authors: I. N. CHRISTIE[1], J. A. WELLS[1], P. S. HOSFORD[1], M. F. FIGUEIREDO[3], A. G. TESCHEMACHER[3], P. VIHKO[4], M. F. LYTHGOE[2], A. V. GOURINE[1], *S. KASPAROV[5]
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Abstract: Cellular mechanisms underlying the blood oxygen-level dependent (BOLD) signal measured in fMRI are poorly understood. There is evidence that astrocytes - the highly abundant glial cells of the CNS - are responsible for triggering changes in local blood flow following bursts of neuronal activity. Here we present data indicating that the principal astrocytic signalling molecule ATP is critically involved in the mechanisms underlying BOLD response. To interfere with ATP-mediated signalling we used a lentiviral vector to drive the expression of a potent ectonucleotidase - transmembrane prostatic acid phosphatase (TMPAP) on the cell surface membranes to accelerate breakdown of extracellular ATP. 21 days prior to MRI experiments, rats underwent bilateral viral injections targeted to the forepaw region of the somatosensory (SSFP) cortex. One hemisphere was transduced to express TMPAP-GFP while the other hemisphere was transduced to express green fluorescent protein (GFP). Bilateral electrical forepaw stimulation triggered significantly smaller BOLD responses in the cortex transduced to express TMPAP-GFP in comparison to the hemisphere transduced to express GFP (n=11) as measured by an experimentator unaware of the nature of transgene expressed on either side. Facilitated ATP breakdown potentially could lead to adenosine accumulation which may affect neuronal activity. However systemic treatment with A1 adenosine receptor antagonist DPCPX (1mgkg-1) further reduced, rather than increased the magnitude of fMRI signal in the hemisphere expressing TMPAP (n=9). To determine whether neuronal responses are affected by TMPAP expression we assessed the minimum amplitude of stimulation required to evoke multiunit activity in the SSFP cortex and also evaluated the amplitude of this response under the same stimulation parameters as used in the fMRI protocol. In both cases, no significant differences were observed between cortices (n=9). In summary, suppression of ATP-mediated signalling results in marked suppression of the BOLD response, indicating that purinergic signalling plays an important role in neurovascular coupling.

Disclosures: I.N. Christie: None. **J.A. Wells:** None. **P.S. Hosford:** None. **M.F. Figueiredo:** None. **A.G. Teschemacher:** None. **P. Vihko:** None. **M.F. Lythgoe:** None. **A.V. Gourine:** None. **S. Kasparov:** None.

Poster

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Topic: B.11. Glial Mechanisms

Support: NSC 102-2321-B-006 -020

Title: Regulation of astrocytic glutamate transporter expression by peroxisome proliferator-activated receptor alpha

Authors: Y.-T. HSIEH[1], W.-T. CHIU[2], *J.-Y. C. HSU[3], S.-F. TZENG[1]
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Abstract: Astrocytes play a crucial role in modulating extracellular glutamate concentrations to maintain low and non-toxic levels in the CNS. Glutamate transporter 1 (GLT-1)/EAAT-2 and glutamate aspirate transporter (GLAST) are the most important glutamate transporter subtypes found in rat astrocytes. The expression of two glutamate transporters can be regulated by microenvironmental factors in developmental and injured brain. Peroxisome proliferator-activated receptor-α (PPARα) can interact with RXRs, and then cooperatively regulate PPARα-target genes. For instance, 9-cis RA and PPARα agonists can downregulate the production of proinflammatory mediators in astrocytes. By examine the promoter sequences of GLAST and GLT-1 through the analysis using the promoter transcription factor binding site prediction software, we found rat GLAST and rat GLT-1 promoter regions contain 2 and 5 RXRα:PPARα binding sites, respectively. In the present study, we found that exposure to the PPARα agonists (WY 14,643 and GW 7647) significantly upregulated GLT-1 mRNA expression in astrocytes in the presence of dibutyryl cAMP (dbcAMP) or inflammagen lipopolysaccharide (LPS). In contrast, the two PPARα agonists reduced GLAST mRNA expression in dbcAMP-treated astrocytes, whereas PPARα agonists caused no significant change in GLAST gene expression by exposure of astrocytes to LPS. Thus, the activation of PPARα upregulated GLT-1 mRNA in astrocytes under the influence of intracellular cAMP rise and inflammagen. The PPARα/RXRα-mediated mechanism in the regulation of GLT-1 expression is currently under investigation.

Disclosures: Y. Hsieh: None. **J.C. Hsu:** None. **S. Tzeng:** None. **W. Chiu:** None.

Poster

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Topic: B.11. Glial Mechanisms

Support: NIH 1P20GM103653-01A1

Title: Role of astrocytes in the development of synchronized bursting behavior in neuronal networks

Authors: K. R. SANCHEZ, N. AGBAZUE, M. HARRINGTON, *M. TEMBURNI
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Abstract: Synchronous oscillations are thought to be necessary for establishing functional neuronal networks for normal vertebrate brain development - although the mechanisms of synchronization are not fully understood. Existing models of synchronous activity assume that it is a process intrinsic to neurons. However, glia have been shown to modulate oscillatory activity in networks of neurons during sleep, during prodromal oscillations of neurons preceding spreading depression, and the slow inward currents (SICs) resulting in synchronous activity in hippocampal neurons, thalamus and nucleus accumbens. Recently glial cells, particularly astrocytes, have been shown to participate in neuronal communication by releasing "gliotransmitters" like glutamate, ATP and D-serine. We hypothesize that astrocyte-neuron interactions are crucial for the development of synchronous activity seen in the developing vertebrate brain. We test this hypothesis by establishing pure and mixed (astrocyte and neuronal) cultures from the developing chicken brain (optic tectum) and recording total neuronal activity using the multi-electrode array system, MED64. Pure neuronal cultures were obtained by treating cultures with the mitotic inhibitor 5-fluorodeoxyuridine (FUdR) which kills mitotically active astrocytes but spares post-mitotic neurons. Neurons were kept alive in the absence of astrocytes by supplementing the culture medium with 50% astrocyte conditioned medium. Typically mixed cultures of astrocytes and neurons show random spiking activity in one week and synchronous activity in two weeks. Our initial results indicate that pure neuron only cultures show random spiking activity without synchronization even after two weeks thus clearly establishing a role for astrocytes in the development of synchronous activity. To further dissect the molecular pathways involved we will target three pathways within astrocytes that have been demonstrated to be crucial for communication with neurons - metabotropic glutamate receptor (mGluR), purinergic (P2Y1) receptor and GABAB receptor. Activation of these G-protein coupled receptors by their respective neurotransmitters mobilizes intracellular calcium release leading to exocytosis of

either glutamate or ATP. Using lentiviral vectors, we propose to express dominant negative peptides designed to disrupt downstream signaling pathways of these receptors and thereby calcium mobilization and exocytosis of gliotransmitters in chick embryo astrocytes.

Disclosures: K.R. Sanchez: None. **N. Agbazue:** None. **M. Harrington:** None. **M. Temburni:** None.

Poster

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UNM-SOM RAC

UNM-Anesthesiology Pain Research Fund

Title: Epidural glutamate produces long-lasting allodynia in rats: Role of glutamate actions in nearby dorsal root ganglia or intervertebral disc nerve endings

Authors: *R. WHITEHEAD, N. LAM, M. SUN, K. TUFFLI, W. ORNATOWSKI, F. HARRINGTON, H. MARTIN, E. MILLIGAN
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Abstract: Low back pain (LBP) is the leading cause of adult disability and arises from chronic intervertebral disc degeneration in the majority of cases. In the absence of physical pressure on nerve roots, the primary excitatory neurotransmitter glutamate is produced from herniated discs via enzymatic breakdown of nucleus pulposus matrix aggrecan proteins, yielding elevated ambient glutamate concentrations in LBP patients. Infusion of epidural glutamate over a 72 hour period results in thermal and mechanical hindpaw hypersensitivity, putatively via focal activation of glutamate receptors expressed on nearby dorsal root ganglia (DRG). However, it is not known whether: (1) epidural glutamate triggers peripheral and/or central sensitization after the time of passive glutamate clearance, (2) if elevated epidural glutamate is a required or chief component of discogenic LBP, and (3) which focal sites glutamate activates following enzymatic breakdown of matrix aggrecan. Here we sought to determine whether a single epidural injection of glutamate in rats would result in prolonged mechanical allodynia. One end of an indwelling catheter was

threaded epidurally to the level L5 DRG, and fastened at midpoint to the L2 spinous process. The opposite end was externalized and contained in a catheter holder sutured to the lumbar dorsal muscle. Following a 7-day recovery and monitoring period, rats received a 10 µl injection over 2-min of either 0.2mM HCL, 0.2mM L-glutamate or equivolume physiological saline. Mechanical allodynia was assessed using a blinded and randomized experimental design by systematically applying calibrated von Frey monofiliments to rat hindpaws to determine 50% threshold responses. Results revealed a single injection of 0.2mM glutamate produced significant bilateral allodynia lasting 10 days (2-way ANOVA: Left $F(2,14)=18.10$, $P<0.0001$; Right $F(2,14)=12.85$, $P=0.002$). Post hoc analysis showed peak effect on day 3 post injection bilaterally ($P<0.0001$; Multiple t-test using Holm-Sidak method). Injection of either 0.2mM HCL or saline failed to generate responses that differed from baseline levels, eliminating injection pressure and acidity as confounds ($P>0.05$). Indwelling catheter tip placements were confirmed at time of tissue harvest. Elevated epidural glutamate produces long lasting bilateral allodynia in rats. Ongoing studies are examining epidural glutamate-induced peripheral and/or central sensitization and excitotoxicity by measuring levels of pronociceptive neurotransmitters (Substance P & calcitonin gene-related peptide), pro-inflammatory mediators, and markers of cell apoptosis and necrosis in discs, DRG and spinal cord.

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Poster

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Topic: B.11. Glial Mechanisms

Support: FONDECYT 1140108

Title: Aldolase C is secreted by forebrain astrocytes in an exosome fraction

Authors: A. LUARTE[1], C. GOMEZ-MOLINA[1], *C. VERGARA[2], U. WYNEKEN[1]
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Abstract: Antidepressant drugs induce slow adaptations at glutamatergic synapses, which are enwrapped by astrocytes in the forebrain. However, the underlying signaling mechanisms involved in these adaptations are not completely understood. Besides their role as glycolitic

enzymes, aldolases have been reported to play secondary, “moonlight” functions mediated by their binding to F-actin and RNA species. Aldolase C (AldoC) is an astrocyte-specific enzyme in the forebrain. We previously showed that repetitive fluoxetine treatment in rats triggered AldoC up-regulation in a microsomal fraction derived from the forebrain. To get insight into the functional meaning of such increases, we performed studies in fluoxetine-treated animals and in astrocyte cell cultures. We found that fluoxetine treatment induces the secretion of AldoC into the extracellular space and that its content increases in the cerebrospinal fluid of fluoxetine-treated animals. Moreover, exosomes isolated from the cerebrospinal fluid contain AldoC. In astrocyte primary cell cultures, AldoC colocalizes with exosome markers. In addition, exosomes isolated from the conditioned medium of astrocyte cultures that express GFP-AldoC contain the recombinant protein. It is possible that AldoC in exosomes play a “moonlight” function, e.g. by regulating the stability or transfer of RNA species. The target cells of such microvesicles remain to be determined and will shed light on signaling mechanisms that participate in fluoxetine-mediated adaptations mediated by astrocytes.

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Poster

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Topic: B.11. Glial Mechanisms

Title: Astrocytic calciium signaling plays an important role for generation of retroaxonal barrage firing in hippocampalNPY interneurons

Authors: *T. DEEMYAD, N. SPRUSTON
Howard Hughes Med. Institute/Janelia Farm, Ashburn, VA

Abstract: A subset of inhibitory GABAergic interneurons in the CA1 area of the hippocampus exhibits persistent firing asa result of integrating action potential firing over the course of tens of seconds to minutes (Sheffield et al. 2011,Krook-Magnuson et al. 2011). Such retroaxonal barrage (RaB) firing could play an important role in regulating circuit excitability in the hippocampus. A few lines of evidence suggest that calcium ions play a role in the generation of RaB firing; namely, a decrease in extracellular calcium concentration or blocking voltage-gated calcium channels inhibit induction of RaB firing (Sheffield et al. 2013). In contrast, loading interneurons

with intracellular BAPTA had no effect on RaB firing. Inhibiting gap junctions also prevented generation of RaB firing, but mice lacking the connexin 36 subunit, which mediates gap junctions between interneurons, exhibited normal RaB firing (Sheffield et al. 2013). Together, these findings suggest a role for calcium signaling outside the interneurons in the induction of RaB firing. We tested the possibility that astrocytes may be the locus of these effects, by using a combination of electrophysiological recording from NPY cells and astrocytes as well as calcium imaging in astrocytes. All experiments were performed in hippocampal slices prepared from mice expressing GFP under the NPY promoter. First, double recordings from an NPY interneuron and a nearby astrocyte indicated that ~30% of astrocytes depolarized >10 mV few seconds before or after the onset of RaB firing (10-40mV, n=8/28). Second, induction of RaB firing was facilitated by depolarizing astrocytes with bath application of 100 uM BaCl2 (30-40 mV depolarization; n=6/6), a selective blocker of inwardly rectifying potassium (Kir) channel in astrocytes (<5 mV depolarization in interneurons), without any affect on the duration and frequency of RaB firing. Third, no RaB firing was observed in interneurons when astrocytic calcium was chelated by intracellular BAPTA during double recording from NPY cells and astrocyte (n=5/5 pairs).Furthermore, in additional experiments, RaB firing was first generated in interneurons and a nearby astrocyte was subsequently patched using pipettes containing BAPTA. In some cases this resulted in complete block ofRaB (n=2/25) or an increase in the number of spikes required for induction of RaB firing (n=5/25). Finally, using a calcium-sensitive dye, we observed, in some astrocytes, an increase in fluorescence preceding the onset ofRaB firing in NPY interneurons (n=3/9). Together, these findings suggest that a subset of astrocytes interact with NPY interneurons in a manner that promotes the generation of RaB firing.

Disclosures: **T. Deemyad:** None. **N. Spruston:** None.

Poster

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NSF IGERT CMMB 0965918 (GNK)

NSF IOS 1354913 (MUG)

Title: Heterogeneous astrocyte dynamics: Diurnal morphological changes in the dentate gyrus and suprachiasmatic nucleus

Authors: S. J. IRVING[1], H. J. ROSENBERG[1], *J. W. MITCHELL[2], G. NASERI KOUZEHGARANI[3], J. L. CHU[2], J. S. RHODES[4], M. U. GILLETTE[2]
[1]Dept. of Mol. and Integrative Physiol., [2]Dept. of Cell and Developmental Biol., [3]Neurosci. Program, [4]Dept. of Psychology, Univ. of Illinois At Urbana-Champaign, Urbana, IL

Abstract: While brain information processing is thought to reside in interactions of complex neuronal circuits, a critical role for astrocytic glia in shaping brain physiology and behavior has emerged. Astrocytes participate at synaptic, cellular, and network levels, and their contributions to brain function by modulating synaptic transmission have received considerable attention. At cellular and network levels, astrocytes modulate the environment and form syncytia that permit rapid trans-network propagation of K+ and small metabolites. When challenged acutely, hypothalamic astrocytes can undergo morphological changes that alter relationships with surrounding cells and coordinate neurohormone release. We asked whether morphological change occurs under resting physiological conditions on a daily basis, the period over which brain physiology normally fluctuates. We evaluated the morphometrics of the astrocyte cytoskeleton, measured via immunofluorescence of the intermediate filament glial fibrillary acidic protein (GFAP), and of cell volume, determined via dye-filling of individual cells. We performed unbiased, computer-based analysis using Imaris software. We found robust diurnal patterns of morphological change in astrocytes of both the hippocampal dentate gyrus (DG) and hypothalamic suprachiasmatic nucleus (SCN). The complexity of GFAP structure and cell volume wax and wane in a rhythmic pattern over the 24-h cycle. GFAP branch terminal-points are significantly more numerous in the SCN than DG, differing by 4-fold at peak times. Terminal-point complexities oscillate in anti-phase, such that GFAP branching peaks at early night in the DG, but at early day in the SCN. In both regions, these daily changes in cytoskeleton architecture are mediated by reorganization of GFAP protein, not turnover. These findings add a new dimension to these cells with the discovery that the morphology of single astrocytes does not reside in a 'resting' basal state. Rather, astrocyte morphology in these regions of hippocampus and hypothalamus is highly dynamic even in unstimulated conditions.

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Poster

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Topic: B.11. Glial Mechanisms

Support: NIGMS SC1089630

Title: Transcriptional regulation of human transforming growth factor-α by NF-κB, Sp1 and YY1

Authors: P. KARKI[1], K. SMITH[1], J. JOHNSON JR[1], D.-S. SON[1], *E.-S. Y. LEE[2]
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Abstract: Transforming growth factor-alpha (TGF-α) play multifunctional roles in the central nervous system including its neuroprotection against excitotoxicity and oxidative stress. TGF-α has also been reported to mediate the neuroprotective effects of estrogen and selective estrogen receptor modulators but the regulation of TGF-α at the transcription level remains poorly understood. We attempted to identify critical transcription factors and signaling pathways for TGF-α regulation in rat primary astrocytes. We identified that the human TGF-αα promoter contains consensus sites for NF-kB, CREB, Sp1, and YY1. The results showed that overexpression of NF-kB p65 increased, whereas mutation in NF-kB binding sites of the TGF-α promoter reduced its promoter activity significantly. Accordingly, pharmacological inhibition of NF-kB abolished TGF-α promoter activity. Dexamethasone (Dx) and dibutyryl-cAMP (dbcAMP) increased TGF-α promoter activity, but their effects were significantly reduced when NF-kB binding sites were mutated. The electrophoretic mobility shift assay revealed that NF-kB binds to the TGF-α promoter directly. Sp1 also appears to be a critical positive regulator of the TGF-α promoter as its overexpression increased, but mutation of Sp1 binding sites decreased TGF-α promoter activity. On the other hand, YY1 is a critical negative regulator of the TGF-α promoter as overexpression of YY1 decreased, but mutation of YY1 binding sites in the promoter increased TGF-α promoter activity. On analyzing the signaling pathways involved in the regulation of TGF-α expression, Dx-enhanced TGF-α promoter activity and mRNA levels were abolished by inhibition of MAPK, PI3K/Akt, and PKA signaling. Taken together, our results suggest that NF-kB and Sp1 are critical positive regulators, whereas YY1 is a critical negative regulator of the TGF-α promoter. Dx enhances TGF-α promoter activity via NF-kB and several signaling pathways. These findings will significantly contribute to identifying the molecular mechanism for transcriptional regulation of TGF-α which induces neuroprotection.

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Poster

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Title: Anatomical arrangement of astrocytes in ferret visual cortex

Authors: *M. LOPEZ-HIDALGO, W. HOOVER, J. SCHUMMERS
Max Planck Florida Inst. For Neurosci., Jupiter, FL

Abstract: There is now abundant evidence that astrocytes are active elements in the proper function of neural circuits. An understanding of how astrocytes interact with the functional organization of different neural circuits will require a description of the anatomical arrangement of astrocytes within each specific neural circuit. Each astrocyte sends densely branching processes which define a territory, or domain, of neural tissue with which it can interact. Within their territory, astrocytes perform metabolic and hemodynamic functions as well as regulate neural activity. In the hippocampus, astrocyte domains have been shown to parcel out the gray matter with minimal overlap between neighboring domains.VIn the present study, we studied the spatial organization and morphology of astrocytes in ferret visual cortex, using a combination of *in vivo* and histological approaches. Astrocyte cell bodies (identified with SR101 *in vivo* or with S100 immunoreactivity in fixed tissue) were more numerous per unit of volume than their counterparts in mouse, through all cortical layers. Analysis of nearest neighbor distances revealed a non-random distribution of astrocyte locations, with a median distance of ~30 microns.VIn order to analyze the morphological characteristics of astrocyte territories, we performed *in vivo* targeted single-cell electroporation with large molecular weight fluorophores to enable high contrast imaging of the extent of astrocyte processes. We were able to image astrocyte morphology *in vivo*, as well as to recover the same astrocytes in fixed tissue for analysis by confocal microscopy. By comparing measurements between ferret and mouse visual cortex, we have found that ferret astrocytes are significantly bigger, more complex and that neighboring astrocytes overlap considerably more. In agreement with this observation, the mean distance of neighboring astrocytes is less than the size of astrocytes excluding the possibility of non-overlapping territories.VThe present study reinforces the growing attention to the diversity of astrocytes and calls into question the extrapolation of data from rodent hippocampus to other species and brain regions.

Disclosures: **M. Lopez-Hidalgo:** None. **W. Hoover:** None. **J. Schummers:** None.

Poster

511. Glial Physiology and Glia-Neuronal Physiology

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Topic: B.11. Glial Mechanisms

Support: NIH Grant R01-NS20212-21

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Title: Astrocytic gq-gpcr signaling contributes to classical conditioning

Authors: ***A. MADAYAG**, K. BOYT, R. LADD, K. D. MCCARTHY
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Abstract: Astrocytes are the most abundant cell type in the mammalian brain. These cells exist in close proximity to other cells of the central nervous system, including neurons. Our laboratory previously reported that astrocytes express Gq G-protein coupled receptors (GqGPCRs) that allow them to sense and respond to neuronal activity with intracellular Ca2+ fluxes. New data from our laboratory posits astrocyte GqGPCR signaling, including Ca2+ fluxes, as a significant regulator of classical conditioning such as fear conditioning, conditioned place preference, and conditioned place aversion. Previous studies have used pharmacological methods to determine astrocytic contributions to behavior. Unfortunately, this approach does not allow for specific targeting of astrocytes as the pharmacological approaches rely on compounds that can directly affect neuronal activity. Accurate investigation into the role of astrocytes in behavior requires the use of highly specific techniques that allow for selective perturbation of astrocyte signaling without direct effects on other cell types. Our laboratory has developed a mouse line that expresses Gq-linked Designer Receptor Exclusively Activated by Designer Drugs (Gq DREADD) in cells expressing glial fibrillary acidic protein (GFAP). With this model, we can activate Gq-GPCR signaling specifically in GFAP+ cells, primarily astrocytes, by injecting the otherwise inert compound clozapine-N-oxide (CNO). In a fear conditioning paradigm, we observe that activating astrocytic GqGPCR by CNO injection (0.5mg/Kg, IP) immediately after conditioning diminishes contextual, but not cue, freezing. Further, activating astrocytic GqGPCR signaling induces conditioned place aversion when the conditioned stimulus (CS+ chamber) is coupled to GqDREADD activation. Astrocytes are unique from other cells of the central nervous

system in part because they mobilize IP3-dependent Ca2+ stores by activating IP3 receptor type II (IP3R2). We sought to determine if mice lacking astrocytic IP3-dependent calcium responses (IP3R2 KO) exhibit deficits in classical conditioning. Indeed, we found that IP3R2 KO mice exhibit almost ablated conditioned place preference to cocaine as well as diminished contextual freezing to fear conditioning. Our findings suggest that intact astrocyte Gq GPCR signaling is essential to stabilizing behavioral output. Future studies should seek to determine mechanisms by which astrocyte Gq GPCR signaling regulates behavior as well as potential roles in neuropathology.

Disclosures: **A. Madayag:** None. **K. Boyt:** None. **R. Ladd:** None. **K.D. McCarthy:** None.

Poster

511. Glial Physiology and Glia-Neuronal Physiology

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Title: TAMRA-conjugated spermine is selectively taken up by astrocytes in the rodent hippocampus

Authors: J. BENEDIKT[1], A. ZAYAS-SANTIAGO[1], Y. RIVERA[1], M. INYUSHIN[1], Y. V. KUCHERYAVYKH[1], L. Y. KUCHERYAVYKH[1], L. A. CUBANO[1], M. J. EATON[1], R. W. VEH[3], *S. N. SKATCHKOV[2]
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Abstract: The polyamines (PAs) spermine (SPM) and spermidine (SPD) are endogenous molecules stored almost exclusively in astrocytes in the adult rodent brain, but not in neurons.

Because of the lack of biosynthetic enzymes for SPM and SPD in glia, we hypothesize that these PAs are taken up by a yet unknown mechanism. To visualize the uptake of SPM in rodent brain slices, we used SPM covalently tagged with fluorescent 5(6)-carboxytetramethylrhodamine (TAMRA). We perfused rat and mouse hippocampal slices with an extracellular solution containing 10 μM SPM-TAMRA and observed robust uptake of fluorescent SPM into astrocytes, but not into neurons or blood vessels. When 10 μM of unconjugated TAMRA was perfused, there was no visible staining of any type of cells in the hippocampus, suggesting the presence of a specific mechanism for SPM uptake. Pre-application of 20 μM Gd3+ did not show any effect on SPM-TAMRA uptake, thus ruling out a glial Cx43 hemichannels as an uptake pathway when calcium and magnesium concentrations were physiological. When 10 μM SPM-TAMRA was injected with the patch pipette into a single astrocyte in the stratum radiatum region of CA1 hippocampus, it rapidly diffused through the astroglial syncytium propagating through the CA1 pyramidal layer into the stratum oriens, and eventually staining hundreds of astrocytes in a 10 min time interval. The same experiments were performed with 10 μM unconjugated TAMRA as control and this resulted in a very low level of dye propagation (33 cells on average). In both cases, pre-application of 200 μM carbenoxolone (gap-junction blocker) effectively abolished the propagation of the fluorescent compounds, implicating a gap-junction mediated mechanism. Taken together, our data suggest that *in vitro*, SPM-TAMRA is specifically taken up by hippocampal astrocytes and rapidly propagated through their syncitium. Because PAs are substrates for organic cation transporters (OCTs) which take up many of the endogenous and synthetic PAs (Sala-Rabanal et al., 2013), using RT-PCR analysis we found OCT1 and OCT3 transporters in astrocytes cultured from rat brain and thus SPM may be taken up by OCTs .

Disclosures: **J. Benedikt:** None. **A. Zayas-Santiago:** None. **Y. Rivera:** None. **M. Inyushin:** None. **Y.V. Kucheryavykh:** None. **L.Y. Kucheryavykh:** None. **L.A. Cubano:** None. **M.J. Eaton:** None. **S.N. Skatchkov:** None. **R.W. Veh:** None.

Poster

511. Glial Physiology and Glia-Neuronal Physiology

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Program#/Poster#: 511.25/R4

Topic: B.11. Glial Mechanisms

Title: Astrocyte mediated synapse model for spike timing-dependent depression

Authors: Y. NISHIMURA[1], Y. KAKIMOTO[1], *O. ARAKI[2]
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Abstract: Recent findings have shown that neuronal activities are influenced by not only neuronal electrical activities but also astrocytic chemical signals. These findings insisted the effect of the astrocyte on variety of cognitive functions. Min and Nevian (2012) reported the contribution of the astrocyte to the memory function. In their study, it was observed that astrocytic calcium transients were induced by endocannabinoids which have been well known as essential material for induction of spike timing dependent LTD (t-LTD) and that disrupting calcium dynamics in astrocyte by calcium clamp inhibits the t-LTD. Based on their experimental results, they proposed a hypothesis of the synapse-astrocyte network for the t-LTD mechanism. In this model, post synaptic action potential leads calcium influx into the postsynaptic neuron. If this calcium influx is followed by a presynaptic action potential, PLC is activated in the postsynaptic neuron. The PLC leads to synthesis of endocannabinoids and these endocannabinoids act on astrocytic calcium transient. This in turn results in glutamate release from the astrocyte, which is thought to induce LTD. The aim of the present study is further investigation to the astrocyte mediated t-LTD by computational simulations. We proposed an astrocyte mediated synapse model constructed by two neurons model which represents dendritic calcium dynamics and emission of the neurotransmitter respectively, and an astrocyte model which represents astrocytic calcium dynamics and emission of the gliotransmitter. We also introduced the PLC activity as the input to the astrocyte. The calcium concentration influx in the postsynaptic neuron is represented by a differential equation, and the PLC activity is proportional to the calcium concentration defined by the temporal difference between postsynaptic and presynaptic action potentials in our model. Simulation results showed that if the postsynaptic action potential was followed by the presynaptic action potential, the PLC was activated and the gliotransmitter was emitted. The PLC activity and the emission of the gliotransmitter were large when the time interval was small. Additionally, they gradually decrease as the time interval increases. These results support that astrocytes are involved in the t-LTD mechanisms and suggest that the astrocytic t-LTD reported by the research of Min and Nevian might be correspondent to the standard STDP graph observed in Bi and Poo (1998).

Disclosures: Y. Nishimura: None. **Y. Kakimoto:** None. **O. Araki:** None.

Poster

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Topic: B.11. Glial Mechanisms

Support: University of Minnesota, Department of Neuroscience

Ministerio de Ciencia e Innovación, Spain (CSD2010-00045)

Title: Endocannabinoid signaling induces lateral long-term potentiation of transmitter release through stimulation of astrocytes

Authors: *A. COVELO[1], M. GOMEZ-GONZALO[2], M. NAVARRETE[2], G. PEREA[2], A. ARAQUE[1]
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Abstract: Endocannabinoids (ECBs) play key roles in brain function, acting as modulatory signals in synaptic transmission and plasticity. They are recognized as retrograde messengers that mediate long-term synaptic depression (LTD) through activation of presynaptic CB1Rs, but unable to induce the long-term potentiation (LTP) of synaptic transmission. Here we show that ECB signaling induces the long-term enhancement of synaptic transmitter release (eLTP) at single hippocampal synapses through stimulation of astrocytes when coincident with postsynaptic activity. This eLTP requires the coordinated activity of the three elements of the tripartite synapse: 1) ECB-evoked astrocyte calcium signal that stimulates glutamate release; 2) postsynaptic nitric oxide production; and 3) activation of presynaptic group I metabotropic glutamate receptors and protein kinase C. Evidence obtained by immunoelectron microscopy confirms the location of group I mGluRs at presynaptic sites. Therefore, while ECBs act as retrograde signals to depress homoneuronal synapses, they serve as lateral messengers to induce LTP in distant heteroneuronal synapses through stimulation of astrocytes. Present results show that endocannabinoids can trigger LTP of synaptic transmission through stimulation of astrocyte-neuron signaling, revealing novel cellular mechanisms of endocannabinoid effects on synaptic plasticity and brain function.

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Poster

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Topic: B.11. Glial Mechanisms

Support: University of Minnesota

MINECO (CSD2010-00045)

Cajal Blue Brain

Title: Column- and layer-specific heterosynaptic depression mediated by astrocytes in neocortex

Authors: A. DIEZ, *A. ARAQUE
Dept. Neuroscience, Univ. of Minnesota, Minneapolis, MN

Abstract: The existence of reciprocal communication between neurons and astrocytes has been demonstrated in different brain areas, including hippocampus, ventrobasal thalamus, cerebellum, retina, etc. However, the presence and properties of this bidirectional communication in the neocortex is poorly known. Moreover, whether functional astrocyte-neuron interactions are widely diffuse or spatially constrained remain unknown. This is especially relevant in the neocortex, where the structural arrangement of cells is highly organized in layers and columns, because the spatially diffuse or synapse-specific astrocytic regulation of synaptic transmission may have important consequences on cortical function. Using combined electrophysiological and calcium imaging techniques in cortical slices from wildtype and transgenic mice, we have investigated the spatial properties of the endocannabinoid-induced astrocyte signalling and their consequences on the spatial regulation of synaptic function by astrocytes in the primary somatosensory cortex. We performed paired recordings from either two layer 5 pyramidal neurons or two layer 4 neurons while recording excitatory synaptic currents evoked by electrical stimulation of layer 2/3. We have found that endocannabinoids (ecbs) released by depolarization of layer 5 pyramidal neurons transiently depressed synaptic transmission in homosynapses as well as heterosynapses in adjacent pyramidal neurons. While homosynaptic depression was directly mediated by presynaptic CB1R activation, heterosynaptic depression required astrocyte calcium elevations and was mediated by activation of presynaptic A1 receptors. Astrocyte-mediated heterosynaptic depression exclusively occurred between layer 5 pyramidal neurons located within the same cortical column but was absent in neurons belonging to adjacent columns. Moreover, heterosynaptic depression recorded between layer 5 pyramidal neurons was never observed between layer 4 neurons located within the same cortical column. These results indicate astrocytes stimulated by an endogenous signal (i.e., ecbs) regulate cortical synaptic transmission through gliotransmitter release and activation of presynaptic purinergic receptors. Furthermore, the astrocyte-mediated heterosynaptic depression is column- and layer-specific, suggesting that astrocytic modulation of cortical synaptic transmission is spatially controlled and synapse-specific, which may have important consequences on the astrocyte regulation of cortical

function. Supported by University of Minnesota, MINECO (CSD2010-00045), and Cajal Blue Brain. AD is a JAE-CSIC predoctoral fellow.

Disclosures: **A. Diez:** None. **A. Araque:** None.

Poster

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Program#/Poster#: 511.28/R7

Topic: B.11. Glial Mechanisms

Title: Regulation of glial Ca2+ signaling in *Drosophila* and its impact on neuronal activity and function

Authors: ***S. WEISS**[1], J. E. MELOM[2], T. J. LITTLETON[1]
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Abstract: Seizures are thought to involve reduced activity in inhibitory networks and increased activity in recurrent excitatory networks, often stemming from abnormalities in voltage-sensitive ion channels or GABAergic signaling. Large-scale screens for TS behavioral mutants to identify *Drosophila* temperature-sensitive (TS) paralytic mutations have been an important tool in the genetic dissection of membrane excitability, endocytosis and exocytosis. The goal of these screens is to generate thermolabile proteins or uncover thermosensitive processes important in neuronal signaling pathways that alter neuronal excitability when disrupted. Previous work in our lab identified Zydeco (Zyd), a glial Sodium-Calcium-Potassium exchanger (NCKX) which is involved in maintaining normal neural excitability in *Drosophila*. Mutation in zyd predisposes the flies to temperature-sensitive seizures. Zydeco was shown to be exclusively expressed in a specific glial subtype called cortex glia. Cortex glia exhibit spatial segregation reminiscent of mammalian astrocytes, with each glial cell ensheathing multiple neuronal soma. The basal intracellular calcium level in zyd mutants was shown to be elevated relative to wt, and near-membrane microdomain calcium oscillation, present in wt *Drosophila* glia, was abolished. Very little is known about the function of cortex glia in the mature nervous system. This work suggests that disruption of glial calcium regulation underlies seizure susceptibility in zyd1 mutants. To test the hypothesis that glial microdomain Ca2+ activity acutely regulates neuronal signaling, we used the zydeco mutation to dissect the mechanism(s) by which glia acutely communicate with neurons to regulate their firing properties. To probe the mechanism by which

altered glial Ca2+ regulation in zyd mutants affects neuronal excitability, we performed an RNAi screen for suppression or enhancement of zyd seizures. We knocked down, specifically in zyd1 glia, ~1000 candidate genes involved in vesicular trafficking and regulation, membrane receptors and secreted signaling ligands, participants in Ca2+ homeostasis and signaling pathways and genes that are enriched in *Drosophila* glia. This screen revealed several genes implicated in vesicle trafficking and in Ca2+ dependent cellular signaling as genetic suppressors, and components of the store operated Ca2+ entry (SOCE) pathway and genes implicated in cellular Ca2+ regulation as genetic enhancers of the zydeco TS phenotype.

Disclosures: S. Weiss: None. **J.E. Melom:** None. **T.J. Littleton:** None.

Poster

511. Glial Physiology and Glia-Neuronal Physiology

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Topic: B.11. Glial Mechanisms

Support: PROMEP 103.5/10/7324

UASLP-FAI C10-FAI-05-55.84

Title: Remodeling of Bergmann glia actin cytoskeleton during glutamatergic neurotransmission in the cerebellum

Authors: *R. R. HERNANDEZ, Y. BASTIÁN, J. MENDEZ
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Abstract: The cerebellum participates, among other things, in fine motor control, motor learning and vestibular function through adaptive synaptic plasticity. The cerebelar functions are coded by the electrical activity of Purkinje cells. These neurons integrate the information received via synapses established with the parallel and climbing fibers within the cerebelar cortex. These incoming synapses are finely wrapped by the lamellar processes of the Bergmann glial cells (BGC). Given that BGC also express ionotropic glutamatergic receptors, during cerebelar glutamatergic neurotransmission BGC also becomes depolarized. Strikingly, inhibition of calcium entry trough AMPA receptors into the BGC, induces a retraction of Bergman glia lamellar processes. This causes glutamate to stay longer within the synaptic cleft and the synchronization of cerebelar glutamate neurotransmission is lost. Therefore, paradoxically the

correct function of Purkinje neuronal transmission depends on the correct functioning of AMPA receptors located onto the Bergman glia. RhoA is a small GTPase protein that regulates changes in the structure of cytoskeleton through the modulation of cofilin. RhoA is highly expressed in BGC and is activated after stimulation of Bergmann glial AMPA receptors. With the goal of determining the signal pathways that modulates the dynamics of actin cytoskeleton in Bergman glia. We stimulated coronal mouse cerebelar slices using 200 μM glutamate. We found that the levels of active RhoA increases after 10 minutes of glutamate treatment. The activation of RhoA was induced by GTP-γ-S as well as prevented with Inhibitor I, a RhoA inhibitor. Moreover, using a fluorescent-labeled Phalloidin we were able to observe changes in both the organization and level of actin polymerization. These results show that the activation of RhoA plays a role in the modulation of actin cytoskeleton in Bergmann glia. We suggest that activation of RhoA depends on PKC, PI-3K or FAK, which become active after AMPA receptor are stimulated in Bergmann glia cells.

Disclosures: R.R. Hernandez: None. **Y. Bastián:** None. **J. Mendez:** None.

Poster

511. Glial Physiology and Glia-Neuronal Physiology

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Topic: B.11. Glial Mechanisms

Support: MH078823

MH099658

T32NS073547

Title: Local astrocyte influences on axonal properties and transmitter synchrony of hippocampal pyramidal neurons

Authors: *C. A. SOBIESKI, X. JIANG, S. MENNERICK
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Abstract: The role of astrocytes in supporting neurotransmission has been extensively characterized at the synaptic level, but less is known about astrocytes' role in upstream events involved in neurotransmission. Furthermore, while global astrocyte factors have been emphasized, much less is known about local astrocytic roles. Recently our lab has uncovered a

novel role of astrocytes in axonal function. We observe large-scale (supraquantal), stereotyped asynchronous glutamate release in autaptic hippocampal pyramidal neurons when local astrocytic support is removed, but no change in GABA release (Sobieski et al., 2013). Here we investigate axonal differences in glutamatergic neurons void of local astrocytic support (-astrocyte neurons) compared to their astrocyte-rich counterparts (+astrocyte neurons) grown nearby that may explain this effect. Immunolabeling for sodium channels and for ankyrin-G showed no difference in the number, length, or intensity of labeling of the axon initial segment between +astrocyte and -astrocyte glutamatergic neurons. Total axon area and labeling intensity, measured by the axonal neurofilament marker SMI312, also did not differ. Because we detected no difference in axonal structural properties and previously detected only subtle differences in somatic excitability, we hypothesized differences in axon excitability. Specifically, we explored differences in Kv7 channels and Kv1 channels, known to be selectively localized to axons of principal neurons. +Astrocyte EPSCs were significantly more sensitive to XE991 (10 μM), a Kv7 antagonist, than -astrocyte EPSCs (17.2 ± 5.6% increase in peak EPSC vs. 2.0 ± 1.6% increase; $p < 0.03$, $n = 6, 8$). Consistent with a deficit in Kv7-like channels, the rheobase of -astrocyte glutamatergic neurons was slightly lower than the rheobase of +astrocyte controls (36.6 ± 5.2 pA vs. 57.4 ± 8.5 pA; $p < 0.05$, $n=10, 9$). By contrast both +astrocyte and -astrocyte neurons exhibited similar EPSC potentiation by another axonal K+ channel (Kv1) inhibitor, dendrotoxin (100 nM, 16.1 ± 6.0 % and 10.4 ± 3.9% increases, respectively, $p=0.44$). We conclude that axonal Kv7 deficits may participate in large-scale asynchrony of glutamate release in the absence of local astrocyte support.

Disclosures: **C.A. Sobieski:** None. **X. Jiang:** None. **S. Mennerick:** None.

Poster

512. Parkinson's Disease: Imaging, Dyskinesia, and Development

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Topic: C.03. Parkinson’s Disease

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Michael J. Fox Foundation

Title: Neuroimaging analysis of the dopamine basis for apathetic behaviors in an MPTP-lesioned primate model

Authors: *L. TIAN, Y. XIA, H. FLORES, M. CAMPBELL, S. MOERLEIN, J. PERLMUTTER
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Abstract: Apathy commonly occurs in Parkinson disease (PD) patients; however, the role of dopamine in the pathophysiology of apathy remains elusive. We previously demonstrated that dopaminergic dysfunction within the ventral tegmental area (VTA)-nucleus accumbens (NAcc) pathway contributes to the manifestation of apathetic behaviors in monkeys treated with the selective dopaminergic neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). In the present study, we identified dysfunction of other dopaminergic pathways that correlate with development of apathetic behaviors. Specifically, we measured the effects of MPTP on monkeys' willingness to attempt goal directed behaviors, which is distinct from their ability to perform tasks. A total of 16 monkeys had magnetic resonance imaging (MRI) and baseline positron emission tomography (PET), using 6-[18F]fluorodopa (FD), [11C]dihydrotetrabenazine (DTBZ), and 2β-[11C]carbomethoxy-3β-(4-fluorophenyl)tropane (CFT). The monkeys received unilateral infusion of different doses of MPTP (0 - 0.31mg/kg), which produced stable hemiparkisonism by 3 weeks. After 8 weeks, PET scans were repeated and animals were euthanized. Apathetic behavior and motor impairments were assessed both pre- and post- MPTP infusion. Apathy scores were compared to motor scores, *in vitro* and *in vivo* dopaminergic measures. Apathy scores increased following MPTP. Apathy scores correlated with the non-displaceable binding potential (BPND) of DTBZ ($r_s = -0.51$, $p < 0.05$) and CFT BPND ($r_s = -0.67$, $p < 0.005$) in the dorsal lateral prefrontal cortex (DLPFC), with CFT BPND ($r_s = -0.73$, $p < 0.005$) in the ventromedial prefrontal cortex (VMPFC), and with DTBZ BPND ($r_s = -0.76$, $p < 0.005$), CFT BPND ($r_s = -0.80$, $p < 0.005$) in the insular cortex (IC), but did not correlate for any tracer in the anterior cingulate cortex (ACC) or the posterior cingulate cortex (PCC). Among all the cortical regions assessed, forward step-wise regression analyses indicated that only VTA cell count predicts IC CFT uptake better than neuropathological changes in the mesocortical network. Our findings suggest that dopaminergic dysfunction within the VTA-IC pathway plays a role in the manifestation of apathetic behaviors in MPTP-lesioned primates.

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Poster

512. Parkinson's Disease: Imaging, Dyskinesia, and Development

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 512.02/R11

Topic: C.03. Parkinson's Disease

Title: *In vivo* MRI reveals early structural changes in a mouse model of α-synucleinopathy

Authors: *K. DEDUCK[1], M. DESCOTEAUX[2], K. C. LUK[3], B. J. BEDELL[1]
[1]Montreal Neurolog. Institute/McGill Univ., Montreal, QC, Canada; [2]Sherbrooke Univ., Sherbrooke, QC, Canada; [3]Dept. of Pathology and Lab. Med., Univ. of Pennsylvania Perelman Sch. of Med., Philadelphia, PA

Abstract: Introduction: Mutant A53T α-synuclein (α-syn) has been linked to familial forms of Parkinson's disease. Mice overexpressing human A53T α-syn under the mouse prion promoter (line M83) develop motor impairment and Lewy-body-like inclusions. While these behavioral and pathological alterations typically do not develop before 6 months of age, it is possible that other alterations may occur earlier. As such, the objective of this study was to use magnetic resonance imaging (MRI) to investigate differences in cortical thickness, regional volume, and white matter microstructure in young M83 mice. Methods: *In vivo* MRI scans were acquired from three month-old, male, homozygous M83 mice (TG) and wild-type littermates (WT). Anatomical scans (N = 9 TG, 7 WT) were acquired using a 3D balanced Steady-State Free Precession (bSSFP) sequence and diffusion tensor imaging (DTI) scans (N = 8 TG, 5 WT) were acquired using a customized pulse sequence. Regional volumes, cortical thickness, and DTI parametric measures (fractional anisotropy (FA) and mean/axial/radial diffusivity) were generated using a fully automated processing pipeline. Results: Regional cortical thickness was significantly reduced in TG mice in most regions, compared to WT mice, with the greatest reductions in auditory cortex, insula, and posterior cingulate cortex ($p \leq 0.005$). Reduced volumes were also observed in several cortical regions, including frontal cortex, insula, and visual cortex ($p \leq 0.05$). Notably, motor cortex was the only cortical region with increased volume in TG mice compared to WT ($p = 0.009$). TG mice also showed significantly increased volumes in the lateral ventricles and brainstem ($p \leq 0.03$). Diffusion imaging revealed significantly reduced FA in TG mice ($p = 0.04$) in the anterior portion of the corpus callosum. Conclusion: The increased lateral ventricular volume, decreased cortical thickness and volume, and reduced FA in anterior corpus callosum indicate cortical atrophy months prior to development of previously reported pathology. Interestingly, the brainstem volume was increased, and this is an area known to develop dense Lewy-body-like pathology in older M83 mice. This early increase in volume may be reflective of neuronal hypertrophy and/or gliosis. Correlative studies with quantitative immunohistochemistry (qIHC) are currently underway to investigate the cellular changes underlying these MRI-defined macrostructural alterations.

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Poster

512. Parkinson's Disease: Imaging, Dyskinesia, and Development

Location: Halls A-C

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Program#/Poster#: 512.03/R12

Topic: C.03. Parkinson's Disease

Title: Voxel-based morphometry, resting-state functional connectivity MRI and histological analysis for evaluating structural and functional changes in a rat model of Parkinson's disease

Authors: ***R. WESTPHAL**[1], C. SIMMONS[1], T. C. WOOD[1], M. B. MESQUITA[1], R. JOULES[1], S. C. R. WILLIAMS[1], A. VERNON[2], D. CASH[1]
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Abstract: Introduction The neurotoxin 6-hydroxydopamine (6OHDA), when administered into the medial forebrain bundle (mfb) of rats, damages the dopaminergic nigrostriatal pathway. This models parkinsonian features such as reduced dopamine in the striatum1. The 6OHDA rat has been widely used as a test-bed for many therapeutic agents. The aim of this study was to further evaluate the 6OHDA rat in terms of structural and functional brain abnormalities in order to identify new biomarkers and targets for therapeutic interventions. Methods & Results Three weeks after unilateral 6OHDA lesioning of the mfb, male adult Sprague-Dawley rats (n=12-16 per group, full lesions were confirmed by apomorphine induced rotations) were imaged *in vivo* by structural (T2 weighted fast spin echo, resolution 0.25x0.125x0.5mm) and functional (gradient echo EPI TR=1s, resolution = 0.5x0.5x1mm) MRI. Structural images were analyzed using automated unbiased voxel-based morphometry2. Resulting statistical parametric maps revealed significant structural differences between the lesioned and sham groups. In particular, there was a reduction of grey matter volume ($p<0.05$, FDR corrected) in the ipsilateral (lesioned) hemisphere in the motor, sensorimotor, cingulate and temporal cortices. Grey matter volume loss was also present in the ipsilateral dorsal striatum and the substantia nigra ($p<0.01$, uncorrected). Functional images were analysed for resting-state connectivity using graph theoretical analysis and pattern recognition technique3. This demonstrated decreased connectivity between the ipsilateral motor and sensory cortex (including S1, S1 UlP and S2) with the contralateral parts of the brain. Dopaminergic neuronal loss and denervation was confirmed post-mortem by tyrosine

hydroxylase immunohistochemistry in the nigrostriatal and cortical areas as highlighted by MRI analysis. Conclusions Studies to identify neuroprotective interventions in Parkinson's disease have been hampered by the lack of clinically relevant animal models and the difficulty to detect brain pathology *in vivo*. This work demonstrates structural and functional brain changes on a topographic scale beyond the nigrostriatal tract in the 6OHDA rat by means of non-invasive and clinically relevant MRI protocols in conjunction with behaviour and histological methods. This highlights the beneficial use of the latest multiparametric MRI in animal models that allows the translational comparison between preclinical and clinical imaging and improves the predictive validity of the 6OHDA model. References 1. Duty et al, 2011, Br J Pharmacol, 2. Suzuki et al, 2013, Neuroimage, 3. Richiardi et al, 2012, Neuroimage.

Disclosures: **R. Westphal:** None. **R. Joules:** None. **C. Simmons:** None. **T.C. Wood:** None. **M.B. Mesquita:** None. **S.C.R. Williams:** None. **A. Vernon:** None. **D. Cash:** None.

Poster

512. Parkinson's Disease: Imaging, Dyskinesia, and Development

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 512.04/S1

Topic: C.03. Parkinson's Disease

Support: Utah Science Technology and Research Initiative Startup Funds

Title: Information theoretic metrics as biomarkers of parkinsonian symptom severity

Authors: ***C. ANDERSON**, A. DORVAL
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Abstract: Deep Brain Stimulation (DBS) is a late-stage surgical intervention used to treat the symptoms of Parkinson's Disease (PD) once the therapeutic window of dopamine replacement has closed. Further probing of the mechanisms of DBS, and how stimulation modifies neural biomarkers of symptom severity, may lead to improved treatments for parkinsonism. Typical biomarkers studied - dopamine concentration, neuronal firing rates, neural coherence, etc - do not correlate strongly with symptom severity. We propose that information theoretic measures, including neuronal entropy and directed information, could serve as robust biomarkers for PD symptom severity. In this work, we use a 6-OHDA rodent model of PD to quantify spike train entropy and directed information within and across the substantia nigra reticulata (SNr) and ventral anterior thalamus (VA), as a function of symptom severity. Long Evans rats were

implanted with microwire recording arrays in the SNr and VA, in addition to microelectrode stimulating arrays in the subthalamic nucleus (STN). Under healthy, hemi-parkinsonian (via 6-OHDA injection to the medial forebrain bundle), and DBS conditions, unit activity and local field potentials were recorded simultaneously in the SNr and VA using a multichannel neural recording system. Symptom severity was measured using free-rotation tasks. Inter-spike intervals were binned logarithmically, entropy was computed for each unit in each condition, and mutual information between all pairs of simultaneously recorded neurons was determined. Spike train entropy in the SNr and VA covaried with symptom severity, while mutual information across all pair types decreased with increased symptom severity. Qualitatively, entropy increases in the PD condition were seen through an increase in bursting, while decreases in the DBS condition follow from unit regularization as the neurons phase lock to stimulation. Information may decrease in the PD condition due to cells being less responsive to inputs during sustained burst-like behavior. Information may increase due to regularization provided by DBS, restoring neuronal ability to respond to synaptic inputs. As the brain shifts from healthy to parkinsonian, neural units in the basal ganglia become more disordered, generating activity that drives symptoms by masking signals that drive healthy behavior. Information transmitted between neurons decreases, making individual neural unit activity more independent. This independence reflects a decrease in transmission of signals from basal ganglia to thalamus that drive healthy behavior, resulting in hypokinetic symptoms of PD, such as akinesia, bradykinesia, rigidity, etc.

Disclosures: C. Anderson: None. **A. Dorval:** None.

Poster

512. Parkinson's Disease: Imaging, Dyskinesia, and Development

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 512.05/S2

Topic: C.03. Parkinson’s Disease

Support: JSPS

Title: Cineradiographic analysis of respiratory function in a murine model mimicking the initial stages of Parkinson’s Disease

Authors: P. S. DE CAMPOS[1], K. HASEGAWA[2], Y. KUMEI[3], *J. L. ZEREDO[1]
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Abstract: Parkinson's disease (PD) is known to cause respiratory alterations including shortening of operational volumes and reduced velocity of respiratory-muscle contraction. It has been proposed that such changes are secondary changes in posture and osteoarticular degeneration, leading into an alteration in the spinal axis that in turn could affect breathing mechanics. In this study, we aimed at testing the hypothesis that respiratory symptoms are associated with primary postural changes in the initial stages of PD.We employed a murine model of a mild hemi-Parkinson's Disease. C57BL/6J mice (n=23) were used. Under surgical anesthesia, PD mice received an injection of 6-OHDA solution (10 µg 6-OHDA in 0.9% NaCl with 0.02% ascorbic acid, infused at 0.5 µl/min) to the left nigro-striatal pathway though a stereotaxically driven microsyringe. These were compared to control mice, which received an injection of saline under the same conditions. Two weeks after surgery, all mice had their respiratory movements recorded by video x-rays on two incidences (lateral and horizontal) without restraint. Conventional behavioral tests were performed to assess the severity of the 6-OHDA lesion (pole test, cylinder test, and nest-building test). As a result, behavioral tests confirmed mild motor impairments in PD mice as compared to controls. Likewise, parameters of respiratory function (diaphragm displacement and ribcage volume) showed mild alterations in the PD group. These results suggest that respiratory alterations in PD may emerge simultaneously to other motor symptoms, and not as a consequence of the latter. Supported by grants from JSPS (Japan) to KH, YK, and JLZ.

Disclosures: P.S. de Campos: None. **J.L. Zeredo:** None. **K. Hasegawa:** None. **Y. Kumei:** None.

Poster

512. Parkinson's Disease: Imaging, Dyskinesia, and Development

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

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Topic: C.03. Parkinson's Disease

Support: FAPESP

CNPq

CAPES (Brazil)

Title: Transient potencial, kinin and purinergic receptors in Parkinson's disease

Authors: *L. M. DATI, A. S. ALVES, A. H. ULRICH, L. R. G. BRITTO
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Abstract: Parkinson's disease (PD) is a progressive neurological disorder characterized by motor and non-motor deficits. Although there are a lot of studies about the mechanisms of Parkinson's disease, the involvement of purinergic, kinin and transient potential receptors (TRP) is still unknown. Here we studied the expression of those receptors in a Parkinson's disease model. Fifty C57BL/6 male mice aged 90 days were stereotaxically injected in to the right striatum (CPu) with 6-hydroxydopamine (6-OHDA) (Parkinson's disease - PD) while the left striatum (CPu) received saline injections (Control). After 15 days the animals were sacrificed and brains were dissected or perfused. The levels of tyrosine hydroxylase (TH), kinin receptors (B2 receptor), purinergic receptors (P2X2, P2X4, P2X7, P2Y1, P2Y4) and TRP channels (TRPM7) were determined in the CPu and substantia nigra (SN) from PD and controls by using both immunohistochemistry and immunoblotting. Data are presented as means ± SEM. The Tukey test was used to compare results from control and PD, with significance set at $p<0.05$. In both SN and CPu we found a 50% decrease in the levels of TH ($p<0.05$) in PD compared to control. The levels of TRPM7 increased around 60% ($p<0.05$) in the SN while in the CPu we observed a significant 80% increase for this receptor. The purinergic receptor P2X2 was increased into SN by about 100% ($p<0.001$); however, no difference was observed in the CPu. Higher levels of P2X4 were found in both SN and CPu (20% and 30%, respectively) in PD brains. The P2X7 receptor levels increased by 45% in the SN of PD ($p<0.05$), but no changes were observed in CPu. There was no difference in the levels of P2Y1 receptor in SN between the groups; however, we observed a 65% ($p<0.05$) increase of this receptor in the CPu. The P2Y4 receptor showed an increase around 230% in the SN of DP group, but no differences were found in the CPu. Finally, the B2 receptor increased by 72% in the SN, but no changes were seen in the CPu of the PD group. The results of this study show that kinin, purinergic and TRP receptors may all be involved in PD, but further studies about their brain localization and exact roles are necessary to better clarify this issue.

Disclosures: L.M. Dati: None. **A.S. Alves:** None. **A.H. Ulrich:** None. **L.R.G. Britto:** None.

Poster

512. Parkinson's Disease: Imaging, Dyskinesia, and Development

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 512.07/S4

Topic: C.03. Parkinson's Disease

Title: A pathophysiological role of aquaporin-9 in parkinson's disease

Authors: K. STAHL, A. PRYDZ, T. B. LEERGAARD, *O. OTTERSEN, M. AMIRY-MOGHADDAM
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Abstract: Parkinson's disease is a relatively new disease, discovered as late as in 1817. It has been found to be clearly associated with environmental toxins, which lead to a selective degeneration of dopaminergic cells in the substantia nigra (SN). To date, however, the factors making these neurons selectively vulnerable are unknown. Aquaporin-9 (AQP9) is an aquaglyceroporin with particularly broad substance permeability, as it is permeable to water, glycerol, monocarboxylates and arsenite, among others (Torres-Avila et al., 2009). The channel is expressed in several tissues, including liver, testis and brain. In mouse brain, AQP9 is selectively expressed on catecholaminergic neurons in the SN, where it is localized both on plasma membranes and inner mitochondrial membranes (Amiry-Moghaddam et al., 2005, Mylonakou et al., 2009). This selective localization together with the broad substance permeability suggest that AQP9 may serve as a gateway for exogenous compounds to enter dopaminergic neurons in the SN. Based on this assumption, we hypothesize that the selective vulnerability of dopaminergic neurons to certain toxins could be attributed to AQP9, implying that this channel may play a role in the pathophysiology of Parkinson's disease. In this study, we investigated whether targeted deletion of the AQP9 gene interferes with 1-methyl-4-pyridinium ion (MPP+) toxicity using stereotaxic surgery. The toxin was infused unilaterally into the striatum of AQP9-/- animals and WT littermates, followed by several behavioral assessments. Following the lesion,all animals displayed a distinct ipsilateral rotational behavior, indicating a disruption of the motoric system. When quantified by means of apomorphine rotation test and cylinder test, AQP9-/- animals showed significantly less rotational behavior and ipsilateral paw preference, respectively, than WT littermates, indicating less motoric disruption in these animals. After seven days, animals were sacrificed by cardiac perfusion with 4 % paraformaldehyde, the brains were dissected out, and the midbrain was stained with antibodies against tyrosine hydroxylase (TH). We are currently using stereological cell counting to quantify whether these behavioral patterns are due to a reduced TH+ cell loss in AQP9-/- animals.

Disclosures: K. Stahl: None. **O. Ottersen:** None. **A. Prydz:** None. **T.B. Leergaard:** None. **M. Amiry-Moghaddam:** None.

Poster

512. Parkinson's Disease: Imaging, Dyskinesia, and Development

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 512.08/S5

Topic: C.03. Parkinson's Disease

Support: MJFF

Title: Impaired dopaminergic neurotransmission and vesicular recycling in human LRRK2-R1441G transgenic mice

Authors: L. LI[1], S. CHOI[2], P. ROY[3], J. J. BALCITA-PEDICINO[3], Y. HUANG[4], C. LI[4], S. R. SESACK[3], *H. ZHANG[1]
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Abstract: Emerging evidence suggests that synaptic dysfunction is an early event in the pathogenesis of Parkinson disease (PD) occurring prior to the onset of symptoms. In order to develop more effective therapeutic strategies, we need a better understanding of the underlying mechanisms of synaptic dysfunction of PD. Mutations in the leucine-rich repeat kinase 2 (LRRK2) gene are the most prevalent causes of familial and sporadic PD, demonstrating an unprecedented significant role in PD pathogenesis. Recently a transgenic (TG) mouse model with over-expression of human LRRK2-R1441G has been shown to recapitulate the robust motor behavioral, neurochemical and pathological features of PD (Li et al., 2009). In this study, we used a battery of tests to characterize evoked dopamine release by fast cyclic voltammetry and amperometry in acute corticostriatal slices of 3, 5 and 10 month old transgenic R1441G-LRRK2 mice and their wild type (WT) littermates. We found age-dependent deficits in dopamine release in the striatum in this model. Specifically, we found that vesicle trafficking and recycling in hLRRK2-R1441G TG mice is slower compared to that of WT and that this deficit starts at 5 months of age prior to the onset of behavior deficits. To determine whether the LRRK2-R1441G mutation resulted in changes to vesicle distribution and density within dopamine axon terminals, we also examined 6 pairs of the 10 month group by electron microscopy in the dorsolateral striatum. To date, no changes have been observed in the mean vesicle density nor in the mean distance of vesicles to the synapse between WT and TG animals. There were, however, some emerging trends for alterations in minimum distance to the synapse and maximal vesicle density. While the data support the conclusion that LRRK2 plays a key role in regulating dopamine release and recycling, further investigations of the underlying mechanisms and how this dysfunction leads to axonal degeneration are ongoing (Supported by MJFF).

Disclosures: L. Li: None. **H. Zhang:** None. **S. Choi:** None. **P. Roy:** None. **J.J. Balcita-Pedicino:** None. **Y. Huang:** None. **C. Li:** None. **S.R. Sesack:** None.

Poster

512. Parkinson's Disease: Imaging, Dyskinesia, and Development

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Topic: C.03. Parkinson's Disease

Support: NRF-2012R1A1A1012435

SBRI, SMX1132521

Title: The biguanide metformin alters phosphoproteomic profiling in mouse brain

Authors: *R. KHANG[1], C. PARK[2], J.-H. SHIN[1]
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Abstract: Metformin, a potent antihyperglycaemic agent is recommended as the first-line oral therapy for type2 diabetes (T2D). Recently, metformin has been reported to be beneficial to neurodegenerative disease models. However, the putative mechanisms underlying the neuroprotective effects of metformin in disease models are unknown. Thus, we applied LC-MS/MS-based pattern analysis and two-dimensional electrophoresis (2DE) -based proteomic approach to understand the global phosphoproteomic alteration in the brain of metformin-administrated mice. Collectively, pattern analysis reveals that 41 phosphoproteins were upregulated and 22 phosphoproteins were downregulated in the brain of metformin-administrated mice. In addition, 5 differentially expressed phosphoproteins were identified upon metformin administration by 2DE coupled with mass spectrometry. The phosphorylation status of metabolic enzymes was decreased while that of mitochondrial proteins was increased by metformin. Taken together, our results might shed light on understanding the pharmacological effect of metformin on brain function.

Disclosures: R. Khang: None. **C. Park:** None. **J. Shin:** None.

Poster

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Topic: C.03. Parkinson's Disease

Support: Boehringer Ingelheim Ulm University BioCenter (BIU) N2

International Graduate School in Molecular Medicine Ulm (IGradU)

Title: Early changes in the endocannabinoid system after 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) treatment in mice

Authors: *N. PASQUARELLI[1,2], C. PORAZIK[1,2], P. WEYDT[1], A. WITTING[1], B. FERGER[2]
[1]Dept. of Neurol., Ulm Univ., Ulm, Germany; [2]CNS Dis. Res., Boehringer Ingelheim Pharma GmbH & Co. KG, Biberach/Riß, Germany

Abstract: The modulation of the brain endocannabinoid system has been identified as an option to treat neurodegenerative diseases including Parkinson's disease (PD). Here, we investigate early changes in the endocannabinoid system preceding neuron loss of tyrosine hydroxylase positive neurons in the substantia nigra using the dopaminergic neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) mouse model of PD. Male adult C57BL/6JRj mice were treated with MPTP (2 x 20 mg/kg MPTP in a 4-hour interval). After one day, changes in striatal gene expression of the cannabinoid receptors (CB_1, CB_2), the endocannabinoid enzymes monoacylglycerol lipase (MAGL) and fatty acid amide hydrolase (FAAH) were determined using qPCR. Additionally, striatal gene expression of tumor necrosis factor α (TNFα) and interleukin 6 (IL6) was quantified. The principal endocannabinoid signaling molecules 2-arachidonoylglycerol (2-AG) and anandamide as well as the endocannabinoid metabolite arachidonic acid were measured by liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). Monoamine neurotransmitter and metabolite levels were determined using HPLC in combination with electrochemical detection. MPTP treatment led to a slight decrease in CB_1 receptor expression and to a pronounced increase in CB_2 receptor expression whereas MAGL and FAAH expression as well as 2-AG, anandamide and arachidonic acid levels were not affected by MPTP treatment. TNFα expression was substantially increased. In contrast, IL6 expression was not affected after MPTP treatment. As expected, MPTP treatment induced a significant decrease in striatal dopamine and dopamine metabolites without affecting serotonin. In summary, our data show that administration of MPTP in mice leads to diverse early changes in parts of the endocannabinoid system and warrants further investigation at later time points.

Disclosures: N. Pasquarelli: None. **C. Porazik:** None. **P. Weydt:** None. **A. Witting:** None. **B. Ferger:** A. Employment/Salary (full or part-time):; Boehringer Ingelheim GmbH & Co. KG.

Poster

512. Parkinson's Disease: Imaging, Dyskinesia, and Development

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Topic: C.03. Parkinson's Disease

Support: PAPIIT IT200813

ICyTDF PICSA12-124

Title: Dihydrotetrabenazine for the assessment of dopaminergic innervation in animal models of Parkinson's disease

Authors: *P. VERGARA-ARAGON[1,2], G. VALVERDE-AGUILAR[5], R. GONZÁLEZ-RIVERA[3], I. E. LÓPEZ-MARTÍNEZ[4], I. SANCHEZ-CERVANTES[4], B. HERNÁNDEZ-TELLEZ[4]
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Abstract: Parkinson's disease (PD) is characterized by the loss of dopamine-producing neurons in the nigrostriatal system. Numerous researchers in the past have attempted to track the progression of dopaminergic depletion in PD. We applied a quantitative non-invasive PET imaging technique to follow this degeneration process in a 6-OHDA-induced rat model of PD. The VMAT ligand DTBZ was used as a radioactive tracer in our imaging experiments to monitor the changes of the dopaminergic system. Intracerebral (caudate nucleus) administration of neurotoxin was delivered to rat to induce hemiparkinsonism. Our results indicate a significant decline in the levels of striatal dopamine. Images obtained by positron emission tomography revealed uptake of DTBZ in the rat striatum. However, reduction in radioligand was evident in the striatum of hemiparkinsonism rats as compared with the control group. Immunohistochemical analysis further confirmed PET imaging results and indicated the progressive loss of dopaminergic neurons in treated animals compared with the control counterparts. Our findings suggest that 6-OHDA model is appropriate to follow the degeneration of dopaminergic system.

Disclosures: P. Vergara-Aragon: None. **G. Valverde-Aguilar:** None. **R. González-Rivera:** None. **I.E. López-Martínez:** None. **I. Sanchez-Cervantes:** None. **B. Hernández-Tellez:** None.

Poster

512. Parkinson's Disease: Imaging, Dyskinesia, and Development

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The Parkinson's Disease Foundation

The Parkinson's Alliance

Title: Ultrastructural localization of Myr-Akt in the nigro-striatal pathway, *in vivo*, following AAV transduction of neurons of the substantia nigra (SN)

Authors: *A.-S. ROLLAND, A. TAGLIAFERRO, T. KAREVA, N. KHOLODILOV, R. E. BURKE
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Abstract: We have observed that increased Akt/mTor signaling in dopamine neurons of the substantia nigra (SN), achieved by transduction with AAV myristoylated-Akt (Myr-Akt), a constitutively active mutant, induces axon re-growth weeks after their destruction by neurotoxin (Kim et al, 2011). While the myristoylation tag has been believed to target Akt to the inner plasma membrane, and this has been confirmed *in vitro*, the subcellular distribution of the mutant Myr-Akt transgene *in vivo* is unknown. To examine its ultrastructural localization, we fused Myr-Akt with either Flag or GFP as a tag and transduced SN neurons by intra-nigral injection. We then performed electron microscopy on the nigro-striatal pathway, e.g. the SN, the medial forebrain bundle (MFB) and the striatum, by immunoperoxidase or gold particle detection. In the neuronal soma we confirmed the presence of Myr-Akt at the plasma membrane, as reported *in vitro*. However, Myr-Akt was also found to be associated with other structures, including the outer membrane of mitochondria, the Golgi apparatus and associated vesicles, the endoplasmic reticulum and with cytoskeleton. We also detected Myr-Akt throughout the nucleus. In dendrites, Myr-Akt was observed to be attached to the cytoskeleton and mitochondria. Myr-Akt was also identified outside the neuronal soma, in the axons of the MFB as well as in the axon terminals in the striatum. We conclude that *in vivo* Myr-Akt localizes not just to the inner surface of the plasma membrane, as emphasized in *in vitro* studies, but also extensively to discrete intracellular organelles. Their identification will shed light on the mechanisms by which Myr-Akt induces axon growth.

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Poster

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Topic: C.03. Parkinson's Disease

Title: Different involvement of opioid receptors in motor control and levodopa induced dyskinesia

Authors: *S. SGROI[1,2], C. CAPPER-LOUP[1], A. KAELIN-LANG[3,4]
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Abstract: Locomotor disorders like bradykinesia are a hallmark of Parkinson's disease (PD). Levodopa (L-DOPA) remains the most effective treatment for PD symptoms although chronic L-DOPA treatment is responsible for the development of motor fluctuations ("ON/OFF" phenomena) and dyskinesia (called "abnormal involuntary movements" AIMs in animals). Opioidergic transmission is involved in basal ganglia function and is mediated by striatal endogenous peptides such as enkephalin (ENK) and dynorphin (DYN) acting through several opioid receptors (μ, δ, κ). Changes in ENK and DYN expression have been described in both untreated and L-DOPA treated PD animals but the role of the striatal opiodergic system in PD symptoms is largely unknown. Based on our previous studies, we hypothesized here that changes in the expression of opioid receptors and in the ENK and DYN mRNA levels are specifically linked with secondary compensatory mechanisms leading to changes in spontaneous motor activity and to the development of dyskinesia in PD rats under L-DOPA treatment. The purpose of this study was thus to investigate whether the opioid receptors expression and the ENK and DYN mRNA levels are correlated with the locomotor activity or the severity of dyskinetic movements. Immunohistochemistry for the anti-μ and anti-δ opioid receptors and *in situ* hybridization analysis were performed in three groups of PD rats, treated with chronic injections of L-DOPA 8 mg/kg, 6 mg/kg and Saline, respectively. The motor activity of all groups was evaluated by an "Open Field test" at several phases of L-DOPA treatment. The rats were also rated for AIMs. The immunohistochemical analysis showed a higher localization of μ receptors

in the striosomes of striatum, compared to a homogeneous distribution of δ receptors in the matrix. All PD rats showed a reduction of μ opioid receptors density in the striosomes of the lesioned striatum, compared to the not lesioned side. The expression of μ receptors was correlated to the motor symptoms, while the expression of δ receptors was correlated to the dyskinetic movements. Differences was also found in the levels of DYN and ENK mRNA between lesioned and not lesioned side, together with a different correlation with motor activity and dyskinetic movements, respectively. We conclude that the locomotor responses and dyskinesia are differently modulated by μ and δ opioid receptors and by their related DYN and ENK in PD rats under L-DOPA treatment.

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Poster

512. Parkinson's Disease: Imaging, Dyskinesia, and Development

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Topic: C.03. Parkinson’s Disease

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NS058830 05

Title: rAAV-Mediated Nurr1 overexpression in striatal neurons results in enhanced levodopa-induced dyskinesias in the 6-OHDA rat model of Parkinson’s disease

Authors: R. C. SELLNOW[1,2], K. STEECE-COLLIER[1], N. M. KANAAN[1], C. E. SORTWELL[1], T. J. COLLIER[1], A. COLE-STRAUSS[1], J. W. LIPTON[1], *F. P. MANFREDSSON[1]
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Abstract: Primary motor symptoms in Parkinson’s disease (PD) arise due to a loss of dopaminergic innervation in the striatum (caudate/putamen). Administration of levodopa (L-DOPA) as a neurotransmitter replacement therapy is widely used to treat such motor symptoms by improving dopamine (DA) signaling. However, a significant proportion of patients receiving

L-DOPA treatment develop a series of debilitating hyperkinetic and dystonic movements known as L-DOPA-induced dyskinesias (LIDs). The molecular mechanism(s) driving LID genesis is not clear, and, it is not currently understood why some patients develop LIDs, but other patients on comparable doses of L-DOPA do not. In an attempt to identify transcriptional differences between LID responders (LID+) and LID non-responders (LID-) in the rat parkinsonian 6-OHDA model, we performed a full genome array to identify differential transcript expression between these two groups. The orphan nuclear receptor, Nurr1, was one transcript was expressed at significantly higher levels--greater than 30 fold--in LID+ animals when compared to LID- animals. In this study, we sought to determine if elevated expression of Nurr1 is a causative factor in dyskinesogenesis, or simply a cellular response to LIDs. Adult male Sprague-Dawley rats were rendered parkinsonian using 6-hydroxydopamine. Following the establishment of a stable lesion (4 weeks) animals received recombinant adeno-associated virus type 2/5 overexpressing either Nurr1 or GFP in the denervated striatum. Four weeks following vector injection, animals received escalating doses (0mg/kg - 24mg.kg) of L-DOPA every other day (M-Fr), and were evaluated for LIDs at 25 min intervals for 2-4 hours using our abnormal involuntary movement (AIM) rating scale. No off-time LID or significant differences in LID severity occurred at low doses of L-DOPA. At doses equal to or exceeding 12mg/kg, rAAV-Nurr1 treated animals exhibited both more severe LIDs as well as LIDs that persisted for longer than their rAAV-GFP control counterparts. Current studies are underway that will determine whether Nurr1 overexpression results in increased frequency of LIDs. These results demonstrate that overexpression of Nurr1 in the DA-depleted striatum of parkinsonian rats has a causative role in the expression of LIDs. These data suggest that the maladaptive upregulation of Nurr1 in striatal neurons is a core event in the formation of LIDs.

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Poster

512. Parkinson's Disease: Imaging, Dyskinesia, and Development

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Michael J. Fox Foundation

Parkinson's Disease Foundation

Title: Striatal cholinergic cell ablation attenuates l-dopa induced dyskinesia in parkinsonian mice

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Abstract: 3,4-Dihydroxyphenyl-L-alanine (L-DOPA)-induced dyskinesia (LID) is a debilitating side effect of long-term dopamine replacement therapy in Parkinson's Disease. At present, there are few therapeutic options for treatment of LID and mechanisms contributing to the development and maintenance of these drug-induced motor complications are not well understood. We have previously shown that pharmacological reduction of cholinergic tone attenuates the expression of LID in parkinsonian mice with established dyskinesia after chronic L-DOPA treatment. The present study was undertaken to provide anatomically specific evidence for the role of striatal cholinergic interneurons by ablating them before initiation of L-DOPA treatment and determining whether it decreases LID. We used a novel approach to ablate striatal cholinergic interneurons (ChIs) via Cre-dependent viral expression of the diphtheria toxin A subunit (DT-A) in hemiparkinsonian transgenic mice expressing Cre recombinase under control of the choline acetyltransferase promoter.Weshow that Cre recombinase-mediated DT-A ablation selectively eliminated ChIs when injected into striatum. The depletion of ChIs markedly attenuated LID without compromising the therapeutic efficacy of L-DOPA. These results provide evidence that ChIs play a key and selective role in LID and that strategies to reduce striatal cholinergic tone may represent a promising approach to decreasing L-DOPAinduced motor complications in Parkinson's disease.

Disclosures: U.J. Kang: None. **Y. Ding:** None. **P. Singh:** None. **L. Won:** None.

Poster

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Title: Role of nitrergic system in L-DOPA-induced dyskinesia in a mouse model of Parkinson's disease

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Abstract: Nitric oxide (NO), a gaseous neurotransmitter synthesized by nitric oxide synthase (NOS), plays a pivotal role in integrating dopamine transmission in the basal ganglia and has been implicated as a key player in the pathogenesis of Parkinson's disease (PD). In order to study the role of nitrergic system on L-DOPA-induced dyskinesia (LID), we assessed the effects of 7-nitroindazole (7-NI, neuronal NOS inhibitor), molsidomine (an NO donor) and zaprinast (cGMP phosphodiesterase 5- PDE5- inhibitor) on LID in Pitx3−/− (aphakia), a well-established mouse model of PD. Our hypothesis was that decreasing the level of NO by 7-NI would reduce LID and increasing NO by either molsidomine or zaprinast will exacerbate LID. To test this hypothesis, aphakia mice were treated with L-DOPA daily (10 mg/kg, ip) for 10 days to induce LID. On the last day of L-DOPA treatment some of these mice were administered 7-NI (30 mg/kg ip) or zaprinast 30 min before L-DOPA, or molsidomine, 10 min before L-DOPA to evaluate their effects on LID. Controls received vehicle. Front paw, hind paw, three paw and four paw dyskinetic movements were evaluated as abnormal involuntary movements, whereas locomotor activity and rotarod test were used as a measure of the antiparkinsonian effect of L-DOPA. Moreover, alterations in FosB, histone (H3) and pERK activation, molecular markers of LID, were examined by immunohistochemistry 1 hour after the last L-DOPA injection. 7-NI decreased LID, without affecting the beneficial antiparkinsonian effect of L-DOPA. In addition, expressions of FosB, pAcH3 and ERK correlated positively with LID. However, 7-NI attenuated FosB and pAcH3 expression only. Surprisingly, increasing the NO/cGMP signaling pathway with molsidomine or zaprinast also significantly diminished LID. However, these drugs also reduced the antiparkinsonian effect of L-DOPA. Thus, here we have demonstrated that NO/cGMP signaling pathway can modify both the behavioral and molecular consequences of L-DOPA treatment. Hence, targeting these molecular pathways might offer a novel pharmacological intervention in treatment of PD. Supported by: This work was supported by grants from the Spanish Ministerios de Economía y Competitividad de Sanidad Política Social e Igualdad, ISCIII: BFU2010-20664, PNSD #2012/071, RedRTA (RD06/0001/1011), CIBERNED ref. CB06/05/0055, and Comunidad de Madrid ref. S2011/BMD-2336

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Poster

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Topic: C.03. Parkinson's Disease

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Field Neurosciences Institute

John G. Kulhavi professorship in Neuroscience

Title: Trans-differentiation of mesenchymal stem cells into dopaminergic neurons via adenoviral transfection

Authors: *R. WELCHKO[1,2], G. SHALL[1,2], S. PARKER[1,2,3], W. HUO[1,2], J. WATTERS[1,2], M. JEAKLE[1,2], L. SIEGEL[1,2,3], N. JONES-CAMP[1,2], D. DUES[1,2], D. DAI[1,2], X. LEVEQUE[1,2], J. ROSSIGNOL[1,2,4], M. LU[1,2,3], G. DUNBAR[1,2,3,5]
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Abstract: Parkinson's disease is a progressive and continuous neurodegenerative disorder, with onset of the disease occurring around 55 years of age. Symptoms are attributed to a loss of dopaminergic neurons within the nigrostriatal pathway, which leads to a significant decrease of dopamine in the patient's striatum. Transplantation of human embryonic dopaminergic progenitors within the striata of Parkinson's disease patients has given the field encouraging results, but ethical concerns and tissue availability limit this approach. The use of mesenchymal stem cells (MSCs) and induced pluripotent stem cells as an alternative cell source for transplantation circumvents the ethical issues, and provides a readily available source of cells, as they are derived from adult tissue. This study explored the use of MSCs as a cell source for DA neuronal induction prior to transplantation as a means to increase integration within the striatum. To this end, our lab developed a novel adenovirus for the polycistronic expression of multiple genes (Ascl1, Lmx1a, and Nurr1) that are involved in dopaminergic differentiation and used gfp to track transfection. MSCs were cultured with the adenovirus, which resulted in morphological changes as well as expression of GFP as evidenced by fluorescence microscopy. The presence of the viral DNA within the transfected cells was confirmed with PCR. Immunocytochemistry and RT-PCR analyses revealed that, cells expressing GFP have nuclear co-labeling of NURR1 and LMX1a, as well as an up-regulation of these genes, along with an up-regulation of downstream

gene targets, such as tyrosine hydroxylase, and the dopamine transporter. These results are indicative of active NURR1 and LMX1a promoting dopaminergic differentiation. Therefore, electrophysiology experiments were performed on these cells to examine their ability to produce action potentials, and dopamine release was quantified utilizing high performance liquid chromatography. Our results suggest that the approach used in this study may provide a new means of facilitating cell replacement therapy.

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Poster

512. Parkinson's Disease: Imaging, Dyskinesia, and Development

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The Parkinson's Disease Foundation

The Parkinson's Alliance

Title: Evaluation of a possible role for the GTPase Rap1B in the induction of new axon growth in the adult nigrostriatal dopaminergic system

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Abstract: A number of extrinsic factors have been found to induce axon growth in dopamine neurons of the substantia nigra (SN) during development, including trophic factors such as GDNF. However, not much is known about the intrinsic mediators of axon growth in this system. Emerging data suggest that the extrinsic and intrinsic control of axon growth during development may also play a significant role in axon growth and regeneration after injury in adulthood. We have previously established a role for such developmental intrinsic mediators, the

kinase Akt and the GTPase Rheb, in the induction of axon growth following axon destruction in a parkinson mouse model. Other signaling pathways have essential roles in a number of aspects of neuronal polarization and axon growth during development. Of these, the Ras-related protein 1 (Rap1B) has been shown to play an important role in axon formation in embryonic hippocampal neurons in culture. We analyzed the expression of Rap1B in dopamine neurons in the SN in adult mice. We detected Rap1B mRNA expression in neurons of the SNpc by in-situ hybridization. Immunoperoxidase staining for Rap1B protein revealed expression in neurons of the SN and double immunofluorescence staining with tyrosine hydroxylase (TH) indicated that it was present in dopamine neurons. In order to investigate the potential of Rap1B to induce axon growth in the adult nigrostriatal dopaminergic system, we generated an AAV2/1 based viral vector containing the constitutively active form of Rap1B (Rap1BG12V) to enhance the activity of Rap1B. Transduction of SNpc neurons with Rap1BG12V caused no toxicity to the dopamine neurons or axons in the medial forebrain bundle (MFB) or striatum as assessed by TH immunostaining. At four weeks post AAV-Rap1BG12V injection into the SNpc, we observed efficient transduction of neurons, demonstrated by immunoperoxidase staining for Flag. We observed expression in the soma of neurons in the SN, in axons in the MFB and in the terminal fields in the striatum. In addition, double immunofluorescence labeling for TH and FLAG demonstrated expression of the transgene within dopamine neurons of the SNpc. Current studies are aimed at testing the role of Rap1B in inducing new axon growth following unilateral destruction of dopaminergic axons in the intra-striatal 6-OHDA mouse model of Parkinson's disease.

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Poster

512. Parkinson's Disease: Imaging, Dyskinesia, and Development

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Topic: C.03. Parkinson's Disease

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FNI

Title: Generation of dopaminergic neurons from rat bone marrow derived mesenchymal stem cells

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Abstract: Parkinson's disease is a neurodegenerative disorder characterized by the loss of dopaminergic neurons from the substantia nigra that innervate the striatum through the nigrostrial pathway. Loss of this pathway leads to significant motor impairments and affective symptoms. Although an effective long-term therapy has not been developed, extensive research focusing on the use of stem cell transplantations as a regenerative therapy for PD is being pursued, including the use of mesenchymal stem cells (MSCs) for this purpose. MSCs can be easily isolated and expanded from different adult tissues, including bone marrow, peripheral blood, vasculature, adipose tissue, and umbilical cord blood. Further, studies have shown that they also have the ability to differentiate *in vivo* and *in vitro* into neural-like stem cells and, more specifically, into dopamine-producing cells using growth factors and morphogens. However, there are discrepancies among studies regarding the optimal time and method for dopaminergic induction *in vitro*. In the current study, we compared the ability of early and later passaged rat bone marrow-derived MSCs to differentiate into dopaminergic-neurons when using two growth-factor based approaches: a single-stage induction (SSI) using sonic hedgehog (SHH) and fibroblast growth factor -8 (FGF-8) and a multiple-stage induction (MSI) using basic fibroblast growth factor (bFGF) epidermal growth factor (EGF), SHH, and FGF-8. Results from flow cytometry, and immunocytochemistry (ICC) analyses indicated that treatment of both early and later passaged MSCs with bFGF and EGF induced neurosphere-like formation, expressing the neural progenitor markers doublecortin (DCX) and nestin. Also, early passaged MSCs that underwent both the SSI and MSI exhibited a change in morphology toward a neuronal phenotype, and exhibited an increase in the expression of the neuronal markers neuron specific nuclear protein (NeuN) and class III β-tubulin (β-III), as determined by flow cytometry, ICC, and reverse transcriptase polymerase chain reaction (RT-PCR). Further, both early and later passaged MSCs, that were exposed to the MSI, showed an increase in the expression of the dopaminergic markers tyrosine hydroxylase (TH), dopamine transporter (DAT), and the nuclear receptor related 1 protein (Nurr1), following treatment with SHH and FGF-8, as evidenced by ICC and RT-PCR. Overall, findings from this study indicate that MSCs have the ability to differentiate into neuronal-like cells, and more specifically, early and later passaged rat MSCs, which have undergone a MSI, have the potential to differentiate into dopaminergic-neurons.

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Poster

513. Parkinson's Disease: Neuroprotection

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Title: Characterization of a novel PPAR-γ agonist as a neuroprotective agent in Parkinson's disease

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Abstract: Evidence has pointed to PPAR-γ as a pharmacological target of disease-modifying drugs in Parkinson's disease. However, currently available PPAR-γ agonists thiazolidinediones (TZDs) present limitations due to a low blood brain barrier permeability and concerns relating to potential increases in cardiovascular or cancer risks. Here, we have characterized the *in vitro* and *in vivo* neuroprotective efficacy of a novel thiobarbituric-based (TBA) compound MDG548, which shows high affinity PPAR-γ activity with increased BBB permeability compared to TZDs. Cell viability assays showed a dose-dependent effect of MDG548 that displayed cytotoxicity in neonate rat cortical neurons only at the high doses (25-50 μM), whilst it was void of effects in the range 100nM-10 μM. Therefore, this dose-range was used to further investigate *in vitro* effects of the compound. First, neuroprotection on H2O2-induced neurotoxicity was evaluated. Rat cortical neurons were pre-exposed for 24 hours to MDG548 prior to H2O2, or were co-treated with MDG548/H2O2 on the same time-point. In both instances, MDG548 dose-dependently increased cell viability as compared to H2O2. Moreover, MDG548 effects on NF-kB activation was investigated in order to assess its anti-inflammatory activity. HEK-Blue-hTLR4 cells were treated with varying concentrations of MDG548 for 24 hours prior to LPS (100 ng/100 ml) stimulation. MDG548 dose-dependently decreased LPS-induced NF-kB activation. Importantly, MDG548 effects in both *in vitro* assays were fully inhibited by PPAR-γ antagonist GW9662. In addition, MDG548 was not genotoxic based on Ames test performed with Salmonella typhimurium TA100 and TA98 strains, with and without metabolic activation (S9). *In vivo*, the neuroprotective effect of MDG548 was tested in a sub-acute MPTP model of

PD in mice. BL/6J mice were treated with MPTP (20 mg/kg i.p. once/day for 4 days) in conjunction with saline, MDG548 (2, 5, 10 mg/kg i.p.) or MDG548 plus GW9662 (10 mg/kg i.p.). Stereological counting showed a reduction in the number of TH-positive cells in the substantia nigra compacta after MPTP treatment, which was abrogated by MDG548 co-administration at all doses tested. Neuroprotection was PPAR-γ-mediated, since GW9662 antagonized the effects. Results suggest that the novel PPAR-γ agonist MDG548 exhibits neuroprotective efficacy *in vitro* and in an *in vivo* model of PD. Being a TBA compound, MDG548 may offer an effective alternative to TZDs in the search for safer PPAR-γ agonists to be utilized as disease-modifying drugs in PD. More investigations are warranted to further assess *in vivo* neuroprotection and molecular mechanisms mediating this effect.

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Poster

513. Parkinson's Disease: Neuroprotection

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RTI International

Title: Evaluation of isradipine for neuroprotection in the MPTP/p mouse model of Parkinson's disease

Authors: *C. J. PRICE[1], M. L. SUTHERLAND[2], J. M. MATHEWS[1], T. R. FENNELL[1], S. R. BLACK[1], S. L. WATSON[1], R. W. SNYDER[1], R. C. SWITZER, III[3], S. O. AHMAD[4]
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Abstract: Isradipine (ISR), a Cav1.3 Ca^{2+} channel blocker and anti-hypertensive, has been previously reported as neuroprotective in rodent neurotoxin models of Parkinson's disease. In the present studies, young male C57BL/6 mice were implanted on study day zero with placebo or ISR-loaded pellets delivering a target dose of 3 mg/kg/day, sc. On day 7, mice were terminated

for analysis of ISR (plasma; brain) or given a single MPTP/p treatment and terminated to assess ISR effects on: (a) MPTP and MPP^+ toxicokinetics (plasma; striatum); and (b) MPTP/p-induced changes in dopamine (DA) neurochemistry (striatum). On day 7, mice assigned to the main neuroprotection study received the 1st of 10 biweekly injections of vehicle or MPTP/p [probenecid (250 mg/kg, ip) followed 30 min later by MPTP·HCl (25 mg/kg, sc)]. Groups (N=12 per group) included: placebo + vehicle; placebo + MPTP/p; ISR + vehicle; ISR + MPTP/p. On day 44, mice were terminated for ISR analysis (plasma) and neuropathology of substantia nigra-pars compacta (SNpc). Immunohistochemical stains included tyrosine hydroxylase (TH) + AgNOR counter stain for DA neurons, GFAP for astrocytes, Iba-1 for microglia, alpha-synuclein (alpha-syn), and pSER129 for phosphorylated alpha-syn. On day 7, the mean plasma ISR was 18.8 ng/mL (range = 9.10 to 31.7 ng/mL) and the brain:plasma ratio was 0.509 (0.335 to 0.934). On day 44, plasma ISR declined to 10.8 ng/mL (8.42 to 16.2 ng/mL) in ISR+vehicle mice or 13.2 ng/mL (8.38 to 18.9 ng/mL) in ISR+MPTP/p mice. On day 7, baseline striatal DA was 47% higher in ISR vs. placebo mice, but ISR did not alter striatal MPP^+ or DA concentrations at 0.5, 1, 2, 3, or 6 h after MPTP. By day 44, mortality in MPTP/p-treated mice (3/12 placebo vs. 1/12 ISR) suggested some ISR-mediated protection against systemic MPTP/p toxicity. Mild body wt deficits and characteristic neurotoxicity occurred in MPTP/p-treated mice. As expected, striatal DA and number of TH^+ neurons in SNpc were reduced, TH staining intensity decreased, and % area stained by GFAP or Iba1 increased in MPTP/p-treated mice. ISR administration, however, failed to attenuate these indicators of MPTP/p-induced neurodegeneration. For alpha-syn and pSER129, staining intensity was not affected. In conclusion, previously reported neuroprotective effects of ISR were not replicated herein, in spite of well-controlled ISR delivery and internal exposures similar to earlier studies. Dose-response studies may be necessary to reliably demonstrate ISR's neuroprotective effect in the MPTP/p mouse model.

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Poster

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FWF P25161

FWF W1206

Title: HDAC inhibition protects dopaminergic neurons in a transgenic mouse model of multiple system atrophy

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Abstract: Within the last years, histone deacetylases (HDACs) have been implicated to play an important role in the pathogenesis of neurodegenerative diseases. It has been shown that their inhibition protects neurons in models of Parkinson's disease (Roy, A. et al., 2012) and amyotrophic lateral sclerosis (Cudkowicz, M. E. et al., 2009). Multiple system atrophy (MSA) is an atypical parkinsonian disorder associated with oligodendroglial alpha-synuclein inclusions, selective neuronal loss and gliosis. MSA has rash progression and lacks treatment. In an attempt to identify new therapeutic targets for this disease in the current project we address the effects of HDAC inhibition by sodium phenylbutyrate (NaPB), a non-selective pan-HDAC inhibitor, in a transgenic mouse model of MSA. Transgenic PLP-alpha-Synuclein MSA mice with targeted overexpression of alpha-synuclein in oligodendroglia received daily intraperitoneal injections of either NaPB (200mg/kg) or saline over a period of two months. Wild type C57Bl/6 mice receiving saline served as healthy controls. Behavioral tests were performed in order to assess progression of motor deficits. Immunohistochemistry and biochemical analysis, including western blotting, were applied to identify the effects of NaPB treatment in selected neurodegenerating regions of the MSA brain. MSA transgenic mice showed reduced acetylation of the nucleosome core protein H3 as compared to healthy wild type mice. NaPB treatment of MSA mice resulted in increased H3 acetylation up to levels of healthy controls. Furthermore, NaPB treatment in the transgenic MSA mouse model was associated with neuroprotection of nigral dopaminergic neurons, reduced oligodendroglial alpha-synuclein inclusion density and a reduction of astrogliosis. We identified that treatment of MSA mice with NaPB led to increased expression of DJ-1, a protein that is an indicator for anti-oxidant and gene-regulatory activity of NaPB. Our data suggest that the pan-HDAC-inhibitor NaPB has a significant neuroprotective effect on nigral dopaminergic neurons of transgenic MSA mice. Epigenetic mechanisms including histone modifications should be investigated further as potential targets in MSA therapy. Acknowledgement: Austrian Science Funds (FWF) F4404 and P25161. We also thank Karin Spiss for excellent technical assistance.

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Poster

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Title: Lovastatin activates SREBP nuclear translocation and modulates dopamine transport system in SH-SY5Y cells

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Abstract: Sterol response element binding protein (SREBP) is a transcription factor regulating the expression of several gene families. Disinhibition of SREBP-dependent gene expression could be at the base of some pharmacological actions attributed to statins such as the lower incidence of Parkinson's disease and dementia in population under statin treatment. Experimental data in Parkinson's models have further shown neuroprotective effects as well as functional modulation of the dopaminergic system. Using immunocytochemistry and high content imaging, we have investigated the effects of HMG-CoA inhibition on the nuclear translocation of SREBP in SH-SY5Y human neuroblastoma cells. The expression of the main presynaptic elements of dopamine (DA) transport system, synaptogyrin-3 (SYNGR3), DA-transporter (DAT) and vesicular monoamine transporter-2 (VMAT-2) were studied using quantitative PCR (qPCR), SDS-PAGE electrophoresis and immunocytochemistry. Changes in the functional activity of the cellular DA transport system were investigated using radio-isotopic method. In undifferentiated SH-SY5Y cells, lovastatin induced a time-dependent and dose-dependent increase of SREBP nuclear translocation with maximal effects observed at 48hr of incubation. Gene expression analysis shows that the treatment of SH-SY5Y cells with lovastatin induced a time-dependent increase of mRNA levels coding for SYNGR3 and VMAT-2 proteins after 6hr incubation. No significant changes of DAT mRNA levels were observed at any time. Western blot analysis showed significant increases of a 75kDa VMAT-2- and a 24kDa SYNGR3-protein bands while no changes were observed for DAT (68kDa) band. DA transport experiments using [^{3}H]DA in whole cells showed a reduction of DA transport uptake activity after 24h of treatment with lovastatin. Our data demonstrate an statin-dependent induction of SREBP translocation into the nucleus concomitant with changes in the gene expression of VMAT2 and SYNGR3 proteins and a modification of the DA-transport capacity in SH-SY5Y

cells. Present findings suggest a potential molecular mechanism of action of statins on the dopaminergic system.

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Poster

513. Parkinson's Disease: Neuroprotection

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Title: Inhibition of the microglial response is essential for the neuroprotective effects of Rho-Kinase inhibitors on MPTP-induced dopaminergic cell death

Authors: A. I. ANA I. RODRIGUEZ-PEREZ[1], A. BORRAJO[1], B. VILLAR-CHEDA[1], R. VALENZUELA[1], M. J. GUERRA[1], *J. L. LABANDEIRA-GARCIA[2,1]
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Abstract: Several recent studies have shown that activation of the RhoA/Rho-associated kinase (ROCK) pathway is involved in the MPTP-induced dopaminergic cell degeneration and possibly in Parkinson’s disease. ROCK inhibitors have been suggested as candidate neuroprotective drugs for Parkinson’s disease. However, the mechanism responsible for the increased survival of

dopaminergic neurons after treatment with ROCK inhibitors is not clear. We exposed primary (neuron-glia) mesencephalic cultures, cultures of the MES23.5 dopaminergic neuron cell line and primary mesencephalic cultures lacking microglial cells to the dopaminergic neurotoxin MPP^+ and the ROCK inhibitor Y-27632 in order to study the effects of ROCK inhibition on dopaminergic cell loss and the length of neurites of surviving dopaminergic neurons. In primary (neuron-glia) cultures, simultaneous treatment with MPP^+ and the ROCK inhibitor significantly reduced the loss of dopaminergic neurons. In the absence of microglia, treatment with the ROCK inhibitor did not induce a significant reduction in the dopaminergic cell loss. Treatment with the ROCK inhibitor induced a significant decrease in axonal retraction in primary cultures with and without microglia and in cultures of the MES23.5 cell line. In conclusion, inhibition of microglial ROCK is essential for the neuroprotective effects of ROCK inhibitors against cell death induced by the dopaminergic neurotoxin MPP^+. In addition, ROCK inhibition induced a direct effect against axonal retraction in surviving neurons. However, the latter effect was not sufficient to cause a significant increase in the survival of dopaminergic neurons after treatment with MPP^+.

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Poster

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FAPESP

CNPq - Programa Ciências sem Fronteiras (Pesquisador Visitante Especial)

CNPq - Cátedra França-USP

Title: Neuronal nitric oxide synthase inhibitor modifies glial reaction trigged by L-DOPA treatment in rat model of Parkinson’s disease

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Abstract: Parkinson's disease is a movement disorder characterized by the dopamine depletion in dopaminergic nigrostriatal system. The most common treatment is with L-3,4-dihydroxyphenylalanine (L-DOPA). However, the use of this drug is severely limited by the motor (dyskinesia) or cognitive (psychosis) side effects it causes. We hypothesize that dyskinesia may be accompanied by alterations of the inflammatory cascade components. To analyze this hypothesis we investigate the inducible nitric oxide synthase (NOS) enzyme, microglia and astrocytes expression in the striatum of rats with L-DOPA-induced dyskinesia. Male Wistar rats were lesioned unilaterally with the 6-hydroxydopamine (6-OHDA), neurotoxin microinjection in the medial forebrain bundle. After 3 weeks, the animals were then treated daily with either the inhibitor of nitric oxide synthase 7-nitroindazole (7-NI, 30mg/kg/day) or vehicle (saline-DMSO 50%) followed (30 minutes later) by L-DOPA (30mg/kg plus benserazide 7.5 mg/kg/ -21 days) or saline. L-DOPA administration brought dyskinesia, which was blocked by 7-NI. An increase in the immunoreactivity of the inducible NOS, the glial fibrillary acidic protein (GFAP) labeling astrocytes and of the anti-CD11b antibody/equivalent protein (clone OX-42) labeling microglia was observed in the lesioned striatum. 7-NI decreased expression of inducible NOS and suppressed astrocytic and microglial activation. Our results provide, for the first time, evidence that glial activation, an actor of inflammatory processes, could play a role on L-DOPA induced dyskinesia in parkinsonian rats. The NOS inhibitor may overcome dyskinesia at least in part via the inhibition of glial cells activation and inducible NOS expression. It furthest settles NOS inhibitor therapeutic potential for control L-DOPA induced dyskinesia.

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Poster

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Parkinson's Disease Foundation

Title: Neuroprotection and neurorestoration by inducible Akt in Parkinson's disease

Authors: *S. PARK[1], O. LEVY[1], A. TAGLIAFERRO[1], T. KAREVA[1], T. FRANKE[2], R. BURKE[1], L. GREENE[1]
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Abstract: Parkinson's disease (PD) is a common progressive neurodegenerative disorder. Existing treatments only help to manage symptoms and do not impede progression of the disease or reverse its course. Thus, there is great demand for treatments that will provide neuroprotection as well as restoration of function. Signaling by the serine threonine kinase Akt, the main effector of the PI3K pathway, has been shown to mediate both neuroprotection and regeneration of axons in PD toxin models. Furthermore, studies of postmortem sporadic PD brains and genetic PD models have suggested that a deficiency in Akt signaling is involved in the neuronal degeneration that is key to progression of PD. Based on these lines of evidence, we have chosen Akt as a promising neuroprotective and neurorestorative target for study in PD models. Because unregulated Akt activity may lead to undesirable side effects such as excess neuronal sprouting and proliferation of non-neuronal cells, we have devised a strategy to deliver a regulatable form of this protein. Here, we utilize an inducible protein stabilization system (Iwamoto et al, 2010) to control the activity of Akt by fusing a constitutively active Akt (CA-Akt, E40K) to a destabilization domain (DD). The DD, derived from E. coli dihydrofolate reductase (DHFR), causes the chimeric DD-CA-Akt to be degraded in proteasomes. The addition of the DHFR ligand, trimethoprim (TMP), rapidly stabilizes the kinase, allowing it to accumulate in the cell and activate downstream signaling cascades. This method thus allows tunable regulation of Akt activity using a ligand that is commonly used as an antibiotic in humans and readily crosses the blood-brain barrier. We have demonstrated that the system successfully confers inducibility of Akt activation at levels comparable to or greater than that of endogenous Akt in HEK293 cells, neuronal PC12 cells, and primary cortical neurons. Additionally, this activation is dose-dependent, reversible, and reaches its maximum within 24 hours of TMP treatment in cell culture. Survival experiments using lentiviral vectors to deliver DD-CA-Akt to neuronal PC12 cells demonstrate that TMP treatment significantly improves survival against the PD-mimetic toxin 6-OHDA, comparable to survival seen with myr-Akt. No protection is seen in absence of TMP or by TMP alone. We are presently testing the inducible system *in vivo* in 6-OHDA treated mouse models. If successful, this inducible system will provide a strategy for gene therapy in PD that has the potential to halt or slow progression of the disease and to lead to functional restoration of damaged neurons.

Disclosures: S. Park: None. **O. Levy:** None. **T. Franke:** None. **A. Tagliaferro:** None. **T. Kareva:** None. **L. Greene:** None. **R. Burke:** None.

Poster

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Topic: C.03. Parkinson's Disease

Title: Fibroblast-derived neurospheres as a therapeutic option to restore dopaminergic neuronal loss in a mouse model of Parkinson's Disease

Authors: *C. D. GUOYNES[1], J. CHO[2], K. LUTFY[3], Y. HONG[2]
[2]Col. of Vet. Med., [3]Col. of Pharm., [1]Western Univ. of Hlth. Sci., Pomona, CA

Abstract: Parkinson's Disease (PD) is characterized by the selective loss of dopaminergic (DA) neurons_as the disease progresses, more and more DA neurons are lost and PD becomes fatal. In order to reduce the progression of the disease and increase patient life expectancy, cells from a patient can be transdifferentiated *in vitro* and injected to restore the lost dopaminergic neurons. Our *in vitro* method involved deriving fibroblasts from mouse ear clip samples and transdifferentiating them into neurospheres using knockout serum replacer (KSR) media supplemented with Sox2 replacer small molecules. Neurospheres were then injected in the substantia nigra of mice with a unilateral 6-hydroxydopamine (6-OH-DA) lesion to assess the viability and transdifferentiation of these cells to dopaminergic neurons. Our results showed that an injection of 6-OH-DA into the striatum caused a significant loss of dopaminergic neurons in the substantia nigra compared to non-lesioned side. Furthermore, animals with unilateral 6-OH-DA lesion exhibited a significant increase in the number of contralateral rotations following apomorphine injection compared to sham-lesioned animals or animals with 6-OH-DA lesions in the nucleus accumbens. Studies are in progress to evaluate the viability and transdifferentiation of neurospheres to dopaminergic neurons in the control and lesioned animals. The results of the current studies will open a new avenue of research for PD cell therapy.

Disclosures: C.D. Guoynes: None. **J. Cho:** None. **K. Lutfy:** None. **Y. Hong:** None.

Poster

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Title: Exercise confers neuroprotection and improved behavior in a sex-dependent manner to the $LRRK2^{R1441G}$ mouse model of Parkinson's disease

Authors: *J. JANG, H. NOH, T. KIM, M. JEONG, J. JEON, H. SEO
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Abstract: Physical exercise has been studied as a potential therapeutic approach to treat patients with Parkinson's disease (PD). In this study, we determined the effects of regular swimming exercise on the PD-related behaviors of male and female $LRRK2^{R1441G}$ transgenic mice (MT). Regular swimming exercise significantly improved the latent periods until falling in the rota rod test in male MT mice. In the open field test, swimming exercise increased the locomotor counts of crossing in both female and male littermate control mice (LM), but did not change the locomotor counts of crossing, rearing, or grooming for either female or male MT mice. Interestingly, swimming exercise increased locomotor counts of wall rearing in female LM mice, but decreased locomotor counts of wall rearing in male LM and MT mice. Regarding spatial learning, swimming exercise did not alter escape latency, as assessed by the Morris swim maze, in both female and male MT mice. Swimming exercise did not change olfactory activity in an odorant avoidance test, in any group of mice tested in this study. Immunohistochemical data supported the neuroprotective effects of swimming exercise, with an increased number of cholinergic neurons observed in the medial septum (MS) of female MT mice, and increased number of dopaminergic neurons in the substantia nigra pars compacta (SNpc) of female and male MT mice after swimming exercise. These data suggest that the therapeutic application of swimming exercise can potentially be considered, in a sex-dependent manner.

Disclosures: J. Jang: None. **H. Seo:** None. **H. Noh:** None. **T. Kim:** None. **M. Jeong:** None. **J. Jeon:** None.

Poster

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Topic: C.03. Parkinson's Disease

Support: Italian Ministry of Health Grant GR-2010-2316671

Title: Acute theta-burst stimulation exerts distinct therapeutic effects in early and late experimental parkinsonism

Authors: *V. GHIGLIERI[1], F. CACACE[1], V. PENDOLINO[1], A. VANNELLI[1], M. MANCINI[1], B. PICCONI[1], P. CALABRESI[2]
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Abstract: In experimental Parkinson's disease (PD), different degrees of degeneration of nigrostriatal dopaminergic neurons produce distinct alterations of dopamine-dependent corticostriatal synaptic plasticity. Several lines of evidence indicate that repetitive transcranial magnetic stimulation (rTMS) induces a selective increase of dopamine in the vicinity of highly active corticostriatal terminals, suggesting that it may alleviate symptoms and improve the response to therapy in PD patients. However, the mechanisms underlying effects of rTMS on subcortical regions are not known and its effects on striatal plasticity have not been clarified either in healthy or in PD brain. Using intracellular recordings from corticostriatal slices of 6-hydroxydopamine (6-OHDA)-lesioned rats, modelling early and late PD, we show that a single session of *in vivo* cortical rTMS, using intermittent theta-burst stimulation (iTBS) protocol, was able to rescue a form of corticostriatal plasticity sensitive to low levels of DA, such as long term depression in fully-lesioned rats. The same treatment was able to induce a recovery of corticostriatal long term potentiation in partially-lesioned rats. These data indicate that acute iTBS may overcome effects of striatal DA denervation and may have a significant impact on neuronal activity of subcortical regions, providing experimental support for its use in clinical settings.

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Poster

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KAKENHI23700479

Title: Zonisamide prevents neurodegeneration in nigrostriatal dopaminergic neurons in a mouse genetic model of Parkinson's disease through brain-derived neurotrophic factor signaling pathway

Authors: *H. SANO[1], M. MURATA[2], A. NAMBU[1]
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Abstract: Parkinson's disease (PD) is a neurodegenerative disorder characterized by the progressive loss of nigrostriatal dopaminergic neurons, and consequent motor dysfunction. Zonisamide (ZNS; 1,2-benzisoxazole-3-methanesulfonamide), which was originally developed as an antiepileptic drug, was shown to also improve motor functions in PD. Although the pharmacological mechanisms behind the beneficial actions of ZNS in PD are not fully understood, some effects, such as the elevation of tyrosine hydroxylase activity, the increase in dopamine turnover and the inhibition of monoamine oxidase B, were suggested. Furthermore, ZNS attenuated cell death induced by seizure and ischemia, and showed neuroprotective effects on nigrostriatal dopaminergic neurons injured by dopaminergic neurotoxins. To clarify the pharmacological mechanisms of ZNS in PD, we have evaluated the neuroprotective effects of ZNS on nigrostriatal dopaminergic neurons of Engrailed mutant mice, which was shown resemble pathological features of PD. Chronic administration of ZNS to Engrailed mutant mice has improved the survival of nigrostriatal dopaminergic neurons compared with those under saline treatment. Although the number of dopaminergic neurons in the substantia nigra of ZNS - treated Engrailed mutant mice have not reached that of control mice, dopaminergic terminals in the striatum and the motor functions have been improved to the levels of those in control mice. To investigate the mechanism of the neuroprotective effect of ZNS, we have measured the contents of neurotrophic factors, such as glial cell-line derived neurotrophic factor (GDNF) and brain-derived neurotrophic factor (BDNF) in the striatum and ventral midbrain after chronic administration of ZNS. BDNF content has been increased in the ZNS-treated mice compared to

saline-treated mice, although GDNF content has remained unchanged. These findings indicate that ZNS prevents nigrostriatal dopaminergic cell death through BDNF signaling and may have similar beneficial effects in human PD patients as well.

Disclosures: H. Sano: None. **M. Murata:** None. **A. Nambu:** None.

Poster

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Title: The deleterious effects of High Mobility Group Box 1 in the MPTP model and Parkinson's disease can be attenuated by glycyrrhizin

Authors: *P. TEISMANN[1], K. SATHE[1], P. STATHAKOS[1], H. L. MARTIN[1], M. A. HOBERT[2], T. W. RATTAY[2], T. GASSER[2], D. BERG[2], K. J. TRACEY[3], W. MAETZLER[2]
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Abstract: High-Mobility Group Box 1 (HMGB1) is a nuclear and cytosolic protein that can be released into the extracellular space from immune and non-immune cells -including microglia and neurons - in response to various stimuli. Extracellular HMGB1 has been shown to contribute to progression of numerous chronic inflammatory and autoimmune diseases which is, among other receptors, mediated by interaction with the receptor for advanced glycation endproducts (RAGE). With regard to Parkinson's disease (PD), there is increasing evidence from in-vitro studies that HMGB1 is a promising candidate for bridging the two main pathophysiological components, i.e. progressive dopaminergic degeneration and chronic neuroinflammation, as a mechanistic basis of PD progression. This study assessed the role of HMGB1 in biospecimen of PD and in the chronic 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) mouse model. We found an increase in HMGB1 protein and RNA expression in the substantia nigra of postmortem PD patients as well as in cerebrospinal fluid and serum. A neutralizing antibody for HMGB1

partly inhibited MPTP-induced dopaminergic cell death, as well as reduction in dopaminergic striatal fibers. In parallel MPTP-induced increase of RAGE and tumor necrosis factor-alpha was attenuated. Glycyrrhizin, a component from liquorice root which can directly bind to HMGB1, reduced MPTP-induced dopaminergic cell death dose-dependently and effectively. This was accompanied by reduction of MPTP-induced protein expressions of HMGB1 and RAGE. Taken together this study provides evidence for a potential role of HMGB1 in the progressive dopaminergic neurodegeneration as seen in PD and the observed chronic neuroinflammation. Thus, HMGB1 might be a suitable and effective target for a potential neuroprotective therapy for PD.

Disclosures: **K. Sathe:** None. **P. Stathakos:** None. **H.L. Martin:** None. **M.A. Hobert:** None. **T.W. Rattay:** None. **T. Gasser:** None. **D. Berg:** None. **K.J. Tracey:** None. **W. Maetzler:** None. **P. Teismann:** None.

Poster

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Title: Therapeutic potentials of human adipose-derived stem cells in mouse model of Parkinson’s disease

Authors: ***H. KIM**[1], H. CHOI[2], Y.-H. SUH[2]
[1]Res. Div., Korea Brain Res. Inst. (KBRI), Daegu, Korea, Republic of; [2]Korea Brain Res. Inst., Daegu, Korea, Republic of

Abstract: Human adipose-derived stem cells (hASCs), a kind of mesenchymal stem cells (MSCs) isolated from adipose tissue, are well known for their pluripotent ability to differentiate into various cell types including adipocytes, osteocytes, cartilage cells, and muscle cells. Furthermore, the differentiation potential of hASCs into neuron-like cells was reported in the recent studies. Autologous hASCs have significant advantages, such as the lack of immune rejection responses, tumorigenesis, or ethical problems. However, for the stability of hASCs transplantation, the consistency and high reliability of the experimental results verified by the animal models of diseases have been considered greatly important factors. Therefore, the aim of

this study is to investigate preventive and therapeutic potential of hASCs for Parkinson's disease (PD). hASCs was intravenously injected into tail vein of Parkinson's disease mouse model induced by 6-hydroxydopamine (6-OHDA). In our previous study, we found that intravenously transplanted hASCs passed through the blood brain barrier (BBB) and migrated into the injuries of the brain. Consequently, the behavioral performances were significantly improved at 3 weeks after the intravenous injection of hASCs in PD mouse model.

Disclosures: H. Kim: None. **H. Choi:** None. **Y. Suh:** None.

Poster

513. Parkinson's Disease: Neuroprotection

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Topic: C.03. Parkinson's Disease

Title: Neuroprotective effects of spinal cord stimulation on Parkinson's disease model of rats

Authors: *A. SHINKO, T. AGARI, T. YASUHARA, M. KAMEDA, A. KONDO, K. SATO, T. SASAKI, S. SASADA, A. TOYOSHIMA, H. TAKEUCHI, I. DATE
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Abstract: In clinical practice, deep brain stimulation (DBS) is effective for the treatment of motor symptoms in Parkinson's disease (PD). However, the mechanisms have not been understood completely. There are some reports that electrical stimulation exerts neuroprotective effects on the central nervous system disorders including cerebral ischemia, head trauma, epilepsy and PD; meanwhile, there are only few reports on neuroprotective effects of spinal cord stimulation (SCS). In the present study, we investigated neuroprotective effects of SCS on PD model of rats. Adult female Sprague-Dawley rats received hour-long SCS (2, 50 or 200Hz) with an epidural electrode at C1-2 level for 16 consecutive days. At 2 days after initial SCS, 6-hydroxydopamine (6-OHDA), a neurotoxin of dopaminergic neurons, was injected into the right striatum of rats. Behavioral evaluations of PD symptoms, including cylinder test and amphetamine-induced rotation test, were performed at 1-2 weeks after 6-OHDA injection. Animals were subsequently euthanized for immunohistochemical investigations. In order to explore neurotrophic and growth factor upregulation induced by SCS, another cohort of rats that received 50Hz SCS was euthanized at 1 and 2 weeks after lesion for protein assays. Behavioral tests revealed that the number of amphetamine-induced rotations decreased in SCS groups. Immunohistochemically, tyrosine hydroxylase (TH)-positive fibers in the striatum were

significantly preserved in SCS groups. TH-positive neurons in the substantia nigra pars compacta were significantly preserved in 50Hz SCS group. The level of vascular endothelial growth factor (VEGF) was upregulated by SCS at 1 week after the lesion. These results suggest that SCS exerts neuroprotection in PD model of rats, at least partially by upregulation of VEGF. SCS is supposed to suppress or delay Parkinson's disease progression and might become a less invasive therapeutic option for PD patients.

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Poster

513. Parkinson's Disease: Neuroprotection

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Topic: C.03. Parkinson's Disease

Title: Lymphocytes improve clinical outcome in the 6-OHDA parkinson mouse model

Authors: *C. IP, S. BECK, J. VOLKMANN
Univ. of Wuerzburg, Wuerzburg, Germany

Abstract: Objective: Neuroinflammation is an important feature of neurodegenerative diseases. The role of microglia in the pathogenesis of Parkinson's disease (PD) has been extensively investigated in human brain tissue and different rodent models. Although lymphocyte populations were found in autopsies material of PD patients and also in the MPTP Parkinson mouse model, there is currently no data on the role of lymphocytes in the 6-OHDA toxin model, which represents the most common rodent model of Parkinson's disease. To evaluate a possible role of the adaptive immune system in the progression of Parkinson' s disease, we used an unilateral 6-OHDA mouse model by injection into the medial forebrain bundle and compared the motor outcome of wt to RAG-1 deficient mice, that do not possess mature lymphocytes. Methods: Clinical examination in 6-OHDA and control mice (4 groups: wt 6-OHDA, RAG-1 -/- 6-OHDA, wt sham, RAG-1 -/- sham) was longitudinally performed by open field and rotarod analysis until day 30 after 6-OHDA injection. Immunohistochemical stainings were performed to analyze lymphocyte infiltration into the striatum. Results: After unilateral 6-OHDA injection RAG-1 -/- mice showed a significantly reduced velocity and exploration path in the open field compared to wildtype mice. Rotarod analysis exhibited a shorter time to fall in RAG-1 -/- mice

during the first 2 weeks after 6-OHDA injection. Wildtype bone marrow reconstitution into RAG-1 -/- recipients led to improvement of the clinical deterioration in RAG-1 -/- 6-OHDA treated animals. Immunohistochemical analysis im wildtype mice demonstrated a significantly higher CD8+ T-cell density in the striatum ispsilateral to the 6-OHDA lesion (right) compared to the untreated hemisphere (left) as well as a higher CD8+ T cell number in the right striatum of 6-OHDA treated mice compared to the sham group (NaCl injection). By contrast CD4+ T-cell number did not change significantly. Conclusion: Our preliminary data indicate that lymphocytes attenuate the clinical effect of 6-OHDA injection into the medial forebrain bundle and thus may play a protective role in this toxic PD mouse model.

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Poster

513. Parkinson's Disease: Neuroprotection

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Topic: C.03. Parkinson's Disease

Title: Nanoparticles as a therapeutic tool to restore lysosomal acidification impairments: implications for Parkinson's disease

Authors: *B. DEHAY[1], M. BOURDENX[1], J. DANIEL[2], E. GENIN[2], M.-L. THIOLAT[1], M. BLANCHARD-DESCE[2], E. BEZARD[1]
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Abstract: Impairment of autophagy-lysosomal pathways, an essential pathway for maintainance of proper protein and organelle quantity and quality within cells, is increasingly regarded as a major pathogenic event in neurodegenerative diseases, including Parkinson's disease (PD). Consequently, the possibility that enhancement/restoratoration of lysosomal-mediated degradation may prove beneficial for PD has been raised. We here report that poly (DL-lactide-co-glycolide) (PLGA) nanoparticles delivered to lysosomes within 24 hours after treatment, lower lysosomal pH and rescue chloroquine-induced cell death, but does not rescue from bafilomycin A-induced lysosomal pH loss. Treatment with PLGA nanoparticles of two disease-related *in vitro* models of PD with profound lysosomal dysfunctions, i.e. ATP13A2-knockdown dopaminergic cell lines and fibroblasts from PD patient carrying ATP13A2 mutations, restores lysosomal function and attenuates cell death. Moreover, PLGA nanoparticles are detected in

neurons after *in vivo* intracerebral injections, suggesting a translational opportunity. Taken together, our results reinforce the concept that restoration of normal lysosomal function might be beneficial in neurological disorders associated with lysosomal impairments.

Disclosures: B. Dehay: None. **J. Daniel:** None. **E. Genin:** None. **M. Blanchard-Desce:** None. **M. Bourdenx:** None. **M. Thiolat:** None. **E. Bezard:** None.

Poster

513. Parkinson's Disease: Neuroprotection

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 513.17/U9

Topic: C.03. Parkinson's Disease

Title: Enhancement of lysosomal biogenesis reverse A53T mutant α-synuclein induced toxicity

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Abstract: Increasing evidence indicates that impairment of lysosomal function may contribute to the pathogenesis of several neurodegenerative diseases, including Parkinson's Disease (PD). Autophagy-lysosome pathways alterations are observed in sporadic and familial forms of PD as well as in toxic and genetic rodent models of PD-related neurodegeneration. In this regard, enhancement or restoration of lysosomal-mediated degradation may prove beneficial for PD. Transcription factor EB (TFEB) has been recently identified as a new master regulator of lysosomal biogenesis and function and its activation has been shown to attenuate 1-methyl-4-phenylpyridinium -induced cell death in an *in vitro* setting. ZKSCAN3, a zinc finger family DNA-binding protein, has been recently characterized as a master transcriptional repressor of autophagy. To investigate the neuroprotective effect of enhancement of lysosomal biogenesis in an *in vivo* model, we used a genetic model of PD based on the overexpression of human mutant A53T synuclein (A53TSyn) by adeno-associated virus serotype 9 (AAV2/9) in the rat midbrain. We here report that unilateral AAV2/9-mediated overexpression of TFEB or repression of ZKSCAN3 through silencing strategy in the substantia nigra pars compacta (SNpc) reverse the toxicity of α-synuclein in the AAV2/9-A53TSyn rat model. Both overexpression of TFEB and silencing of ZKSCAN3 restored left paw use in cylinder test. Histological analyses performed at

the level of SNpc dopaminergic neuron cell bodies and striatal dopaminergic terminals were used to assess extent of dopaminergic lesion as well as occurrence of α-synuclein pathology. In order to assess the translational value of such strategies, the neuroprotective effect was also tested in a primate model of PD.

Disclosures: **M. Bourdenx:** None. **S. Dovéro:** None. **M. Bastide:** None. **G. Porras:** None. **Q. Li:** None. **A. Ballabio:** None. **E. Bezard:** None. **M. Vila:** None. **B. Dehay:** None.

Poster

513. Parkinson's Disease: Neuroprotection

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Program#/Poster#: 513.18/U10

Topic: C.03. Parkinson's Disease

Support: CONACYT fellowship 350320 YAV

Title: Beta-estradiol protects against 1-methyl-4-phenylpyridinium induced-Parkinsonism in rat by activation of endogenous antioxidant system

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Abstract: Steroids are bio-synthetized from cholesterol in different tissues including the brain. Specifically, estradiol (E2) can be produced from circulating testosterone or cholesterol by neurons and astrocytes. It is well known that E2 is involved in regulating neural development, synaptic plasticity and cell survival. Therefore, it has been linked with protection in neurodegenerative diseases including Parkinson disease (PD). However, the mechanism by which E2 protects dopaminergic neurons in PD it is not clear. PD is characterized by the progressive degeneration of dopaminergic neurons in substantia nigra pars compacta probably as a result of increased oxidative stress. The objective of the present work was to determine whether estradiol (given as beta-estradiol-3-benzoate) protects dopaminergic neurons through mechanisms related to antioxidant enzymes such as glutathione peroxidase (GPx), superoxide dismutase (SOD) and paraoxonase (PON) in a Parkinson's disease rodent model. Male Wistar rats (200-250 g) were gonadectomized and 30 days later, divided into 4 experimental groups: control group (C), MPP+ group (M), beta-estradiol-3-benzoate group (B) and beta-estradiol-3-

benzoate/MPP+ group (BM). The estrogen was dosed 100 µg every 48 h for 11 days (s.c.) in corn oil as vehicle. On day 6 of estradiol treatment, animals were infused with the neurotoxin MPP+ (10 µg/8 µl) or saline into the right striatum. Twenty four hours after the last dose of estradiol, rats were administered with apomorphine (1 mg/kg, s.c.) to evaluate circling behavior. The following day, animals were killed to determine dopamine in the right striatum. For the determination of antioxidant enzymes, rats were sacrificed 2 h after MPP+ injury. Estradiol treatment prevented by 80 % the decrease of striatal DA levels induced by MPP+. It also diminished by 50 % the turns induced by apomorphin in parkinsonic animals. Estradiol partially preserved glutathione (GSH) levels from oxidation by MPP+. These preliminary results suggest that estradiol protects from oxidative damage in the MPP+ PD experimental model.

Disclosures: Y. Aguirre-Vidal: None. **S. Montes:** None. **N.L. Chávez-García:** None. **A. Monroy-Noyola:** None.

Poster

513. Parkinson's Disease: Neuroprotection

Location: Halls A-C

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Program#/Poster#: 513.19/U11

Topic: C.03. Parkinson’s Disease

Support: Japan Society for the Promotion of Science Grant, B; 21380188

Japan Society for the Promotion of Science Grant, C; 22580339

Title: Botulinum neurotoxin A subtype 2 reduces pathological behaviors more effectively and confers greater safety than subtype 1 in a rat Parkinson's disease model

Authors: *M. ITAKURA[1], H. NAKAJIMA[1], T. KOHDA[2], T. KUBO[1], Y. SEMI[1], K. NISHIYAMA[1], Y.-T. AZUMA[1], S. KOZAKI[2], T. TKEUCHI[1]
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Abstract: Botulinum neurotoxin type A (BoNT/A) acts on cholinergic neurons, cleaves presynaptic protein SNAP-25 (a 25 kDa synaptosomal-associated protein) and interrupts the release of acetylcholine. The type A organisms have been classified into five subtypes (A1 to A5). Among them, BoNT/A1 and BoNT/A2 cleave SNAP-25 more efficiently than that of other subtypes. Recent studies imply the clinical benefits of BoNT/A in neurological disorders. Parkinson’s disease (PD) is characterized by imbalanced cholinergic hyperactivity in the

striatum. Some reports suggest the interruption of acetylcholine release by intrastriatal injection of BoNT/A in a rat Parkinson's disease model. It is, however, not clear which subtype of BoNT/As has the greatest efficacy for treatment with PD. Therefore, we compared the effect of BoNT/A1 with that of BoNT/A2 on pathologic rotation behavior which was induced by the treatment with methamphetamine in the 6-hydroxydopamine-induced PD rat model. Intrastriatal treatment with BoNT/A1 or BoNT/A2 significantly reduced the pathologic rotation behavior in a dose-dependent manner. While the highest tested dose of BoNT/A1 (1 ng) resulted in significant reduction of the pathologic behavior, all of tested does of BoNT/A2 (0.1, 0.5 or 1 ng) did well. We next examined the localization of cleaved SNAP-25 in the BoNT/A-treated striatum by immunohistological analysis. Treatment with 0.1 ng BoNT/A1 did not change the level of cleaved SNAP-25 in the striatum compared with that of the vehicle-treated group. In contrast, treatment with 0.1 ng BoNT/A2 significantly increased the level of cleaved SNAP-25. Interestingly, SNAP-25 cleaved by BoNT/A2 was strictly localized to the striatum on the injected side although SNAP-25 cleaved by BoNT/A1 diffused contralaterally. Furthermore, treatment with BoNT/A1 caused a significant reduction in body weight, while BoNT/A2 treatment did not. Taken together, these findings suggest that BoNT/A2 is beneficial for clinical application against Parkinson's disease, compared to BoNT/A1

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Poster

513. Parkinson's Disease: Neuroprotection

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Topic: C.03. Parkinson's Disease

Support: NIH/NINDS

Parkinson's Disease Foundation

Title: Mithramycin A blocks the induction of the pro-apoptotic gene Trib3 and protects from cell death induced by the Parkinson's disease mimetics 6-OHDA and MPP+

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Abstract: Parkinson's disease (PD) is characterized by the progressive loss of several neuronal populations, including dopaminergic neurons of the substantia nigra pars compacta (SNpc). We have reported in abstract form that the pro-death gene Trib3 is elevated in a subset of dopaminergic neurons of the SNpc of PD patients and that Trib3 is up-regulated in cellular models of PD, including neuronal PC12 cells and postnatally-derived dopaminergic ventral midbrain (VM DA) neurons treated with 6-OHDA, 1-methyl-4-phenylpyridinium (MPP+) or alpha-synuclein fibrils (aSYN). Trib3 overexpression is sufficient to induce death of neuronal PC12 cells and VM DA neurons. In cellular models of PD, Trib3 is also necessary to trigger neuronal death since Trib3 knock-down protects neuronal PC12 cells and VM DA neurons from 6-OHDA, MPP+ or aSYN and VM DA neurons from Trib3 null mice are partially protected against PD toxins. Taken together, our previous work suggests that Trib3 contributes to neuronal death in PD models. Understanding the regulation of Trib3 induction by PD-relevant stressors may identify novel pathways to target in order to reduce Trib3 levels. In the present work, we found multiple putative binding sites in the Trib3 promoter for Specificity protein 1 (Sp1) and related family members. Sp1 and its family members are zinc finger transcription factors that bind to GC-rich motifs of many promoters. Sp1 protein levels and DNA-binding activity are increased during oxidative stress. Mithramycin A (mTm) is an anti-neoplastic agent that blocks the binding of Sp1 family members to DNA and that has been shown to play a protective role against stress in different cellular models. We therefore tested the ability of mTm to block Trib3 induction and to protect in cellular models of PD. We found that mTm blocks activation of the Trib3 promoter and completely abolishes the increase of Trib3 mRNA levels induced by PD toxins in neuronal PC12 cells. We also demonstrated that mTm treatment protects VM DA neurons from cell death induced by 6-OHDA and MPP+. These data indicate that Sp1 binding sites in the Trib3 promoter are required for induction of Trib3 in cellular models of PD. Furthermore, interventions that interfere with Sp1 family binding, such as mTm, may be a potential therapeutic strategy to block Trib3 induction and arrest neuronal degeneration in Parkinson's disease.

Disclosures: **P. Aime:** None. **O. Levy:** None. **A.V. Rao:** None. **L.A. Greene:** None.

Poster

513. Parkinson's Disease: Neuroprotection

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Topic: C.03. Parkinson's Disease

Support: Northeastern University Tier 1 Interdisciplinary Grant

Michael J. Fox Foundation for Parkinson's Research

Title: Widespread expression of GDNF throughout rat brain after intranasal delivery of hGDNF plasmid nanoparticles

Authors: *A. E.-E. ALY[1], B. T. HARMON[1], O. SESENOGLU-LAIRD[2], L. PADEGIMAS[2], M. J. COOPER[2], B. L. WASZCZAK[1]
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Abstract: Glial cell line-derived neurotrophic factor (GDNF) gene therapy is a promising therapeutic approach for CNS disorders such as Parkinson's disease (PD) since it could provide a renewable source of GDNF within the brain while avoiding the need for repeated dosing. Current approaches largely rely on viral vectors to carry the gene into cells and most require direct intracranial injection. The immunogenicity of viral vectors and the invasiveness of surgical intervention are serious limitations. Thus, we are investigating the intranasal route of administration of PEGylated lysine 30-mer (PEG-CK30) DNA nanoparticles (NPs) encoding hGDNF. These NPs developed by Copernicus Therapeutics, Inc. compact single molecules of the expression plasmid and have minimal cross-sectional diameters of ~10 nm. We have shown that DNA NPs co-expressing enhanced green fluorescent protein (eGFP) and hGDNF (pUGG) successfully transfect neural cells *in vitro* and brain cells *in vivo*. The goal of this study was to assess *in vivo* transfection in brain after intranasal administration of Copernicus' hGDNF DNA NPs. GDNF ELISA was carried out on a series of rostral-caudal brain slabs 7 days post-treatment. Results showed significant increases in GDNF levels throughout the brains of rats given intranasal pGDNF relative to controls. To examine cellular transfection patterns, eGFP immunohistochemistry (IHC) was performed on brain sections from rats that received intranasal doses of pUGG NPs, naked pUGG, or saline. The number of cells expressing eGFP was significantly higher across brain regions in rats given intranasal pUGG NPs versus saline controls, with the highest number of eGFP positive cells in the midbrain. Double label IHC was also carried out for both eGFP and either rat endothelial cell antigen (RECA-1) or glial fibrillary acidic protein (GFAP). Most of the eGFP positive cells found in brain 7 days after intranasal delivery of pUGG NPs were immediately adjacent and abluminal to capillary endothelial cells staining for RECA-1. Preliminary results show a similar distribution of eGFP positive cells adjacent to capillaries enwrapped by GFAP positive astrocytic endfeet. This pattern suggests that pericytes may have been preferentially transfected after intranasal administration of these DNA NPs, consistent with distribution by the perivascular transport system. Collectively, these results confirm successful delivery and transfection of cells in rat brain after intranasal administration of Copernicus' DNA NPs, and they support the use of intranasal delivery of hGDNF NPs as a non-invasive means of gene therapy for PD and other CNS disorders.

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Poster

514. Motor Neuron Disease Therapeutics

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Program#/Poster#: 514.01/U14

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NIH grant R01NS073118-02

Burroughs Wellcome Fund CASI Award 1007274 (to S. V. Sarma)

NSF CAREER Award 1055560 (to S. V. Sarma)

Title: Neural restoration via loop-based reinforcement: A mechanism of therapeutic high frequency stimulation in Parkinson's disease

Authors: *S. SANTANIELLO[1], M. M. MCCARTHY[2], E. B. MONTGOMERY, Jr[3], J. T. GALE[4], N. KOPELL[2], S. V. SARMA[1]
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Abstract: Deep brain stimulation (DBS) is a clinically recognized treatment for movement disorders in Parkinson's disease but its mechanisms remain elusive. Two questions have hampered our understanding of the mechanisms of DBS so far: (1) Why is DBS therapeutic only when the frequency of stimulation belongs to a specific high range (i.e., 130-180 Hz)? (2) What is the fundamental mechanism that keeps high frequency DBS therapeutic even if the stimulation target is moved across distinct (and physiologically different) structures in the brain? To answer these questions, we developed a detailed computational model (880 single-compartment neurons) of the direct pathway in the motor loop, including the motor cortex, ventro-lateral thalamus, striatum, and internal globus pallidus, and we validated the neuronal activity of each structure with single unit recordings from non-human primates and rats, both in normal and parkinsonian conditions. Then, we used the model to study the effects of several DBS settings

(i.e., DBS frequency ranged from 20Hz to 180Hz, both regular and irregular DBS pattern) via numerical simulations. Regarding to 1), we show that, differently from what is currently hypothesized, the therapeutic effects of DBS do not entirely stem from local changes of the neuronal activity in the stimulation target but they also depend, in part, on the fact that the motor loop is a closed reentrant system. Due to the closed-loop nature, the perturbations induced by consecutive DBS pulses may travel along the system both forward and backward through multiple pathways, they can rendezvous, and positively overlap if the pulses are constantly spaced (i.e., DBS is regular) and close enough one to one another (i.e., DBS is high frequency). This suggests that (i) DBS globally impacts the entire loop, (ii) the therapeutic merit of clinically-used DBS settings depends on the anatomical properties of the treated system, and (iii) DBS in different individuals may require slightly different settings, which is consistent with clinical practice. Regarding to 2), we show that the rendezvous of consecutive DBS-elicited perturbations occurs in the striatum and may determine a dominant discharge pattern that percolates through the basal ganglia, projects towards the thalamus, and restores the normal function of the thalamo-cortical sub-system, which is primarily involved in the selective dis-inhibition of motor commands. Because the rendezvous exploits the closed-loop nature of the system and the fact that perturbations move both forward and backward, we show that the therapeutic effects may be preserved even though the entry point of the DBS input is moved along the loop.

Disclosures: S. Santaniello: None. **M.M. McCarthy:** None. **E.B. Montgomery:** None. **J.T. Gale:** None. **N. Kopell:** None. **S.V. Sarma:** None.

Poster

514. Motor Neuron Disease Therapeutics

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 514.02/U15

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: Judith and Jean Pape Adams Charitable Foundation

Prize4Life

Title: Effect of molecular tweezers treatment in a transgenic mouse model of amyotrophic lateral sclerosis

Authors: *C. V. FONTANILLA, B. CHAN, R. ROSALES, M. CHATTOPADHYAY, T. T. VU, A. ATTAR, J. S. VALENTINE, M. H. WIEDAU-PAZOS, G. BITAN
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Abstract: Amyotrophic lateral sclerosis (ALS) is an incurable, neurodegenerative disease characterized by progressive motor neuron loss, occurring in both sporadic and familial forms and leading to paralysis and ultimately death within 2–5 years after symptom onset. Currently, there are no effective treatments for ALS. Abnormal protein aggregation is thought to play an important role in the underlying pathogenic mechanisms of ALS, yet whether protein aggregation actually causes motor neuron death is an open question. The objective of this study was to examine whether prevention of protein aggregation leads to improvement in disease symptoms of ALS or lifespan. In this context, the best-characterized system has been the assembly of Cu/Zn-superoxide dismutase 1 (SOD1) into neurotoxic oligomers and aggregates. The current study investigated the effect of the "molecular tweezer" (MT), CLR01 – a broad-spectrum, "process-specific" inhibitor of amyloidogenic proteins' self-assembly and toxicity, in a transgenic mouse model. Initial data show that CLR01 inhibits both wild type and mutant SOD1 aggregation, dissociates pre-formed SOD1 fibrils and attenuates cell death in a neuronal cell line expressing the SOD1-G93A variant. Based on these data, we evaluated the effect of CLR01 administration on symptom onset, survival, limb muscle strength, and pulmonary function in male and female SOD1-G93A mice in a gender-balanced, investigator-blinded study. Daily treatment with CLR01 (0.5 or 5 mg/kg) from 50 days of age until death did not extend lifespan significantly in CLR01-treated mice as compared to saline-treated animals, regardless of sex. Around the time of onset, differences appeared among the three treatment groups, yet they disappeared after several days and overall, the differences in limb muscle strength, as assessed by wire test and Rotarod, or pulmonary function, as measured using unrestrained, whole-body plethysmography, were not statistically significant. The data suggest that although CLR01 treatment did not result in overall improvement in the animals over time, the treatment may have an effect on the onset of disease rather than its progression. Source of research **Support:** Judith and Jean Pape Adams Charitable Foundation and Prize4Life.

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Poster

514. Motor Neuron Disease Therapeutics

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Program#/Poster#: 514.03/U16

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: Crystal Ball for a Cure, Inc.

Title: Pur alpha: A potential therapy for ALS due to the C9ORF72 expanded repeat

Authors: *E. W. GODFREY[1], J. ORIANS[2], E. M. JOHNSON[3], D. C. DANIEL[3]
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Abstract: Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease that results in paralysis due to loss of motor neurons in the cortex and spinal cord. The most common genetic cause of ALS is an expanded hexanucleotide repeat (GGGGCC) in a non-coding region of the C9ORF72 gene, which encodes a protein involved in endocytosis and autophagy. Patients with this mutation have hundreds to thousands of copies of this repeat sequence. Repeat sequence RNA aggregates in nuclear foci that can be detected by fluorescent *in situ* hybridization (FISH). The mechanism by which the C9ORF72 repeat causes ALS is unknown, but a prominent hypothesis is that the repeat sequence in the RNA sequesters RNA-binding proteins. Disturbing the content of RNA-binding proteins in the cell can affect transcription, translation, and splicing of many mRNAs, resulting in expression of altered RNAs and reduction in RNA content. We hypothesize that overexpression of Pur-alpha, an abundant RNA- and DNA-binding protein that binds to GGGGCC repeats, or a peptide with a generic Pur family repeat may be therapeutic in C9ORF72 ALS. Overexpression of Pur alpha in *Drosophila* or neuronal cells with 30 copies of the repeat resulted in a reduction in neurodegeneration (Xu et al., 2012). We are using cells from C9ORF72 ALS patients to test the effects of Pur alpha on cellular pathology. We labeled autophagosomes in virally transformed lymphocytes from patients and controls with an antibody to p62, a protein that binds to the autophagosome membrane. These organelles are involved in removal of proteins that misfold and aggregate in the cytoplasm of neurons in ALS. Lymphoblasts from C9ORF72 patients had significantly more p62-labeled organelles than control cells. Transfection of Pur alpha or treatment of cells with the peptide resulted in a significant reduction in p62-positive puncta in C9ORF72 lymphoblasts, but did not reduce the amount of p62 protein in cells. These results suggest that Pur proteins and the peptide may reduce autophagy and ameliorate cellular pathology in this form of ALS. Additional experiments are underway to test the efficacy of the peptide in reducing cellular pathology in patient fibroblasts and in motor neurons differentiated from iPS cells of C9ORF72 ALS patients.

Disclosures: E.W. Godfrey: None. **J. Orians:** None. **E.M. Johnson:** None. **D.C. Daniel:** None.

Poster

514. Motor Neuron Disease Therapeutics

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Support: The ALS Association

The Muscular Dystrophy Association

Department of Defense ALS Research Program

Maryland Stem Cell Research Foundation

Michael S. and Karen G. Ansari ALS Center for Cell Therapy and Regeneration Research

NIH 5U01NS062713

Title: Role of the ALS spinal cord environment on human astrocyte engraftment and differentiation

Authors: *A. M. HAIDET-PHILLIPS[1], A. DORESWAMY[1], X. P. TANG[1], S. K. GROSS[1], J. T. CAMPANELLI[2], N. J. MARAGAKIS[1]
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Abstract: Amyotrophic Lateral Sclerosis (ALS) is a neurodegenerative disease characterized by the death of upper and lower motor neurons. Recent studies have highlighted a role for not only neurons, but also glia in disease pathogenesis. Therefore, strategies which replace or supplement the diseased astrocyte population with healthy astrocytes may protect against motor neuron degeneration. Our studies have sought to evaluate astrocyte replacement using glial-restricted progenitors (GRPs) which are lineage-restricted precursors capable of differentiating to astrocytes after transplantation. We have previously demonstrated that rodent GRPs can provide therapeutic efficacy in the ALS rat model. However, in all of our previous studies, the GRPs were transplanted into the diseased ALS spinal cord environment. The goal of our current study was to evaluate how transplantation to the diseased ALS spinal cord versus a healthy, wild-type spinal cord may affect the engraftment and differentiation of human GRPs. Human fetal-derived GRPs were transplanted into the spinal cord of either the ALS SOD1 G93A mouse model (n=19) or wild-type littermate mice (n=17). Mice were sacrificed for analysis at either the onset of ALS (90 days of age) or at the endstage of disease (>125 days of age). Immunohistochemical analyses

were used to quantify survival, migration, proliferation, and differentiation of the transplanted GRPs. No gross differences were found for any of these measures; however, proliferation of the human GRPs was slightly enhanced in the ALS spinal cord versus transplantation into the wild-type spinal cord. Between 80-90% of the transplanted GRPs differentiated into glial fibrillary acidic protein (GFAP)+ astrocytes with no differences found after transplantation to the ALS spinal cord versus the wild-type spinal cord. NanoString® gene profiling was used to analyze human-specific gene expression from the transplanted human cells in the mouse spinal cord. A panel of genes expressed by astrocytes as well as genes expressed by other cell lineages present in the central nervous system was assessed. No major differences were found in gene expression from the engrafted cells in the ALS spinal cord versus the wild-type spinal cord. Overall, these results suggest that the diseased ALS spinal cord environment does not inherently change the engraftment and differentiation of human GRPs. These findings are of interest given that human GRPs are currently in clinical development for spinal cord transplantation into ALS patients.

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Poster

514. Motor Neuron Disease Therapeutics

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Support: GACR 14-10504P

GACR 304/11/0189

GACR P304/12/G069

CZ.1.07/2.3.00/30.0018

Title: Amyotrophic lateral sclerosis affects the extracellular matrix: detection of its components could serve as a disease marker

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Abstract: Aims: 1) To study changes in the spinal extracellular matrix (ECM), particularly the perineuronal nets (PNNs), of transgenic rats harboring the superoxide dismutase 1 (SOD1) gene, during the course of ALS and after the application of human stem cells (SCs); 2) To evaluate the expression of ECM molecules in the cerebrospinal fluid (CSF) of rats and human patients (ALS and non-ALS). SOD1G93A rats were used as an *in vivo* model of ALS. Motor function of the rats was tested behaviorally during the whole course of the disease. Immunohistochemistry was used to visualize spinal PNNs and their components and to study the fate of the delivered human SCs. Western Blot was used to analyze spinal cord and CSF PNNs components at the presymptomatic, symptomatic and terminal stages of the disease in transgenic rats and wild-type (WT) littermates. The mRNA expression of PNN compounds were analyzed using RT-qPCR. Spinal cord tissues were used for *in situ* hybridization with several DIG-labeled chondroitin sulphate proteoglycans (CSPGs). Human CSF and serum were used for the evaluation of CSPGs content. SOD1G93A rats have an abnormal PNN structure around their spinal motoneurons, which appears with the onset of initial disease symptoms; this is indicated by disorganized Wisteria floribunda agglutinin staining and different immunohistological and western blot profiles of CSPGs. The spinal cord of SOD1 animals had a different expression of CSPG genes (Versican, Hapln1, Neurocan and Tenascin-R), whereas Aggrecan and Brevican profiles remained unchanged. The application of SCs preserved PNN structure and resulted in significantly better survival of motoneurons accompanied with higher motor activity. SC-treated rats lived significantly longer compared to sham-treated littermates. We found that CSPGs could be detected in the CSF of healthy and SOD1-rats, however at the symptomatic stage transgenic rats had a significantly higher amount of spinal and CSF CSPGs compared to the presymptomatic or age-related WT animals. At the terminal stage animals lost considerably more CSPGs in the spinal cord. We evaluated CSPGs in the CSF of patients with a confirmed diagnosis of ALS and non-ALS controls. Our results confirmed that CSPGs are present in the human CSF. We can conclude that the ECM is involved in the pathogenesis of ALS in SOD1-transgenic rat models of the disease. Detection of CSPGs in the CSF could potentially serve as biological markers for the diagnosis, assessment of treatment efficacy and prognosis of ALS. The administration of SCs safeguards PNNs and remodels the recipients' pattern of CSPGs gene expression. Human CSF contains CSPGs and some of them could serve as a biological marker of ALS.

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Poster

514. Motor Neuron Disease Therapeutics

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 514.06/U19

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: Fidelity Biosciences Research Initiative

Karyopharm Therapeutics, Inc.

Title: Preservation of forelimb function by UPF1 gene therapy: Potential regulation of UPF1 in TDP-43 toxicity

Authors: K. L. JACKSON[1], R. D. DAYTON[1], S. E. LOPEZ[1], E. A. ORCHARD[1], L. E. MAQUAT[2], S. JU[3], D. RINGE[4], G. A. PETSKO[5], *R. L. KLEIN[1]
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Abstract: Nonsense mediated mRNA decay is an RNA surveillance mechanism that requires upframeshift protein one (UPF1). We demonstrate here that UPF1 expression exerts protective effects in a rat paralysis model based transactive response DNA binding protein 43 kDa (TDP-43). TDP-43 is an RNA binding protein involved in the processing of RNA and DNA. In amyotrophic lateral sclerosis (ALS), TDP-43 abnormally aggregates into pathological lesions. Here we use an adeno-associated virus vector (AAV9) to express TDP-43 throughout the spinal cord of rats to induce reproducible paralysis of the limbs mimicking paralysis in ALS. We selected UPF1 for therapeutic testing based on results obtained from a genetic screen of yeast. The overexpression of human TDP-43 or UPF1 in the spinal cord was titrated to less than twofold the level of their endogenous rat counterpart. AAV9 UPF1 clearly improved overall motor scores in rats also expressing TDP-43. The gene therapy effect of UPF1 was specific and reproducible when compared to groups receiving TDP-43 combined with either an empty vector or a green fluorescent protein (GFP) vector, which controlled for vector dose or foreign transgene. The UPF1 treatment was relevant in that it maintained forelimb motor function in rats that would otherwise become quadriplegic. We observed a reduction in UPF1 expression in the spinal cord by TDP-43, suggesting that UPF1 dysfunction may have a role in TDP-43's toxic action. This work helps validate UPF1 as a novel therapeutic target for ALS and other TDP-43-related diseases and hints UPF1 and NMD involvement in underlying disease mechanisms.

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Poster

514. Motor Neuron Disease Therapeutics

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 514.07/U20

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: CAPES

CNPQ

DECIT/MS

FAPERJ

Title: Cell therapy in a murine model of amiotrophic lateral sclerosis: Functional and histological approach

Authors: ***I. B. PEREIRA**, F. GUBERT, A. DECOTELLI, M. SANTIAGO, R. MENDEZ-OTERO
Univ. Federal Do Rio De Janeiro, Rio De Janeiro, Brazil

Abstract: Amyotrophic lateral sclerosis is a fatal neurodegenerative disorder which affects motorneurons primarily leading to axonal retraction and cell death. The symptoms are tremors, spasms, loss of muscular strength and tonus until total loss of movement.Patient death occurs from 3 to 5 years after diagnosis. About 90% of the cases are sporadic, with unknown causes, and 10% have a genetic origin. In this work, we used the animal model of the disease and tested a possible therapy using bone-marrow mononuclear cells injected intravenously and/or intramuscular. These animals were evaluated in order to assess their motor capacity and their survival. We performed histological analyses to quantify the number of motorneurons, microglia and the denervation of motor plates one week after the last injection or at the end stage of the disease in order to evaluate if the therapy had any effect on these parameters. The cells remained on the injection site at least until seven days after the procedure, when a mild injury was made on

the target muscle. The therapies were able to delay the motor deficit with no effect on survival. The protocol combining on intravenously and intramuscular injections was able to reduce the motor plate denervation and microglial cell numbers, although it did not had any effect on neuronal survival. We concluded that the injection of bone-marrow mononuclear cells following the protocols studied was capable to reduce motor plate denervation, reduce microglial cell numbers and delay the motor deficit in this model. However, more studies need to be conducted in order to investigate whether the intravenous injection is required for a better result of the treatment.

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Poster

514. Motor Neuron Disease Therapeutics

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Program#/Poster#: 514.08/U21

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: Telethon foundation

Title: iPSC-derived neural stem cells act via kinase inhibition to exert neuroprotective effects in SMARD1

Authors: C. SIMONE[1], M. NIZZARDO[1], F. RIZZO[1], M. RUGGIERI[1], G. RIBOLDI[1], S. SALANI[1], M. BUCCHIA[1], P. RINCHETTI[1], F. PORRO[1], N. BRESOLIN[1], G. P. COMI[1], *S. CORTI[2]
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Abstract: Currently there is no cure for spinal muscular atrophy with respiratory distress type 1 (SMARD1), which is a hereditary motor neuron disease caused by mutations in the IGHMBP2 gene. The aim of this study was to demonstrate that neural stem cells (NSCs) derived from human induced pluripotent stem cells (iPSCs) have therapeutic potential in the context of SMARD1. Using human skin fibroblasts, iPSC cell lines were derived with a non-viral non integrating method, based on the expression of reprogramming factors with episomal vectors. The iPSCs were differentiated based on a protocol to stimulate neural stem cell and motor neuronal fate. The phenotype of these cells was analyzed by morphological, gene expression, and protein analysis. Finally, nmd mice (an animal model of SMARD1) were injected with iPSC-

purified NSCs by intraspinal cord injection. NSCs from iPSCs are self-renewing, multipotent, and can differentiate *in vitro* into the three neuroectodermal lineages, as well as into motor neurons. We show that, upon transplantation, NSCs can appropriately engraft and differentiate into the spinal cord of SMARD1 animals and, thus, ameliorate their disease phenotype by protecting their endogenous motor neurons. The effect of NSCs in the context of human disease was evaluated using human SMARD1-iPSCs motor neurons with significantly reduced survival and axon length. Notably, the disease features were ameliorated when they were co-cultured with NSCs. This observation is attributed to the production of neurotrophic factors and their dual inhibition of GSK-3 and HGK kinases. Our data support the role of iPSCs as a SMARD1 disease model and potential for cell-mediated therapies in motor neuron disorders using pluripotent stem cells.

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Poster

514. Motor Neuron Disease Therapeutics

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: Motor Neurone Disease Association Biomedical Research Project Grant

Thierry Latran Foundation

Title: Restoring function to paralysed muscles by transplanting optogenetically-active embryonic stem cell-derived motor neurons in mice

Authors: *J. BRYSON[1], C. BARCELLOS MACHADO[3], I. LIEBERAM[3], L. GREENSMITH[2]
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Abstract: Traumatic brain and spinal cord injury (SCI), as well as diseases that cause degeneration of motor neurons, such as amyotrophic lateral sclerosis (ALS), can block the transmission of motor signals from the CNS to skeletal muscles resulting in paralysis. Currently,

strategies to repair neurological damage or halt neurodegenerative diseases are extremely limited and any muscle paralysis that ensues from these disorders is typically permanent. We have recently developed a novel strategy that employed a synthesis of stem cell-derived neural tissue replacement and optogenetics, which results in restoration of controllable function to muscles that had been paralyzed (Bryson, *et al*. 2014). Following ligation of the sciatic nerve in mice, to model permanent muscle denervation, we transplanted stem cell derived motor neurons into specific nerve branches, distal to the injury site. These motor neurons had been modified to express the light-sensitive ion channel, channelrhodopsin-2 (ChR2) to enable optogenetic control of their activity, as well as glial derived neurotrophic factor (Gdnf) to promote their survival *in vivo*. The transplanted motor neurons not only robustly reinnervated the muscles that lie distally along the specific nerve branches, but, importantly, upon optical activation with a blue LED light-source, these neurons were capable of initiating finely controllable muscle contraction. We are currently developing this approach to enable long-term *in vivo* stimulation, using an implantable fiber-optic nerve cuff to chronically activate the muscle in awake mice and to promote maturation of neuromuscular junctions (NMJs), thereby preventing muscle atrophy. A similar approach has been shown to be effective in rats following viral expression of ChR2 in motor neurons (Towne, *et al*. 2013). Additionally, we are also investigating whether these modified motor neurons are capable of surviving and reinnervating muscles when transplanted into post-symptom onset $SOD1^{G93A}$ mice, which model ALS. These investigations are essential to advance the translational potential of this novel strategy to restore function to paralyzed muscles.

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Poster

514. Motor Neuron Disease Therapeutics

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: Boehringer Ingelheim Ulm University BioCenter (BIU) N2

International Graduate School in Molecular Medicine Ulm (IGradU)

Title: Therapeutic application of the monoacylglycerol lipase inhibitor KML29 in the SOD1G93A mouse model of amyotrophic lateral sclerosis

Authors: *A. WITTING[1], N. PASQUARELLI[1,2], J. HANSELMANN[1], D. WIESNER[1], C. PORAZIK[1,2], C. VOLANI[1], M. KARSAK[3], P. WEYDT[1], B. FERGER[2]
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Abstract: Amyotrophic lateral sclerosis (ALS) is a devastating neurodegenerative disease of the motor neuron system. Apart from riluzole, an anti-glutamatergic drug which prolongs survival of ALS patients up to 3 months, no treatment is available so far. Endocannabinoids like 2-arachidonoylglycerol (2-AG) induce neuroprotective and anti-inflammatory effects through cannabinoid receptor (CB) signaling. In ALS, 2-AG levels increase and CB_2 expression increases on activated microglia suggesting that these disease-associated changes might counteract the occurring neurodegeneration. Therefore, enhancing the disease-related 2-AG increase by pharmacological inhibition of the 2-AG-degrading enzyme monoacylglycerol lipase (MAGL) might be desirable for the treatment of ALS. In addition, inhibition of 2-AG degradation by MAGL is linked to decreases in arachidonic acid and prostaglandins, possibly leading to further benefits. In our study, we pharmacologically inhibited MAGL by the highly selective MAGL inhibitor KML29 in the low-copy $SOD1^{G93A}$ (B6SJL-Tg(SOD1*G93A)^{dl}1Gur/J) mouse model of ALS. For this purpose, we orally treated male and female mice with 10 mg/kg KML29 three times a week from postnatal day 150 until disease end stage. Furthermore, we monitored running wheel activity from postnatal day 140 and body weight from postnatal day. Post mortem, we investigated the endocannabinoid system and the inflammation by quantifying the expression of CB_1 and CB_2, the endocannabinoid-degrading enzymes MAGL and fatty acid amide hydrolase (FAAH), cytokines like tumor necrosis factor α (TNFα) and interleukin 6 (IL6) by qPCR. We also analyzed the principal endocannabinoid signaling molecules 2-AG and anandamide as well as the metabolites arachidonic acid and prostaglandins by liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). In female $SOD1^{G93A}$ mice, KML29 delayed the disease onset by 35 days, the occurrence of first pareses by 49.5 days and the disease end stage by 33 days. In male mice, the effect of KML29 on onset, first pareses and end stage was lower, producing a delay ranging from 16 to 18 days. Furthermore, KML29 had a beneficial effect on the disease-related body weight reduction. In addition, we could show that KML29 increased 2-AG levels particularly in the spinal cord, the diseased tissue in ALS. In summary, our therapeutic application of KML29 in the $SOD1^{G93A}$ mouse model evidences MAGL as a valuable target for the treatment of ALS.

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Poster

514. Motor Neuron Disease Therapeutics

Location: Halls A-C

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: KAKENHI 24659024

Title: Methyl pyruvate rescues mitochondrial degeneration by sigma-1 receptor mutation in amyotrophic lateral sclerosis

Authors: *K. FUKUNAGA, H. TAGASHIRA, Y. SHINODA
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Abstract: [Objective] Recently, sigma-1 receptor mutation (Sig-1R^{E102Q}) was discovered in gene of amyotrophic lateral sclerosis (ALS) patient. The Sig-1R^{E102Q} aggravated ER stress-induced motor neuronal cell death (Ann Neurol 2011;70:913). We here addressed whether treatment with methyl pyruvate, a mitochondrial substrate rescues the mitochondrial damages in neuro2A cells overexpressed mutant of Sig-1R^{E102Q}. **[Methods]** Neuro2A cells overexpressed Sig-1R^{E102Q} were treated with methyl pyruvate for 48 hrs and were assessed aggregation of Sig-1R^{E102Q} and cytoplasmic accumulation of TDP-43. **[Results]** Since σ_1R is critical for mitochondrial Ca^{2+} transport through IP_3 receptor and mitochondrial ATP production (Shioda and Fukunaga: J Biol Chem 2012;287:23318), overexpression of Sig-1R^{E102Q} decreased mitochondrial Ca^{2+} transport and ATP production. Dissociation of Sig-1R^{E102Q} from IP_3 receptor promoted the accumulation and aggregation of Sig-1R^{E102Q} in neuronal cytoplasm. We hypothesized that the impaired mitochondrial ATP production by the mutation causes neuronal cytoplasmic accumulation and inclusions of TDP-43. The methyl pyruvate treatment with 5 μM improved the ATP production and cytoplasmic accumulation of TDP-43 under ER stress condition. The methyl pyruvate treatment also prevented ER stress-induced apoptosis. **[Conclusion]** The decreased ATP production by Sig-1R^{E102Q} mutation is causative for the cytoplasmic accumulation and inclusions of TDP-43 under ER stress condition. The methyl pyruvate rescues the mitochondrial damages induced by Sig-1R^{E102Q}. **[Acknowledgements]** This work was supported by KAKENHI 24659024 (KF).

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Poster

514. Motor Neuron Disease Therapeutics

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NHI Grant RO1HD069562

Title: Microglia migration and interactions with dendrimer in brain in the presence of neuroinflammation

Authors: *F. ZHANG[1,2], E. NANCE[2,3], Y. ALNASSER[3], K. RANGARAMANUJAM[2], S. KANNAN[2,3]
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Abstract: Introduction: Neuroinflammation mediated by activated microglia has been implicated to play a crucial role in the pathology of neurodevelopmental disorders such as cerebral palsy (CP). There is no effective cure for cerebral palsy currently. We have previously reported hydroxyl terminated PAMAM dendrimer can target activated microglia in a rabbit model of cerebral palsy. Understanding the dynamics of microglia in the presence of CP and their interactions with dendrimer can help improve and sustain the efficacy of dendrimer-based nano-therapeutics. The objective of this study is to understand microglia function in a rabbit model of CP by evaluating their morphology change, migration pattern and their dendrimer uptake, through an *ex vivo* study of rabbit brain slices. **Materials and Methods:** We established brain slice culture platform from postnatal day 1 (PND1) rabbits to analyze microglial migration and morphology. Live imaging technique was then used for observing the morphology, migration and dendrimer interaction. We also quantitatively measured the microglial cells migration and interaction with dendrimer using MATLAB and Imaris Software. **Results and Discussion:** The measurement of LDH level and microglial surface to volume ratio indicated brain slices from healthy/CP neonatal rabbit have good viability and retained their physiologically activated/resting state in the first day of incubation, when all other experiments were conducted. Our study showed the microglia cells from the CP brain slices have higher mean square displacement <MSD> when comparing with the microglia from healthy brain slices, indicating the impairment of their normal function that can be related to surveillance under in the presence of brain injury. In the dendrimer uptake assessment, activated microglia cells from CP brain slices show not only a faster uptake during the first 4 hours, but also 2.5 fold higher uptakes after (at 1 hour) and 4 fold higher uptake (at 4, 12 hours) after exposure to dendrimer,

when comparing with those from healthy controls. These results suggest pro-inflammatory or 'activated' microglia in the CP brain has an increased ability of phagocytizing xenobiotics. **Figure 1.** (A) Mean square displacement <MSD> of microglia cells (B) Representative trajectories. **Conclusions:** This study showed that pro-inflammatory or 'activated' microglia may have an impairment of their normal function that can be related to surveillance. These results may also explain why dendrimers show selective uptake and retain *in vivo*, delivering drugs to the activated microglia cells, producing dramatic motor function improvements.

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Poster

514. Motor Neuron Disease Therapeutics

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: Margaret Q. Landenberger Research Foundation

Title: AAV-based GLT1 overexpression in the SOD1G93A mouse cervical spinal cord does not protect respiratory phrenic motor neurons, preserve histological or functional diaphragm innervation, or extend disease phenotype

Authors: *M. URBAN[1], K. LI[1], T. J. HALA[1], D. J. POULSEN[2], M. C. WRIGHT[3], A. LEPORE[1]
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Abstract: Amyotrophic lateral sclerosis (ALS) is characterized by relatively rapid degeneration of both upper and lower motor neurons, with death normally occurring 2-5 years following diagnosis primarily due to respiratory paralysis resulting from phrenic motor neuron (PhMN) loss and consequent diaphragm denervation. In ALS, cellular abnormalities are not limited to MNs. For example, decreased levels and aberrant functioning of the major central nervous system (CNS) glutamate transporter, GLT1, occur in spinal cord and motor cortex astrocytes of both humans with ALS and in SOD1G93A rodents, a widely studied ALS animal model. This results in dysregulation of extracellular glutamate homeostasis and consequent glutamate

excitotoxicity, a primary mechanism responsible for MN loss in ALS animal models and in the human disease. Given these observations of GLT1 dysfunction in areas of MN loss, as well as the importance of testing therapeutic strategies for preserving PhMNs in ALS, we evaluated intraspinal delivery of an adeno-associated virus type 8 (AAV8)-Gfa2 vector to the cervical spinal cord ventral horn of SOD1G93A ALS mice for focally restoring intraspinal GLT1 expression. AAV8 was specifically injected into the ventral horn bilaterally throughout the cervical enlargement at 90 days of age, a clinically-relevant time point coinciding with phenotypic/symptomatic disease onset. Intraspinal delivery of AAV8-Gfa2-GLT1 resulted in robust transduction primarily of GFAP+ astrocytes that persisted until disease endstage, as well as a 2-3-fold increase in total intraspinal GLT1 protein expression in the ventral horn. Despite this robust level of astrocyte transduction and GLT1 elevation, GLT1 overexpression did not protect PhMNs, preserve histological PhMN innervation of the diaphragm NMJ, or prevent decline in diaphragmatic respiratory function as assessed by phrenic nerve-diaphragm compound muscle action potential (CMAP) recordings compared to control AAV8-Gfa2-eGFP injected mice. In addition, AAV-based increase in GLT1 expression in the cervical spinal cord did not delay forelimb disease onset, extend disease duration (i.e. time from either forelimb or hindlimb disease onsets to endstage) or prolong overall animal survival. These findings suggest that focal restoration of GLT1 expression in astrocytes of the cervical spinal cord using AAV delivery is not an effective therapy for ALS.

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Poster

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: Department of Defense Therapeutic Idea Award

Title: Liposome-encapsulated H-ferritin improves survival in a SOD1 mutant mouse model of amyotrophic lateral sclerosis

Authors: *A. M. SNYDER[1], A. B. MADHANKUMAR[1], E. B. NEELY[1], E. RIZK[1], O. M. HESS[3], Z. SIMMONS[2], J. R. CONNOR[1]

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Abstract: The misregulation of iron and subsequent oxidative stress are consistent features shared between humans with Amyotrophic Lateral Sclerosis (ALS) and in animal models of the disease. The iron sequestration protein H-ferritin has ferroxidase activity and limits the toxic potential of iron, making it an attractive therapy to pursue in ALS. One of the disadvantages of most systemically-delivered treatments for neurological diseases is that they exert their biological effects not only at their target sites but also at peripheral tissue and cells. This often results in dilution of the agent below therapeutic levels to the target tissue; a way to increase efficacy and reduce the amount of agent administered is to utilize liposomal drug carriers. The objective of this work is to determine if infusion of liposome-encapsulated iron-poor H-ferritin has neurorescue properties in a murine model of ALS. Two types of liposomes were used: those that were not directed to any specific cell type and those that were directed to microglia by the presence of lipopolysaccharide (LPS). At 90 days of age, mice with the SOD1G93A mutation underwent surgery allow for continuous infusion of liposome-encapsulated H-ferritin into the lateral ventricle. Disease onset was assessed by performance on the rotarod apparatus, and endpoint was determined by the inability of the animal to right itself. A significant impact on lifespan occurred in mice treated with H-ferritin encapsulated by non-targeted liposomes, resulting in a 10.5 day extension of survival as compared to untreated SOD1G93A mice (median values). Specific delivery of H-ferritin to microglia was of limited benefit in extending lifespan. A plausible explanation for this outcome is over-stimulation of microglia by accessing them through the TL4 receptor. Indeed, histological examination of lumbar spinal cord sections indicated less extensive microglial activation at end-stage in the group of mice treated with non-targeted liposomes carrying H-ferritin as compared to the group of animals treated with the LPS-directed liposomes. Furthermore, the motor neurons that remained at end-state in the non-targeted liposomal group had thick, extensively branched projections: features not seen in the LPS-targeted group. Our intervention in the animal model is of particular relevance to the clinical population because our intervention occurs at the peri-symptomatic stage of the disease; this would correlate to the time in which human patients would begin to notice symptoms and seek treatment in the clinic. Therefore, our therapy may be of greater clinical benefit than those compounds tested while animals are asymptomatic.

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Poster

514. Motor Neuron Disease Therapeutics

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NIH Grant NS081426

NIH Grant NS069616

Title: Post-mortem assessment of potential ALS biological predictors: An analysis of 51 patient autopsies

Authors: *G. COAN[1], J. GLASS[2], C. S. MITCHELL[1]
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Abstract: In an effort to identify both biological predictors for the onset and progression of Amyotrophic Lateral Sclerosis (ALS) as well its clinically relevant cellular underpinnings, we perform a retrospective meta-analysis of 51 autopsies conducted on patients with a confirmed diagnosis of ALS. All autopsies were performed at Emory University Hospital. Both the Internal Review Boards of Georgia Institute of Technology and Emory University approved this study. The analysis was focused on 71 distinct parameters, ranging in nature from the temporal (patient survival past the onset of the first symptom of ALS, survival past the date of ALS diagnosis, ALS symptom onset age, etc.), to the microscopic (severity of loss of neurons in a variety of regions, findings of numerous immunohistochemical tests, severity of loss of Purkinje cells, cytoplasmic accumulation of TDP-43, etc.), to the gross anatomical (localized atrophy of the brain and spinal cord, occlusions, etc.). Numerical scales were developed for the classification of the non-quantitative parameters, such as the severity of the loss of neurons in a given region of the brain. A cross-correlational analysis was performed in order to identify and prioritize possible relationships among assessed measures and immunohistochemistry test outcomes. A number of unique and potentially insightful relationships were identified that could be of potential future research interest.

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Poster

514. Motor Neuron Disease Therapeutics

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NUS

MOE

Title: Dose-dependent neurotoxicity in mice expressing wild type or ALS-linked mutants of FUS/TLS is mediated by disruption in protein and RNA homeostasis

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Abstract: Abstract Mutations in two ubiquitously expressed nucleic-acid binding proteins, TDP-43 (TAR-DNA binding protein-43 KDa) and FUS/TLS (fused in sarcoma/translocated in liposarcoma), cause amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). Abnormal aggregates of these two proteins are present in inherited and sporadic ALS, FTD, and other neurodegenerative diseases, independent of mutations in either gene. We show here that broad expression within the nervous system of wild type or either of two ALS-linked mutants of human FUS/TLS produces progressive motor phenotypes accompanied by characteristic ALS-like pathology. FUS/TLS levels are demonstrated to be autoregulated, as expression of wild type or ALS-linked mutants of human FUS/TLS downregulates endogenous mouse FUS/TLS expression at both mRNA and protein levels. A modest increase in expression of FUS/TLS achieved by saturating the autoregulatory mechanism in homozygote or double heterozygote transgenic mice produces rapidly progressive neurological phenotypes and dose-dependent lethality. Increased expression of FUS/TLS inhibits autophagy and causes splicing defects and reduction of long pre-mRNAs similar to the alteration observed in knockdown of FUS/TLS. Genome-wide expression analysis reveals mis-regulation of genes involved in ALS pathogenesis. Taken together, our results demonstrate that (1) mice expressing FUS/TLS develop progressive motor deficits and early mortality, (2) a surprisingly small increase in increased expression of wild type FUS/TLS sharply accelerate neurodegeneration, providing a basis for FUS/TLS involvement in pathogenesis in the absence of mutation, and (3) disturbance in both protein homeostasis and RNA processing contribute to FUS/TLS-mediated toxicity.

Disclosures: S. Ling: None. **S. Da Cruz:** None. **S. Dastidar:** None. **S. Tokunaga:** None. **P. Parone:** None. **H. Ilieva:** None. **O. Platoshyn:** None. **D. Swing:** None. **L. Tessarollo:**

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Poster

514. Motor Neuron Disease Therapeutics

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Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 514.17/U30

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: Judith and Jean Pape Adams Charitable Foundation

The Paul and Harriett Campbell Fund for ALS research

Zimmerman Family Love Fund

The Robert Luongo ALS Fund

Title: H63D HFE modifies disease pathophysiology in animal models of amyotrophic lateral sclerosis

Authors: *W. NANDAR[1], E. B. NEELY[1], Z. SIMMONS[2], J. R. CONNOR[1]
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Abstract: The H63D HFE gene variant is present in as many as 30% of individuals with amyotrophic lateral sclerosis (ALS). H63D HFE is associated with disease processes implicated in ALS such as iron dyshomeostasis and oxidative stress. Thus, H63D HFE is proposed to be a genetic modifier for the risk of ALS. To determine how H63D HFE impacts ALS pathogenesis, we generated a double transgenic mouse line (SOD1/H67D) carrying the H67D HFE (homologue of human H63D) and SOD1(G93A) mutations. The double transgenic mice have shorter survival and accelerated disease progression. To determine mechanisms underlying accelerated disease observed in double transgenic mice, we examined parameters in the lumbar spinal cord of double transgenic mice at 90 days (presymptomatic), 110 days (symptomatic) and end-stage. Transferrin receptor and L-ferritin expression, both indicators of iron status, were altered in double transgenic and SOD1 mice starting at 90 days, indicating iron dyshomeostasis in these mice. However, double transgenic mice had higher L-ferritin expression than SOD1 mice suggesting higher iron in double transgenic mice. In addition to increased L-ferritin, double transgenic mice exhibited increased Iba-1 immunoreactivity and caspase-3 levels, indicating

increased microglial activation. Although both SOD1 and double transgenic mice had increased GFAP expression, the magnitude of the increase was higher in double transgenic mice at 110 days, suggesting increased gliosis in these mice. Increased hemeoxygeanse-1 and decreased nuclear factor E2-related factor 2 levels in double transgenic mice strongly suggest the accelerated disease process could be associated with increased oxidative stress. There was no evidence of TDP-3 mislocalization to the cytoplasm in double transgenic mice. However, there was evidence suggesting neurofilament disruption, which has been reported in ALS. Our findings indicate H63D HFE modifies ALS pathophysiology via pathways involving oxidative stress, gliosis and disruption of cellular function. Thus, we hypothesize that H63D HFE increases the risk of ALS by promoting the convergence of disease processes implicated in ALS.

Disclosures: W. Nandar: None. **E.B. Neely:** None. **J.R. Connor:** None. **Z. Simmons:** None.

Poster

514. Motor Neuron Disease Therapeutics

Location: Halls A-C

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NIH Grant NS081426

NIH Grant NS069616

Title: A meta-analysis of rotarod experimental protocols in the G93A SOD1 transgenic ALS mouse

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Abstract: The rotarod is a frequently used tool for the evaluation of disease progression in the G93A superoxide dismutase-1 (SOD1) transgenic mouse model of Amyotrophic Lateral Sclerosis (ALS). The methods employed by researchers in the field are not homogenous, which makes it difficult to directly compare results from different studies. The ability to compare ALS transgenic mouse studies, including cellular mechanistic studies and assessments of potential ALS treatment, is critical to forward progress in the field. By performing a meta-analysis from a pool of over 250 peer-reviewed articles, we assess rotarod as a predictor for disease onset, loss of

motor function, and survival for the G93A transgenic mouse. Moreover, we quantify the effect and dependency of experimental factors such as mouse sex, transgenic strain, and rotarod experimental parameters (actual speed, accelerating rotarod versus constant rotarod, etc.) on systemic output variables. We propose a regression model capable of comparing mouse survival times from different G93A experimental protocols, including mouse age at disease onset, rotarod parameters, and rotarod latency at a particular age.

Disclosures: S. Pfohl: None. **C.S. Mitchell:** None. **M. Halicek:** None.

Poster

514. Motor Neuron Disease Therapeutics

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: Judith and Jean Pape Adams Charitable Foundation

Paul and Harriett Campbell Fund for ALS research

Zimmerman Family Love Fund

Robert Luongo ALS Fund

Title: HMG-CoA reductase inhibitors negatively impact amyotrophic lateral sclerosis via mitochondrial mechanisms

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Abstract: Background: HMG-CoA reductase inhibitors (statins) may increase ALS risk or accelerate disease progression. Mitochondrial dysfunction contributes to ALS, and statins are known to perturb mitochondrial enzymes. Objectives: To determine if statins accelerate disease progression or decrease survival in ALS mice, and if statin-induced mitochondrial dysfunction mediates these effects. Methods: SOD1 G93A and wild type (WT) mice balanced for gender were used in accordance with IACUC guidelines. For the survival study, animals were administered daily 2 mg/kg simvastatin or vehicle beginning at disease onset, which was determined by rotarod, and allowed to reach endstage. Disease progression was measured by gripstrength. Plasma cholesterol and ferritin levels were measured by colorimetric assay or

ELISA. Western blot of mitochondrial fractions from gastrocnemius muscle and lumbar spine was conducted. For the mechanism study, animals were administered simvastatin in a similar fashion, gastrocnemius muscle and lumbar spine was collected at the symptomatic 120-day timepoint, and Western blots were conducted as above. For the rescue study, animals were administered daily 2 mg/kg simvastatin, 10 mg/kg coenzyme Q10, or the combination beginning at disease onset and allowed to reach endstage. SAS 9.3 or NCSS 9 was used for statistical analyses. Results: Animals administered simvastatin had physiologically relevant decreases in cholesterol. ALS mice had increased ferritin levels compared to WT mice. ALS mice administered simvastatin had accelerated disease progression as measured by gripstrength and decreased survival by Kaplan-Meier analysis compared to ALS mice administered vehicle. At 120 days and endstage, ALS mice had significantly decreased levels of complexes I and IV of the electron transport chain; cytochrome c; and the VDAC1 mitochondrial anion channel in gastrocnemius muscle and lumbar spine compared to WT mice. At 120 days, mice administered statin had increased complex I and VDAC1, as well as decreased cytochrome c, in lumbar spine compared to mice administered vehicle. At endstage, there were no statin-induced differences in ALS mice, possibly reflecting a floor effect at this advanced timepoint. Coenzyme Q10 administration did not rescue the statin-induced decrease in survival in ALS mice. Conclusions: Statins accelerate disease progression and decrease survival in ALS mice, effects mediated by perturbations in mitochondrial enzymes and which are not reversed by coenzyme Q10 supplementation. These results suggest statin therapy in patients with ALS should be investigated for a possible negative effect on disease progression.

Disclosures: X.W. Su: None. **W. Nandar:** None. **E.B. Neely:** None. **Z. Simmons:** None. **J.R. Connor:** None.

Poster

514. Motor Neuron Disease Therapeutics

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: Grant-in-Aid for Scientific Research on Innovative Areas from The Ministry of Education, Culture, Sports, Science and Technology (MEXT)

Title: Mechanism-based gene therapy for ALS using sporadic ALS model mice

Authors: *T. YAMASHITA[1], H. CHAI[1], S. TERAMOTO[1], S. TSUJI[2], K. SHIMAZAKI[3], S.-I. MURAMATSU[4], S. KWAK[1,5]
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Abstract: Motor neurons of patients with sporadic amyotrophic lateral sclerosis (ALS) express abundant GluA2 that has glutamine at the glutamine/arginine (Q/R) site. All of the GluA2 expressed in the central neurons have arginine at this site due to adenosine to inosine conversion (RNA editing), which is specifically catalyzed by adenosine deaminase acting on RNA 2 (ADAR2), and motor neurons devoid of ADAR2 activity express abnormally Ca^{2+}-permeable AMPA receptors that contain Q/R site-unedited GluA2. We developed conditional ADAR2 knockout (AR2) mice, in which the ADAR2 gene was targeted in cholinergic neurons including spinal motor neurons. Because lack of ADAR2 causes death of motor neurons by failure to edit this site in AR2 mice, it is likely that ADAR2 underactivity is involved in ALS-like progressive motor dysfunction resulting from death of motor neurons. Therefore, enhancement of ADAR2 activity in the motor neurons would be a therapeutic strategy for ALS, and we attempt to deliver the ADAR2 gene to the mouse motor neurons using an adeno-associated virus serotype 9 (AAV9) vector. One-shot intravenous injection of AAV9-ADAR2 vector rescued ALS-like progressive motor dysfunction and effectively prevented motor neurons from death in AR2 mice. Moreover, ALS-like TDP-43 pathology observed in motor neurons of untreated AR2 mice was rescued and motor neurons that expressed exogenous ADAR2 exhibited exclusively nuclear TDP-43 immunoreactivity in the treated AR2 mice. This AAV9-mediated ADAR2 gene delivery may therefore enable the development of a gene therapy for ALS.

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Poster

514. Motor Neuron Disease Therapeutics

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NIH grant 1 R15 AG022908-01A2

NSF grant DBI 0552517

Title: Effect of exercise training on skeletal muscle GDNF content and neuromuscular physiology in a mouse model of amyotrophic lateral sclerosis

Authors: *N. C. CARPP[1], A. M. GYORKOS[1], M. J. MCCULLOUGH[2], L. R. ROSARIO[3], J. M. SPITSBERGEN[1]
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Abstract: Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease accompanied by the loss of motor neurons, leading to paralysis and death. Glial cell line-derived neurotrophic factor (GDNF) promotes neuron health and function and has been proposed as a therapeutic treatment for ALS. GDNF protein expression in skeletal muscle is regulated by physical activity. The aim of this study was to determine if low intensity exercise would increase GDNF expression in skeletal muscle and slow degeneration of motor neurons in a mouse model of ALS. Following the first sign of disease onset, transgenic ALS mice were randomly assigned to one of three groups: sedentary control, involuntary low intensity exercise, and involuntary low intensity exercise with anti-GDNF injections, twice daily. Anti-GDNF injections were administered to determine if neutralizing GDNF inhibited the beneficial effects of exercise on the motor nervous system. Neurological score was tested daily throughout the exercise protocol, and animals were euthanized at 115 days of age. Lumbar spinal cord and soleus, extensor digitorum longus (EDL), and pectoralis major muscles were removed and analyzed for GDNF content by enzyme-linked immunosorbant assay. Immunohistochemical analysis of spinal cord was performed to assess motor neuron cell body count. Histological analysis of skeletal muscle was performed to examine endplate area and location of GDNF. Onset of neurological symptoms appeared to be delayed in the exercise group, when compared to the sedentary control and exercise with anti-GDNF treatment groups, although this was determined not to be statistically significant. GDNF content was not significantly affected in spinal cord, soleus, EDL, or pectoralis major, although there was a trend towards an increase in the exercised group compared to the control and anti-GDNF group. Histological analysis of spinal cord sections revealed a significant ($p<0.05$) increase in motor neuron number in the exercised animals (6.7 ± 0.7 ChAT positive cells per section) compared to the anti-GDNF treated animals (4.0 ± 0.6 ChAT positive cells per section). These results suggest that exercise protects against motor neuron loss and that neutralization of GDNF blocks this protective effect. This suggests that the neuroprotective effect of exercise may be related to the activity dependent expression on GDNF and that GDNF may have implications as a therapeutic agent in neurodegenerative disease. This work was supported by NIH grant 1 R15 AG022908-01A2 and NSF grant DBI 0552517.

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Poster

514. Motor Neuron Disease Therapeutics

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NIINDS

NUS

MOE

Title: Age-dependent and mutant-enhanced synaptic deficit caused by als-ftd-linked fus/tls

Authors: *S.-H. TYAN[1,3], Q. WU[2], D. W. CLEVELAND[4,5,3], E. H. KOO[1,3], S.-C. LING[2,3,4]
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Abstract: Amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD), two adult-onset neurodegenerative diseases, have traditionally felt to be different disorders with different mechanisms. Recent studies have shown that they share overlapping clinical, genetic, and pathological features. For example, mutations in FUS/TLS cause a disease with features of both ALS and FTD and where pathological inclusions contain FUS/TLS irrespective of the presence or absence of FUS/TLS mutations. In the companion abstract (Ling et al.,), we showed that transgenic mice expressing wild type or either of two ALS-linked mutants of human FUS/TLS produce ALS-like pathology and phenotype. To investigate the cortical phenotypes expected of these mutations, we extend here our analysis of hippocampus synaptic plasticity and found that FUS/TLS play a role in synaptic plasticity and contribute to synaptotoxicity. Specifically, we analyzed the basal synaptic transmission, pair-pulse facilitation (PPF) and long-term potentiation (LTP) in acute hippocampal slices of wild type and FUS mutants in young (6 months) and older (12 months) mice. Similar to the ALS-like phenotype and pathology, the mice expressing moderate levels of wild-type human FUS/TLS demonstrated age-dependent synaptic dysfunction (e.g., basal transmission and LTP) in hippocampus but were more severe in those hippocampi of mice expressing FUS mutants. Morphological and behavioral analyses of these mice are currently underway. Taken together, our results demonstrate that mice overexpressing wild type and mutant FUS/TLS develop age-dependent synaptic deficits that would be predicted based on the cortical symptoms seen in the human disease.

Disclosures: **S. Tyan:** None. **Q. Wu:** None. **D.W. Cleveland:** None. **E.H. Koo:** None. **S. Ling:** None.

Poster

514. Motor Neuron Disease Therapeutics

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Title: Kit is important for ALS mouse survival independent of mast cells

Authors: *K. A. STAATS[1,2,3], S. SCHONEFELDT[2], M. VAN RILLAER[2], L. VAN HELLEPUTTE[3], Y. LAMPI[2], W. ROBBERECHT[3], L. VAN DEN BOSCH[3], A. LISTON[2]
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Abstract: Amyotrophic Lateral Sclerosis (ALS) is a devastating neurodegenerative disease leading to progressive and lethal paralysis. The disease is multi-factorial and is characterized with specific motor neuron degeneration. Increasing growth factors in ALS can increase survival of ALS mice. C-kit is a growth factor, also present in the spinal cord, of which its role in ALS mouse survival is unknown. To dissect the role of c-kit in ALS we interbred SOD1G93A mice with kitw-sh/w-sh mice that have an approx. 70% decrease of kit gene expression in the spinal cord. kitw-sh/w-sh SOD1G93A mice survive shorter than SOD1G93A mice, while the amount of (motor) neurons at end stage is similar. By means of grip strength and nerve conductance analysis we show that kitw-sh/w-sh mice are less strong and have a slightly impaired CMAP latency, although the number of (motor) neurons is similar across genotypes. To exclude whether the decrease in survival of SOD1G93A mice by kitw-sh/w-sh is (additionally) by the lack of mast cells, we administered ketotifen to ALS mice. This did not alter disease survival. Decreasing kit gene expression in ALS mice is detrimental and our results imply that this is independent of mast cells. Additionally, we detect a decrease in strength and motoric EMG abnormalities in kitw-sh/w-sh mice. These mice are often used to assess the role of mast cells, also in neurodegenerative disorders. To conclude, our data confirms the protective role of growth factors in ALS, as decreasing kit by approx. 70% is detrimental in ALS mice.

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Poster

514. Motor Neuron Disease Therapeutics

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: Northwestern Weinberg Grant (MJS)

The Milton Safenowitz Post-Doctoral Fellowship from the ALS Association (JHJ)

NUCATS Translational Innovation Award (PHO,MCB)

Les Turner ALS Foundation (PHO)

Wenske Foundation (PHO)

Title: Specific transduction of corticospinal motor neurons by AAV2 upon direct motor cortex injection

Authors: *M. J. STANFORD[1], J. H. JARA[2], M. W. TU[2], Y. ZHU[3], M. C. BOHN[6], S. H. DEVRIES[6], P. H. OZDINLER[4,5,7]
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Abstract: The application of adeno-associated virus (AAV) in gene therapy has multiple advantages due to its long-term expression in the central nervous system (CNS) and low immunoreactivity in humans. Gene therapy strategies in CNS include Canavan's disease, Alzheimer's disease and motor neuron diseases such as amyotrophic lateral sclerosis (ALS). Targeting only the vulnerable neuron populations without affecting other neuron types within the cerebral cortex is a major obstacle for translational neuroscience. This applies to ALS, in which the corticospinal motors neurons (CSMN; a.k.a upper motor neurons) show selective vulnerability and progressive degeneration. In this study, seven different AAV serotypes that harbor the eGFP gene were tested for their ability to transduce CSMN upon direct injection into the layer V of the motor cortex. CSMN transduction was confirmed by immunocytochemistry to CTIP2 and with red fluorescent microsphere labeling of CSMN after injection into the corticospinal tract (CST). Large pyramidal neurons located in layer V showed higher tropism for

AAV2-2. In an effort to increase the selective transduction of CSMN by AAV, we used capsid proteins that are engineered, and different promoters to drive the eGFP expression. Our ongoing studies suggest that the choice of the promoter is critically important to enhance selectivity of gene expression in CSMN. Identification of AAV serotypes that transduce only a select set of neuron populations, even upon direct cortical injection is critically important to develop effective and long-term gene therapy approaches in the cerebral cortex.

Disclosures: **M.J. Stanford:** None. **J.H. Jara:** None. **M.W. Tu:** None. **Y. Zhu:** None. **M.C. Bohn:** None. **S.H. DeVries:** None. **P.H. Ozdinler:** None.

Poster

514. Motor Neuron Disease Therapeutics

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 514.25/V2

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NIH Director's Challenge Award

Title: A high-throughput genome-wide RNAi screen for novel modifiers of survival of motor neuron (SMN) protein levels

Authors: ***E. S. ARNOLD**[1], R. M. GIBBS[1], D. Y. KWON[1], S. E. MARTIN[2], E. BUEHLER[2], R. HUANG[1], B. REDAN[2], K. H. FISCHBECK[1], B. G. BURNETT[3]
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Abstract: Spinal muscular atrophy is caused by a deficiency of the survival of motor neuron (SMN) protein due to deletion or loss of function mutations in the survival of motor neuron 1 (SMN1) gene. The severity of the disease is modulated by the number of copies of the SMN2 gene, a near duplicate of the SMN1 gene that is present in humans. SMN2 produces an unstable, rapidly degraded gene product, but higher copy numbers of SMN2 produce sufficient full length SMN protein to rescue the SMA phenotype. The mechanisms that regulate SMN protein stability remain largely unknown. In collaboration with NCATS, we conducted a genome-wide RNAi screen to identify protective modifiers of SMN protein levels using a cell line stably expressing an SMN2 mini-gene that produces an SMN-luciferase fusion protein. We identified 106 genetic modifiers whose depletion increased the level of SMN-luciferase activity. These modifiers

represent genes involved in multiple cellular processes. In particular, we find high representation of genes involved in RNA processing, trafficking, and transport. Additionally, we identified genes in other functional categories, including post-translational modifications. Based on previous work from our group and others, SMN is known to be ubiquitinated and degraded by the UPS. The identification of additional genes involved in the ubiquitination of proteins may yield further insights into the post-translational regulation of SMN protein stability.

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Poster

514. Motor Neuron Disease Therapeutics

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 514.26/V3

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: AFM

Title: Regulation of SMN and other key pathogenetic events in Spinal Muscular Atrophy (SMA): Moving to RNA-Based treatment strategies

Authors: ***M. BUCCHIA**[1], M. NIZZARDO[1], C. SIMONE[1], F. RIZZO[1], G. ULZI[1], S. DAMETTI[1], A. RAMIREZ[1], E. FRATTINI[1], S. PAGLIARANI[1], N. BRESOLIN[1], F. PAGANI[2], G. P. COMI[1], S. CORTI[1]
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Abstract: SMA is a genetic motor neuron disorder caused by mutations of the survival motor neuron gene (SMN1). Currently, there is no effective treatment; however, regulation of SMN levels through antisense mechanisms has shown promising results. Induced pluripotent stem cell (iPSC) lines were reprogrammed from SMA/wild-type human fibroblasts, using a non-viral non-integrating method or lentiviral constructs. The iPSCs were differentiated based on a protocol to stimulate motor neuronal commitment. The phenotype of these cells was analyzed by morphological, functional, gene expression, and protein analysis. SMN levels and Fas activation were modulated *in vitro* in iPS and differentiated cells by antisense morpholino or engineered U1 small nuclear RNA (U1). Compared with wild-type subject iPSC lines, we confirm that SMA cultures present progressively fewer and smaller motor neurons, features that recapitulate human

disease. In the SMA motor neurons treated with antisense morpholino or U1, which increase SMN levels, these phenotypeswere rescued. During motor neuron development, SMA lines exhibited an up-regulation of Fas ligand-mediated apoptosis and increased caspase-8 activation. Importantly, this could be mitigated by Fas silencing. Our data support the utility of SMA iPSCs as an*in vitro* disease model and suggest that RNA-based therapies can be considered in the treatment of SMA.

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Poster

514. Motor Neuron Disease Therapeutics

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: ALS Canada

Title: Single chain antibodies against TDP-43 for treatment of ALS

Authors: ***S. POZZI**, K. DUTTA, C. GRAVEL, J. KRIZ, J.-P. JULIEN
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Abstract: TDP-43 represents a major component of ubiquitinated cytoplasmic inclusions of brain and spinal cord in sporadic ALS cases (1). Although different mutations have been identified in ALS patients, nowadays the real cause of TDP-43-induced pathology is still unknown thereby necessitating further studies. Recently, it has been reported that TDP-43 can induce inflammatory events by interacting with p65, the main subunit of NF-kB (2). The two proteins interact in spinal cords of sporadic ALS patients and of mice overexpressing the human WT or a mutated TDP-43 protein. Co-transfection experiments demonstrated that, by interacting with p65 through its RRM1 domain, TDP-43 acts as a co-activator of NF-kB. Moreover, it is now emerging that TDP-43 proteinopathy is mainly determined by RRM1 domain alterations. Indeed, oxidation (3) or misfolding (4) of the RRM1 domain trigger irreversible protein aggregation, impaired nucleic acid binding activity together with TDP-43 cytosolic mislocalization and motor neuron toxicity. The project is aimed to develop a possible therapy that would involve delivery of single chain antibodies (scFv), enclosed in viral vectors (5), that

can target specifically the cytoplasmic and nuclear RRM1 domain of TDP-43 with the dual function of (i) inhibiting TDP-43-mediated NF-kB hyperactivation during the early stages of the disease and (ii) preventing the cytoplasmic aggregation by the end stage in TDP-43 mutant mice. Among nine different clones of anti-RRM1 monoclonal antibodies analyzed, three of them are able to recognize TDP-43 and immunoprecipitate both TDP-43 and phospho-p65 in nuclear extract of LPS-activated microglial cells and, more importantly, to inhibit their interaction. Vectors, encoding for single chain antibodies obtained from the selected clones, have been produced by cloning the variable heavy (VH) and light (LH) chains together with Igk secretory signal and myc reporter. Single chain antibodies can be efficiently released in the medium of transfected cells. Moreover, their signal can be detected in cells treated with ScFv antibody containing media, confirming the capacity of these antibodies to cross the cellular membrane. ScFv antibodies against the RRM1 domain of TDP-43 will be used to establish a novel antibody-based therapy that specifically targets TDP-43-induced inflammation and proteinopathy. Moreover, it will constitute a new and useful tool to better elucidate the mechanisms of TDP-43 toxicity. **References** (1) Neumann M, et al. Science 2006. (2) Swarup V, et al. JEM 2011. (3) Chang C, et al. FEBS Letters 2013. (4) Shodai A, et al. JBC 2013. (5) Patel P, et al. Mol. Ther. 2013.

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Poster

515. Rett Syndrome

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 515.01/V5

Topic: C.06. Developmental Disorders

Support: NIH Grant NS081026

Rett Syndrome Research Trust Grant

Title: Mecp2: A novel regulator of tissue-resident macrophage function and survival

Authors: *J. CRONK[1], N. C. DERECKI[2], I. SMIRNOV[2], E. JI[2], G. T. NORRIS[2], N. CODDINGTON[2], S. UPADHYAY[2], Y. WOLF[3], T. H. HARRIS[2], S. JUNG[3], J. KIPNIS[1]
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Abstract: Methyl-CpG-binding protein 2 (MeCP2) is an important regulator of gene transcription, and mutations in MeCP2 are the major cause of Rett syndrome. Rett features prominent neurologic sequelae, thus research has been focused on the role of MeCP2 in neuronal function. Recent studies, however, have suggested the importance of glial cells in Rett syndrome. Our lab previously showed that wild type bone marrow transplant into Mecp2-null mice, including engraftment of wild type microglia-like cells into the brain, significantly improves lifespan and disease outcomes. Here we report Mecp2 as a novel regulator of function and survival in microglia and other tissue-resident macrophages. We found that multiple tissue-resident macrophage populations express high levels of Mecp2. Mecp2-null mice at the peak of disease display a 30% to 50% diminution of several tissue-resident macrophage populations, including microglia. In the periphery, impairment of tissue-resident macrophages in bone marrow is associated with neutrophilia, and eventual hematopoietic stem cell niche collapse. Attenuation of neutrophil load by anti-GCSF treatment slows down peripheral disease progression, extending lifespan by an average of about two weeks, but does not provide full rescue. In the brain, microglia are significantly reduced in numbers as disease progresses. Microglia from Mecp2-null mice exhibit abnormal morphology, as observed in fixed sections and confirmed with *in vivo* live imaging by two-photon microscopy. Selective expression of Mecp2 in tissue-resident macrophages using CX3CR1Cre in cross with Mecp2lox-stop results in a surprisingly robust disease arrest, significantly improving lifespan and disease scores, in addition to full rescue of somatic growth deficits. Further, specific rescue of microglia using tamoxifen-inducible CX3CR1CreER crossed to Mecp2Lox-stop results in significant increase in lifespan, implicating microglia specifically in the pathogenesis of Mecp2 deficiency. Together, these findings suggest a novel role for Mecp2 in tissue-resident macrophage survival and function, which may in part explain pathologies seen in Rett syndrome.

Disclosures: **J. Cronk:** None. **N.C. Derecki:** None. **I. Smirnov:** None. **E. Ji:** None. **G.T. Norris:** None. **N. Coddington:** None. **S. Upadhyay:** None. **Y. Wolf:** None. **T.H. Harris:** None. **S. Jung:** None. **J. Kipnis:** None.

Poster

515. Rett Syndrome

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Support: Simons Foundation

Translational Research Program- Boston Children's Hospital

IRSF- HeART

IDDRC

NIH

IRSF-Mentored Training Fellowship Program

Title: Chronic ketamine treatment ameliorates cortical function in Rett Syndrome mouse model

Authors: *N. PICARD[1], A. PATRIZI[1], A. J. SIMON[1], G. GUNNER[2], N. A. ANDREWS[2], M. FAGIOLINI[1]
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Abstract: Mice deficient in Mecp2 recapitulate many of the symptomatic features of Rett Syndrome (RTT), a devastating neurodevelopmental disorder affecting 1 in 10,000 girls. Visual circuits rapidly decline after an initial apparent normal development that directly correlates with the onset of the RTT phenotype. Genetic reduction of the excitatory NMDA receptor subunit GluN2A prevents such regression including the hyper-connectivity of parvalbumin-positive (PV) interneurons, loss of spontaneous and evoked neuronal activities associated with the decline in visual cortical function. Acute ketamine administration is sufficient to restore basal c-fos level in the forebrain of KO mice. Together, these findings suggest that targeting NMDA receptor may provide a new therapeutic approach for RTT. Here, we tested whether chronic ketamine treatment at low dosage (8mg/kg/day, i.p.) was sufficient to attenuate RTT visual cortical defects. Pharmacokinetic analysis of ketamine administration from plasma and brain samples demonstrated brain penetration (TMax=5min; Cmax=12uM post i.p. injection) and a rapid elimination from plasma (below LOD after 120 min). Two paradigms of administration were conducted: one starting from P15, when PV hyper connectivity first emerges, and one from P30, when visual function begins to regress and general RTT phenotypes appear. Both treatments were effective in ameliorating visual circuit deficits at adulthood (P55). *In vivo* single-units recordings of pyramidal cells, in response to visual stimulation, revealed that spontaneous and maximal evoked activities were restored to WT levels after ketamine treatment. Moreover, ketamine treated-Mecp2 KO mice exhibited improved optomotor visual acuity compared to controls. Notably, both treatments improve some of the RTT general phenotypes (like hindlimb clasping) in a subset of KO treated mice. However, only the ketamine treatment starting from P30 to adulthood enhanced performance on the accelerating rotarod test in KO mice. Overall, our results support the use of a therapeutic treatment targeting NMDA receptors to prevent or delay regression in a mouse model for RTT.

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Poster

515. Rett Syndrome

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Topic: C.06. Developmental Disorders

Support: NIH Grant MH085802

Simons Foundation Autism Research Initiative

Title: An isogenic human induced pluripotent stem cell model of Rett Syndrome reveals early alterations in microRNA expression patterns and downstream neuronal maturation

Authors: *D. A. FELDMAN[1], N. MELLIOS[1], S. D. SHERIDAN[2], S. KWOK[1], B. ROSEN[1], B. CRAWFORD[1], S. HAGGARTY[2], M. SUR[1]
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Abstract: Rett Syndrome (RTT) is an X-linked developmental disorder that is predominantly caused by mutations in methyl CpG-binding protein 2 (MECP2). Clinical features of RTT include a period of normal development lasting up to 6-18 months followed by stagnation of both neurological and general growth development. As this is a disorder of early development, it has proven difficult to elucidate phenotypes and/or molecular signatures at a pre-symptomatic stage. We have generated human induced pluripotent stem cell (iPSC) lines in which to study molecular and functional phenotypes *in vitro* at various stages of neural development. We took advantage of clonal X-inactivation from RTT patient lines in addition to shRNA knockdown of MECP2 in control lines to generate pairs of isogenic cell lines. Neural progenitors were generated from iPSCs via dual-SMAD inhibition and subsequently differentiated into cortical neurons, which exhibited intrinsic membrane excitability and spontaneous post-synaptic activity as observed via whole-cell patch clamp and functional calcium imaging. In this study, we aimed to identify key miRNA and molecular signatures at early stages of neuronal differentiation and in neural progenitors using these isogenic patient-derived and MECP2 knockdown cell lines. We screened for affected miRNAs and discovered a distinct miRNA family of interest that is robustly augmented in RTT patient-derived cell lines. Our results were subsequently confirmed

in MECP2 knockdown cell lines and embryonic mouse brain tissue. In parallel, components of the mitogen-activated protein kinase (MAPK) pathway that are known targets of the upregulated miRNA family were found to be misregulated in RTT lines. Such pathways are known to converge on key processes of neuronal development, thus leading to observed maturation deficits in early RTT neurons. Downregulation of aforementioned miRNAs in MECP2 knockdown neural progenitors ameliorated downstream neuronal maturation. Ongoing experiments are focused on elucidating the mechanisms of disease-related impairments of neurogenesis in a mouse model of RTT. Our data highly supports the hypothesis that the expression of a key miRNA family is altered in a human model of RTT and has significant effects on downstream signaling pathways implicated in RTT pathogenesis.

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Poster

515. Rett Syndrome

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Topic: C.06. Developmental Disorders

Support: Simons Foundation

Title: Gabaergic contributions to neural deficits in a genetic model of rett syndrome

Authors: J. M. MOSSNER, R. BATISTA-BRITO, *J. A. CARDIN
Dept. of Neurobio., Yale Univ., New Haven, CT

Abstract: Autism Spectrum Disorders (ASDs) affect approximately 1% of the world's population and are associated with cognitive deficits in perception, social interaction, and communication, all functions served by the cerebral cortex. Recent evidence has suggested an association of these disorders with a disruption of GABAergic inhibitory interneurons in the brain. Genetic studies of ASD patients have identified several candidate genes including MeCP2, a transcriptional regulator that plays a role in synaptic development. Mutations in MeCP2 are strongly associated with Rett Syndrome, an X-linked ASD characterized by loss of language skills, deficits in social interaction and sensory processing, repetitive movements, and mental retardation. MeCP2 is strongly expressed in GABAergic cells in the brain, and deletion of MeCP2 specifically from inhibitory interneurons replicates many behavioral phenotypes of the

global knockout, including repetitive behaviors, impaired social interactions, deficits in spatial learning, and increased PPI. However, the cell type-specific role of MeCP2 signaling and the contributions of specific interneuron populations to these phenotypes remain unclear. Using Cre-dependent conditional deletion of MeCP2 from targeted neural populations, we compared mice lacking MeCP2 specifically in parvalbumin- (PV) and somatostatin- (SOM) expressing interneurons. To assess the role of MeCP2 signaling in cortical activity patterns, we recorded spontaneous and visually evoked activity in primary visual cortex (V1) of awake behaving mice. In each experiment, we used tetrode arrays to record local field potentials (LFP) and isolated single units across all layers of cortex. In a subset of experiments, we used optogenetic tagging to record the activity of identified interneurons from target populations. We further used a series of behavioral assays to elucidate the contributions of each interneuron population to previously reported behavioral deficits in this genetic model. Our preliminary data suggest that deletion of MeCP2 from PV and SOM interneurons may contribute to discrete elements of the overall phenotype observed in this model of Rett Syndrome. We find that PV and SOM deletion mice exhibit different patterns of cortical activity and seizure incidence. Additionally, these groups show distinct patterns of locomotor activity during behavioral tests. Overall, our data suggest that MeCP2 signaling contributes to the function of the two largest populations of inhibitory interneurons, and deletion of this gene impacts both cortical network activity and behavior in a cell type-specific manner.

Disclosures: **J.M. Mossner:** None. **J.A. Cardin:** None. **R. Batista-Brito:** None.

Poster

515. Rett Syndrome

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Topic: C.06. Developmental Disorders

Support: NIH T32 AG000222-22

Simons Foundation

Title: A mass spectrometry based analysis of NMDAR regulation in Rett Syndrome

Authors: ***A. J. SIMON**[1], W. MAIR[2], H. CHEN[2], J. STEEN[2], M. FAGIOLINI[2]
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Abstract: Mutations in the transcriptional regulatory gene, MECP2, generate Rett Syndrome (RTT), a neurodevelopmental disorder characterized by an initial normal developmental period, followed by stagnation and regression at 12-18 months. We recently discovered that Mecp2 deletion engenders sensory processing defects in the visual system, including reduced spontaneous and evoked single-unit activity in visual cortex. Additionally, mRNA transcripts from visual cortex reveal a significant increase in the ratio of the NR2A to NR2B subunit of the N-methyl-D-aspartate receptor (NMDAR) in Mecp2 KO mice. Normalizing the NR2A/NR2B ratio genetically by creating a Mecp2 KO / NR2A-heterozygous mouse model is sufficient to rescue visual cortical defects. Importantly, NMDAR density in cortical tissue of post-mortem RTT patients is also increased. Together these data support the hypothesis that NMDAR subunit dysregulation may contribute to the sensory deficits of Rett Syndrome. To create a comprehensive picture of NMDAR subunit dysfunction in Rett Syndrome, we took a high resolution, unbiased, mass spectrometry-based approach. By immunoprecipitating (IP) the NR2A and NR2B subunits from visual cortex homogenate and using spectral counting techniques and/or heavy isotope labeled peptides for absolute quantification (AQUA), we analyzed protein quantity, post-translational modification (PTM), and interacting partners at the onset of regression in a Mecp2 KO mouse (postnatal day 30, P30). We found that, in the absence of Mecp2, NR2B and NR1 protein levels remained unchanged compared to WT levels while NR2A expression was increased. In addition, among the 60 C-terminal peptides that we were able to measure, 10% of them were differentially modified in the KO visual cortex. Interestingly, their PTM profile was renormalized in the Mecp2 KO / NR2A-heterozygous rescue mouse similar to a wild-type status. Finally, NR2A and NR2B co-immunoprecipitate analysis detected multiple kinases and proteins known to be localized at the synapse. Several proteins common to both the Mecp2 KO NR2A and NR2B co-IPs showed opposite abundance trends when compared to the WT levels. Overall, our data indicates that Mecp2 deletion induces a differential regulation of NR2A and NR2B subunits and postsynaptic composition. Using small molecules to target kinases and phosphatases that modify NR2A and NR2B could represent a new therapeutic approach to Rett Syndrome.

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Poster

515. Rett Syndrome

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Topic: C.06. Developmental Disorders

Support: Rett Syndrome Research Trust

AstraZeneca Pharmaceuticals

Title: Remacemide eliminates apneic breathing in a mouse model of Rett syndrome

Authors: R. J. MATHER[1], I. ADAMS[2], M. LANG[2], *J. DUNLOP[1], E. ABERG[3], M. C. QUIRK[1], D. M. KATZ[2]
[1]Astrazeneca - Neurosci. Innovative Med. Unit, Cambridge, MA; [2]Neurosci., Case Western Reserve Univ. Sch. of Med., Cleveland, OH; [3]Astrazeneca - Innovative Med. Unit, Stockholm, Sweden

Abstract: Patients with Rett syndrome (RTT), a severe disorder caused by mutations in the MECP2 gene exhibit postnatal neurological regression leading to motor and cognitive impairments, respiratory and autonomic dysregulation, seizures and autism-like behaviors. Studies in the Katz laboratory demonstrated that a sub-anesthetic dose of the non-selective NMDA antagonist ketamine acutely reverses cortical hypofunction in Mecp2 null and heterozygous mice (Kron et al., 2012), suggesting the potential utility of NMDA antagonists for treatment of RTT. Therefore, the present study was designed to evaluate more selective NMDA antagonists in rodents for their ability to alleviate symptoms likely to be of clinical relevance for RTT patients. In particular, we examined remacemide and its active metabolite, desglycinyl remacemide, which are moderate to low affinity (0.5 to 68 uM) NMDA channel blockers previously in clinical development for the treatment of epilepsy and ischemic brain damage. Behavioral and physiological testing in wildtype rats and mice (seizure suppression, EEG-biomarker, anxiety/depression) and Mecp2tm1.1Jae mutant mice (plethysmographic analysis of breathing) were used to characterize the potential utility of remacemide for the treatment of RTT symptoms. Studies in wildtype animals demonstrated that remacemide and desglycinyl remacemide attenuate electroconvulsive-induced seizures (MES-test) and reduce the acute stress response in a forced-swim test at doses (8-30 mg/kg) associated with measurable changes in cortical gamma band EEG. Furthermore, both compounds acutely reverse the apneic breathing phenotype in Mecp2 mice at doses corresponding to clinically relevant and well tolerated exposures. Specifically, a single intraperitoneal injection of remacemide (60 mg/kg) or desglycinyl remacemide (30 mg/kg) in 12-16 week old female heterozygous Mecp2tm1.1Jae mice reduced spontaneous apneas (defined as respiratory pauses longer than twice the average duration of expiration) to wildtype levels within 3 hours of treatment. These preliminary studies, combined with human data demonstrating positive effects of remacemide as an adjunctive treatment for refractory epilepsy (Baesag et al., 2001; Chadwick et al., 2002; Devinsky et al., 2002) suggest that remacemide or related compounds may have therapeutic value in the treatment of RTT patients. Supported by grants from the Rett Syndrome Research Trust and AstraZeneca Pharmaceuticals to DMK

Disclosures: **R.J. Mather:** A. Employment/Salary (full or part-time):; AstraZeneca. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); AstraZeneca. **J. Dunlop:** A. Employment/Salary (full or part-time):; AstraZeneca. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); AstraZeneca. **M.C. Quirk:** A. Employment/Salary (full or part-time):; AstraZeneca. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); AstraZeneca. **D.M. Katz:** 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.; AstraZeneca. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); AstraZeneca. **I. Adams:** None. **M. Lang:** None. **E. Aberg:** A. Employment/Salary (full or part-time):; AstraZeneca.

Poster

515. Rett Syndrome

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Topic: C.06. Developmental Disorders

Support: RSRT

HHMI

Title: Reversibility of symptoms in a mouse model of MECP2 duplication syndrome

Authors: *E. SZTAINBERG[1,2], H.-M. CHEN[3,4], J. W. SWANN[3,4], H. Y. ZOGHBI[1,2,4,5]
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Abstract: *MECP2* duplication syndrome is a condition that manifests almost exclusively in males and is characterized by autism, intellectual disability, anxiety, hypotonia, epilepsy, recurrent respiratory tract infections and premature death. The disease is the result of duplications of genetic material on chromosome Xq28 with minimal region of overlap spanning

MECP2 and *IRAK1*. Mice overexpressing human *MECP2* at 2X (*Mecp2*-Tg1) replicate many phenotypes of the duplication syndrome including hypoactivity, increased anxiety, decreased social behavior, and abnormal motor coordination. Reversibility in adult mice has been demonstrated in a handful of mouse models of neurodevelopmental disorders including Rett syndrome, but whether the duplication is treatable remains unknown. To determine whether the phenotypes of *MECP2* duplication are reversible upon normalization of MeCP2 levels, we generated and characterized a new mouse model that over-expresses a conditional allele of *Mecp2* that could be deleted in the adult animal. Upon normalization of MeCP2 in 8 weeks symptomatic mice, several phenotypes were rescued including hypoactivity in the open field (n = 10-15, $P = 0.0016$), anxiety in the elevated plus-maze test (n = 10-15, $P = 0.03$), social behavior in the 3-chamber test (n = 10-15, $P = 0.05$), and motor coordination in the rotarod (n = 10-15, $P = 0.0003$). In addition, genes abnormally expressed in the brain of *MECP2*-overexpressing mice such as Crh, Gamt, Agrp, Prl2c2 and Rcor2, were normalized. These data demonstrate that developmental overexpression of MeCP2 in mice does not irreversibly damages neural function, and suggests that correcting the expression level of MeCP2 can potentially reverse some aspects of the *MECP2* duplication syndrome pathology. Funding: Rett Syndrome Research Trust (RSRT) and Howard Hughes Medical Institute (HHMI).

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Poster

515. Rett Syndrome

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Support: Kakenhi 23500381

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The Japan Epilepsy Research Foundation Research Grant

The Mother and Child Health Foundation Research Grant

Title: Funcitonal analyses of the CDKL5, a causative gene for neurodevelopemental disorders

Authors: *T. TANAKA[1], A. WATANABE[1], M. HAGIWARA[1], T. MURAKAMI[1], M. MIZUGUCHI[1], K. TAKAO[2], T. MIYAKAWA[2], S. KOBAYASHI[3], T. MANABE[3], M.

FUKAYA[4], H. SAKAGAMI[4], K. OKUDA[1]
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Abstract: The Cyclin-dependent kinase-like 5 (CDKL5) gene encodes for a serine-threonine kinase sharing homology to Mitogen-activated kinases (MAPKs) and Cyclin-dependent kinases (CDKs). Recently, mutations in the CDKL5 gene have been identified in the patients with neurodevelopmental disorders associated with epilepsies, such as West syndrome or atypical Rett syndrome, suggesting its critical role in neurodevelopment. However, neither its molecular functions or pathomechanisms caused by its mutations are largely unknown. Aiming to elucidate these problems, we have taken multidimensional strategies, combining an unbiased interactome approach and a targeted loss-of-function (LOF) approach. For the interactome approach, we performed the yeast two-hybrid screening and identified several CDKL5 interacting proteins. For the LOF approach, we have generated the Cdkl5 knockout mouse and analyzed the neurological phenotypes. The combination of these approaches suggested us possible mechanisms of CDKL5 regulating neural functions during development.

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Poster

515. Rett Syndrome

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Support: IRSF Basic Biomedical

NSERC Discovery

Title: Altered nicotinic receptor expression and nicotinic receptor mediated changes in open field locomotor behaviour in a mouse model of Rett syndrome

Authors: D. MCPHEE, A. RENDA, J. LEUNG, *K. R. DELANEY, R. NAHSMI
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Abstract: Rett syndrome (RTT) results from loss of function of the X-linked transcription factor MECP2. In the Jaenisch RTT mouse model, a deletion of exon 3 eliminates the methylated DNA binding domain, leading to loss of MeCP2 function. Several studies have indicated that cholinergic hypofunction is one of the consequences of MeCP2 mutation in mice and humans. In WT mice, acute nicotine (NIC) decreases the open field movement (OFM) of males and females in a dose-dependent manner, with reduced movement evident at 1 mg/kg and immobility at 2 mg/kg. We therefore tested whether injection of NIC would induce similar behavioural effects in RTT mice. NIC injected male RTT mice showed significantly increased OFM compared to saline injection at 0.5 mg/kg, no difference at 1 mg/kg, with a large decrease at 2 mg/kg. Responses in presymptomatic (≈ 6 wks) female RTT mice were variable but nonetheless showed less suppression of locomotion at 1mg/kg compared to WT females. qRT-PCR of thalamus and midbrain tissue of male mice showed a 3.6-fold reduction in α4 nAChR expression in the RTT brain compared to WT littermates ($p=0.0054$, n= 5 WT; 5 RTT). We therefore tested the effect of TC-2559 (Tocris), an agonist with selectivity for α4 and α6 containing receptors. WT OFM was suppressed by TC-2559 above 1 mg/kg with saturating effects at 2.5 mg/kg but, unlike NIC, complete locomotor suppression was not produced by doses up to 10 mg/kg. Analysis of locomotor paths taken by WT in the test chamber revealed significant changes in pattern. Normally, mice circulate mainly around the perimeter of the test chamber reversing direction occasionally and crossing the centre at 10-20 sec intervals. WT mice injected with TC-2559, in addition to walking slower and stopping more, increased the proportion of time spent in the centre, away from the walls. RTT mice injected with 2.5 mg/kg showed a dramatic doubling of average velocity during the 5 min post injection test period with no change in the pattern of locomotion. Seizure activity was not observed in response to NIC or TC-2559 at the doses used. Our data suggest that the pathophysiology of RTT may be influenced by a decreased expression of the α4 (and/or α6) nAChR subunits resulting in altered response to nAChR agonists in the RTT brain.

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Poster

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Title: Unstable dendritic spines in a mouse model of CDKL5 disorder

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Abstract: The X-linked gene cyclin-dependent kinase-like 5 (CDKL5) is mutated in many severe neurodevelopmental disorders, including some forms of atypical Rett syndrome. It has been suggested that CDKL5 could interact with synaptic proteins important for the organization of the postsynaptic density and dendritic spine morphology, however an *in vivo* analysis of the role of CDKL5 in dendritic spine dynamics and synaptic molecular organization is still lacking. To answer these question we performed *in vivo* two-photon imaging of dendritic tufts of layer V pyramidal neurons in the somatosensory cortex of male CDKL5 null mice. We found that adult mutant mice (imaged from P50 until P80) show a significant reduction in spine density that remained substantially stable during the observation period. Short-term spine turnover was unaffected, however the percentage of stable spines persisting for one month was significantly reduced. Spine deficits were accompanied by synaptic deficits consisting in a reduction of the expression of synaptic PSD95 and impaired LTP maintenance. The analysis of young CDKL5 null mice (P27-P28) revealed that spine density and levels of synaptic PSD95 already begin to be reduced at this age. Moreover, repeated *in vivo* imaging showed a dramatic increase in short-term spine elimination but normal spine formation, suggesting a crucial role of CDKL5 in spine stability in developing mice. To explore a possible therapeutical approach to reverse synaptic deficits, we administered IGF1 to juvenile mutant mice and we found that both spine density and spine loss rate were rescued to control levels. These data demonstrate that dendritic spine stabilization is a cellular process dramatically affected by CDKL5 deletion. Loss of stabilization, albeit with different time constants, is present both in juvenile and adult animals suggesting that CDKL5 role in synaptic maintenance is not restricted to development. Moreover, our data suggest that IGF1 treatment could be a promising candidate for clinical trials in CDKL5 patients.

Disclosures: G. Della Sala: None. **E. Calcagno:** None. **M. Giustetto:** None. **G. Chelini:** None. **T. Pizzorusso:** None. **E. Putignano:** None. **G. Ratto:** None. **E. Amendola:** None. **C. Gross:** None.

Poster

515. Rett Syndrome

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 515.11/V15

Topic: C.06. Developmental Disorders

Support: NIH Grant NS057398

Autism Speaks

Title: Abnormal arousal to auditory stimulation in Mecp2 mutant mice is reversed by treatment with LM22A-4, a small molecule partial TrkB agonist

Authors: *D. M. KATZ[1], M. LANG[1], I. ADAMS[1], F. M. LONGO[2]
[1]Dept Neurosciences, Case Western Reserve Univ., Cleveland, OH; [2]Dept. of Neurol. and Neurolog. Sci., Stanford Univ. Sch. of Med., Stanford, CA

Abstract: Complex respiratory disturbances are a prominent feature of Rett syndrome (RTT), a severe neurodevelopmental disorder caused by mutations in the MECP2 gene. Moreover, the severity of breathing dysfunction in RTT patients is strongly influenced by behavioral state, as respiratory phenotypes worsen when patients are agitated and improve with relaxation or sleep (reviewed in Ramirez et al., 2013). However, mechanisms that underlie behavioral state-dependent respiratory dysfunction in RTT are unknown and no treatments are currently available. We therefore sought to determine if breathing abnormalities associated with behavioral arousal could be modeled in Mecp2 mutant mice and if so, whether such abnormalities might be useful endpoints for preclinical evaluation of potential RTT therapeutics. To approach this issue we compared the respiratory component of the orienting reflex (OR) in wildtype (Wt) and Mecp2tm1.1Jae null mice. The OR, which is elicited by sensory stimuli that are sub-threshold for evoking a startle response but sufficient to draw attention to the stimulus is normally accompanied by a transient increase in breathing frequency (Nalivaiko et al., 2012). 6-8 week old animals were exposed to a brief auditory stimulus (50 ms, 80 dB white noise) during plethysmographic recording of respiration. Wt mice exhibited a transient increase in respiratory frequency in response to the auditory stimulus that returned to baseline within a few seconds. In contrast, null mice exhibited an increase in frequency that was significantly larger than Wt and remained significantly elevated above baseline for at least one minute after the stimulus. We therefore sought to determine whether or not the arousal breathing phenotype in nulls could be ameliorated by activation of the BDNF/TrkB signaling pathway, which we previously showed reduces excitability in the brainstem respiratory network (Katz, 2014). To approach this issue,

mice were treated with saline or LM22A-4 (150 mg/kg, i.p.), a BDNF loop domain mimetic with partial TrkB agonist activity (Massa et al., 2010), one hour prior to testing. LM22A-4 treatment completely restored the breathing response in nulls to Wt levels and had no effect in Wt mice. These data demonstrate that Mecp2 mutants exhibit an arousal breathing phenotype reminiscent of behavioral disruptions of breathing in RTT and suggest that treatments aimed at restoring BDNF/TrkB signaling may be effective at normalizing state-dependent changes in respiration in RTT patients. COI: F.M. Longo has intellectual property interest in LM22A-4 and equity interest in PharmatrophiX, a company developing small molecule ligands.

Disclosures: D.M. Katz: None. **M. Lang:** None. **I. Adams:** None. **F.M. Longo:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); PharmatrophiX.

Poster

515. Rett Syndrome

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 515.12/V16

Topic: C.06. Developmental Disorders

Support: NSC99-2320-B-004-001-MY2

NSC101-2320-B-004-003-MY2

Title: Loss of MeCP2 in forebrain GABAergic neurons modulates striatal dopamine synthesis in a region-specific and non-cell autonomous manner

Authors: *W.-L. LIAO, F.-C. KAO, Y.-B. HUANG
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Abstract: The methyl-CpG binding protein 2 (MeCP2) is highly expressed in mature neurons and is involved in regulating target gene transcription. Mutations of *MECP2* gene cause more than 90% cases of Rett syndrome (RTT), a neurodevelopmental disorder featured by striking psychomotor dysfunction. Mice lacking MeCP2 recapitulate symptoms of RTT, including late onset hypoactivity and deficits in motor coordination and motor skill learning. We previously found that psychomotor deficits in *Mecp2*-null mice are associated with aberrant dopamine content in the striatum, which is composed of more than 98% GABAergic neurons. To investigate the causal roles of MeCP2 in modulation of striatal dopamine content, we selectively

removed MeCP2 in the forebrain GABAergic neurons, predominantly in the striatum, and measured dopamine content by HPLC. We uncovered that the amount of dopamine was significantly reduced in the rostral striatum (ST-r), increased in the caudal striatum (ST-c), but not altered in the ventral midbrain (VMB) of *Mecp2* $^{flox/y;Dlx5/6\text{-}Cre}$ (cKO) mice compared to littermate controls. To study the mechanism underlying the dopamine alterations, we next examined the protein expression of the rate-limiting enzyme for dopamine synthesis, tyrosine hydroxylase (TH) and its active form, phosphorylated TH at serine 40 (pTH-Ser40), in the striatum and VMB. We found that the total amount of TH protein remained unchanged in the ST-r, but increased in the ST-c of cKO mice. The level of pTH-Ser40 that indicates TH activity, however, was significantly reduced in the ST-r but increased in the ST-c of cKO mice. No change was found in total TH or pTH-Ser40 levels in the VMB of cKO mice compared to controls. Given that the phosphorylation of TH is inhibited by activation of dopamine D2 receptor (DRD2), we also examined the expression of DRD2 protein in the striatum of cKO mice. Consistently, the DRD2 expression was selectively elevated in the ST-r of cKO mice. Together, these results suggest that loss of MeCP2 in forebrain GABAergic neurons leads to region-specific alterations of protein expression of DRD2 and TH, as well as altered enzymatic activity of TH and ultimate changes of dopamine content in the striatum, without changing the dopamine features in the VMB. Therefore, MeCP2 in the striatal GABAergic neurons non-cell autonomously modulates dopamine synthesis in a sub-region specific manner.

Disclosures: W. Liao: None. **F. Kao:** None. **Y. Huang:** None.

Poster

515. Rett Syndrome

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Program#/Poster#: 515.13/V17

Topic: C.06. Developmental Disorders

Support: NIH Grant NS072128

Title: Identification of a novel pathway involved in MECP2 disease-relevant cellular phenotypes using *Drosophila*

Authors: *A. WILLIAMS[1], C. DUCH[1,2]
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Abstract: Methyl-CpG binding protein 2 (MECP2) is a widely abundant, multifunctional regulator of gene expression with highest levels of expression in mature neurons. In humans, both loss-and gain-of-function mutations of MECP2 cause mental retardation and motor dysfunction classified as either Rett Syndrome (RTT, loss-of-function) or MECP2 Duplication Syndrome (MDS, gain-of-function). There are currently no effective treatments for these conditions. At the cellular level, misregulation of MECP2 leads to both synaptic and dendritic defects; however, the underlying molecular pathways and cellular mechanisms are unknown. Here, we have used *Drosophila* as a model system to investigate the mechanisms affected by MECP2 gain-of-function involved in the regulation of dendritic growth. We have previously shown that expression of human MECP2 (hMECP2) in *Drosophila* leads to impairments in dendritic branching in an identified flight motoneuron (MN5), and used RNA-sequencing technology to determine genes differentially expressed with hMECP2 expression that may be involved in this process. We have subsequently identified Kibra, a gene associated with learning and memory in humans, as a top target from this screen. Kibra also activates the Hippo kinase cascade, a pathway involved in the regulation of dendritic growth in both *Drosophila* and mammals. We used quantitative PCR to confirm upregulation of kibra with expression of hMECP2, and have found that RNAi mediated knockdown of kibra partially rescues hMECP2 induced dendritic defects in MN5. We find that kibra knockdown does not increase dendritic growth independently of hMECP2 expression. Furthermore, over-expression of kibra alone does not phenocopy the hMECP2 induced cellular phenotype in MN5. Thus, we conclude that increased expression of kibra is necessary for the dendritic reduction with hMECP2 over-expression, but not sufficient to disrupt dendritic morphology alone. We are currently working to validate these findings in mouse primary neuron culture. The investigation of MECP2 targets identified in *Drosophila* may ultimately lead to novel therapeutic strategies for RTT and MDS.

Disclosures: **A. Williams:** None. **C. Duch:** None.

Poster

515. Rett Syndrome

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 515.14/V18

Topic: C.06. Developmental Disorders

Support: The Health Sciences Centre Foundation (HSCF)

Scottish Rite Charitable Foundation of Canada 10110 (SRCFC)

Title: MeCP2 isoforms and neurological disorders

Authors: *M. RASTEGAR
Biochem. and Med. Genet., Univ. of Manitoba, Winnipeg, MB, Canada

Abstract: MeCP2 is an important protein in brain, and its altered expression causes neurodevelopmental disorders including Rett syndrome and autism. Alternative splicing of a single gene gives rise to two protein isoforms (MeCP2E1 and MeCP2E2). Deregulation of either isoform is implicated in neurological complications and impaired brain function. Currently, MeCP2-associated disorders have no cure and the mechanisms by which MeCP2 isoforms are controlled are not fully understood. We were the first group to develop isoform-specific MECP2 gene therapy vectors and to study the functional role of MeCP2E1 and MeCP2E2 in neuronal maturation. By developing isoform-specific anti-MeCP2E1 and anti-MeCP2E2 antibodies, we reported that MeCP2E1 and MeCP2E2 expression are cell type-, and brain region-specific. Further, we showed that DNA methylation impacts MeCP2 isoform-specific expression in differentiating brain-derived neural stem cells during brain development and in the adult murine brain in a brain-region specific manner. MeCP2 expression is important in different brain cell types, and disease-associated phenotypes are proven to be cell type-specific. Here, we will discuss our recent results on the MeCP2 isoform-specific regulation and functional role in neurons. We have exciting novel data on the epigenetic control of MeCP2 isoforms in brain-derived neural stem cells and different brain cells. Our results will have significant implications for future therapeutic strategies of MeCP2-associated neurological disorders.

Disclosures: M. Rastegar: None.

Poster

515. Rett Syndrome

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 515.15/V19

Topic: C.06. Developmental Disorders

Support: Dorrance Center for Rare Childhood Disorders at TGen

Barrow Neurological Foundation

Title: Development of an *in vitro* assay using cerebellar granule neurons for therapeutic screens in Rett syndrome

Authors: *S. RANGASAMY[1], S. OLFERS[4], H. YIN[2], V. NARAYANAN[1,3,4]
[1]Dorrance Ctr. for Rare Childhood Disorders, Translational Genomics Res. Inst., PHOENIX, AZ; [2]Cancer and Cell Biol. Div., [3]Neurogenomics Div., Translational Genomics Res. Inst., Phoenix, AZ; [4]Barrow Neurolog. Inst., Phoenix, AZ

Abstract: Rett syndrome (RTT) is a neurodevelopmental disorder that is caused primarily by mutations in the X-linked gene encoding methyl-CpG binding protein 2 (MeCP2). Pathological studies of human RTT cases have all shown a reduction in brain size, increased cell packing density, and aberrant dendrite structure. The major barrier toward developing an effective therapy for RTT is the lack of neuronal phenotypes to test in discovery platforms capable of supporting high throughput screening (HTS). In our studies with a unique mouse model (MeCP2-A140V "knock-in" human mutation) developed in our laboratory, we have identified decreased dendritic branching of cortical layer III pyramidal neurons, and increased cell packing density in several brain regions. Here, we detail the measurements of neuronal soma and nuclear size in cultured neurons from A140V and wild type (WT) mice. Our previous observations in cultured hippocampal neurons indicated that there was significant reduction in neuronal soma size, which was observable as early as 3 DIV. Further, the reduction in soma size was associated with decrease in nuclear size. To further validate this observation in a homogenous cell population, we quantified soma and nuclear size in primary cerebellar granule neuronal (CGN) cultures from wild type and MeCP2 A140V mutant mice. We found that the soma size of CGN (measured as cross-sectional area) was significantly smaller in mutant MeCP2 mice (55.94 ± 2.849 μm2) compared to wild type mice (65.06 ± 2.5 μm2). In addition, we also found that CGN nuclear size was decreased in MeCP2 A140 mice (28.50 ± 1.076 μm2) compared to wild type mice (31.97 ± 1.068 μm2). In our studies, we also found that mTORC2 pathway is significantly downregulated in MeCP2-A140V model, suggesting MeCP2 influences neuronal size via the mTORC2 pathway. The "soma size" phenotype in cultured neurons was rescued by treatment with recombinant IGF-1 *in vitro*. Neuronal size in such homogeneous CGN cultures is a predictable neuronal phenotype. Our findings suggest that soma/nuclear size can be used as biomarker in a high throughput screening assay for identification of potential therapeutic agents for Rett syndrome.

Disclosures: S. Rangasamy: None. **S. Olfers:** None. **H. Yin:** None. **V. Narayanan:** None.

Poster

515. Rett Syndrome

Location: Halls A-C

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Topic: C.06. Developmental Disorders

Support: IRSF 2916

NIH R01 NS075062

Title: Alterations in astrocytic gene expression in pre-symptomatic MeCP2-deficient mice

Authors: *N. L. PACHECO[1], M. L. OLSEN[2]
[1]Univ. of Alabama At Birmingham, Birmingham, AL; [2]Univ. of Alabama at Birmingham, Birmingham, AL

Abstract: Rett syndrome (RTT) is an X-linked neurodevelopmental disorder characterized by a decline in motor and language functions (6-18 months), breathing abnormalities and seizures. RTT is caused by mutations in the methyl CpG binding protein 2 (*MECP2*) gene, a transcriptional regulator. Expression of *MeCP2* specifically in astrocytes in a *MeCP2*-deficient mouse model ameliorated deficits associated with RTT. This coupled with findings that *MeCP2*-deficient astrocytes induced a RTT phenotype in wild type neurons suggests that astrocytes contribute to RTT pathology. We recently demonstrated that MeCP2 is a positive transcriptional regulator of the glial specific inwardly-rectifying potassium channel Kir4.1. Kir4.1 is highly expressed in gray matter astrocytes and is implicated in extracellular potassium homeostasis. Furthermore, Kir4.1 contributes to the hyperpolarized resting membrane potential and high resting K+ conductance observed in gray matter astrocytes. The importance of this channel for normal brain functioning is underscored by studies in Kir4.1 knock-out mice which develop ataxia, seizures, show altered synaptic plasticity and die during early postnatal development. Strikingly similar to individuals with RTT, knockout or mutation of *KCNJ10*, the gene that codes for Kir4.1, causes seizures, ataxia, and developmental deficits in humans alike. Our previous study demonstrated that *MeCP2*-deficient mice have decreased Kir4.1 protein and mRNA levels, which correlated with aberrant Kir4.1 function in astrocytes and increased neuronal excitability. However, this study focused exclusively on symptomatic *MeCP2*-deficient mice. It is unclear if reduced levels of Kir4.1 protein are a result of the disease process or contribute to the pathogenesis in *MeCP2*-deficient mice. Here we aim to address this by examining Kir4.1 expression and function in pre-symptomatic animals.

Disclosures: **N.L. Pacheco:** None. **M.L. Olsen:** None.

Poster

515. Rett Syndrome

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 515.17/V21

Topic: C.06. Developmental Disorders

Support: Deutsche Forschungsgemeinschaft (CNMPB)

International Rett Syndrome Foundation

Title: Aberrant redox homeostasis in RETT syndrome affects cytosol and mitochondria

Authors: K. CAN[1], J. TOLÖ[2], S. KÜGLER[2], *M. MUELLER[1]
[1]Zentrum Physiologie & Pathophysiologie, [2]Univ. Goettingen, Goettingen, Germany, Germany

Abstract: Rett syndrome is associated with mitochondrial impairment, chronic oxidant challenge, and aberrant cellular redox homeostasis. Since mitochondria are partly uncoupled and show increased respiratory turnover rates, they may underlie the oxidative burden in MeCP2-deficient (Mecp2-/y) brain tissue. Since these alterations manifest early in life, they may facilitate or even underlie disease progression in Rett syndrome. Earlier we reported exaggerated responses of Mecp2-/y hippocampus to redox challenge and mitochondrial inhibition, and showed that extramitochondrial ROS production is not intensified. To decipher the molecular causes of redox imbalance in more detail, we now took advantage of viral constructs expressing the genetically-encoded optical redox sensor roGFP1 specifically in neurons. For subcellular analyses in hippocampal cell and slice cultures, roGFP1 was targeted to either the cytosol or the mitochondrial matrix. To enable quantitative recordings, the ratiometric response ranges of mito-roGFP1 and cyto-roGFP1 were calibrated to full oxidation and reduction. The response dynamics clearly differed between dissociated cell cultures and the more complex organotypic slices. Genotypic differences occurred especially in the slice cultures: both mitochondria and cytosol showed slightly more oxidized redox baselines in Mecp2-/y than in wildtype neurons. In addition, the redox balance of both compartments was less stable in Mecp2-/y hippocampus. Severe hypoxia caused more pronounced reducing shifts and acute oxidant challenge by 200 µM H2O2 elicited more intense oxidizing transients in Mecp2-/y neurons. Block of superoxide dismutase evoked only dampened oxidizing responses in Mecp2-/y cytosol and mitochondria. In conclusion, mito-roGFP1 and cyto-roGFP1 respond reliably to oxidation and reduction, thereby allowing analyses of subcompartmental redox dynamics. Genotypic differences among wildtype and Mecp2-/y mice are evident not only in the cytosolic but also the mitochondrial compartment. Since mitochondria are the primary cellular source of reactive oxygen species, this supports our hypothesis that it is especially the mitochondrial dysfunction which underlies the oxidative burden in Rett syndrome.

Disclosures: **K. Can:** None. **M. Mueller:** None. **J. Tolö:** None. **S. Kügler:** None.

Poster

515. Rett Syndrome

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 515.18/V22

Topic: C.06. Developmental Disorders

Title: Loss of MeCP2 affects normal development of hippocampal glutamatergic neurons by altering axonal and dendritic outgrowths

Authors: *C. SAMPATHKUMAR, T. TRIMBUCH, C. ROSENMUND
Charité - Universitätsmedizin Berlin, Berlin, Germany

Abstract: Methyl CpG – binding protein 2 (MeCP2) is a transcriptional regulator whose loss-of-function mutations result in neurodevelopmental disorders such as Rett syndrome. The objective of the study is to understand the role of MeCP2 in regulating synapse formation in hippocampal glutamatergic neurons during development. *Mecp2*$^{Null/y}$ and *Mecp2*^{Tg1} male mice were used and hippocampal neurons were plated on glial micro islands to study synapse formation at a single-cell level. We characterized MeCP2 wild type (WT) expression levels in autaptic murine glutamatergic neurons from the hippocampus and found that MeCP2 endogenous levels correlated to the number of synapses formed during peak synaptogenesis and synapse maturation. Additionally, we observed a developmental defect in glutamatergic MeCP2-null neurons. Loss of MeCP2 resulted in reduced axonal outgrowths as well as shorter dendritic structures compared to their WT littermates. Furthermore, the soma size of these neurons was significantly reduced. Interestingly, the MeCP2 overexpressing neurons (Tg1) did not display any of these developmental defects and were identical to WT neurons although synapse number was significantly increased. Restoring MeCP2 to WT levels in MeCP2 null neurons resulted in rescue of dendritic outgrowth comparable to WT neurons. In order to study the importance of MeCP2 during various stages of synapse formation, MeCP2 null neurons were treated with Cre recombinase at various time points during synaptogenesis to rescue MeCP2 to WT levels and synaptic output was measured. We observed that evoked excitatory postsynaptic current amplitude, pool size of readily releasable vesicles and frequency of spontaneous miniature events were significantly rescued to WT levels when MeCP2 was restored at early stages of synapse formation. Moderate rescue was seen also when MeCP2 was restored during synapse maturation. Taken all together, this study shows that abnormal axonal and dendritic outgrowths may contribute in part to the pathophysiology of the Rett syndrome. Furthermore, MeCP2 is critical throughout synapse formation as well as maintenance.

Disclosures: C. Sampathkumar: None. **T. Trimbuch:** None. **C. Rosenmund:** None.

Poster

515. Rett Syndrome

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 515.19/V23

Topic: C.06. Developmental Disorders

Title: Analysis of whole-brain X-chromosome inactivation within MeCP2 mutant and wild type mice

Authors: *E. SZELENYI, Y. KIM, K. U. VENKATARAJU, P. OSTEN
Cold Spring Harbor Lab., Cold Spring Harbor, NY

Abstract: Rett syndrome (RTT) is an X-linked neurodevelopmental disorder caused by mutations in the methyl-CpG-binding protein 2 (MECP2) gene. Unfavorable skewing of X chromosome inactivation is proposed to contribute to the severity of clinical symptoms in females with RTT. Here we examine X chromosome skewing of MeCP2 using whole-brain imaging of MeCP2-GFP reporter allele (Lyst et al., 2013, Nat Neurosci 16(7):898-902) crossed from the maternal or paternal side into wild type or MeCP2 mutant genetic background. The brains are imaged as 280 coronal section datasets by serial two-photon (STP) tomography (Ragan et al., 2012, Nat Methods 9(3): 255-258), the location of MeCP2-GFP+ cells is detected by convolutional neural network and watershed based algorithms, and the datasets are registered to the a 2-photon based Allen Mouse Brain Atlas with whole brain anatomical segmentation. This analysis revealed a lateralization-based skewing in some wild type mice, replicating a recent finding from different X-chromosome reporter mice (Wu et al., Neuron, 2014. 81(1): 103-19). Currently, our analysis focuses on mapping the distribution of MeCP2-GFP+ cells in the MeCP2 mutant background in female mice MeCP2GFP/null and MeCP2GFP/308X with the GFP allele introduced either from the maternal or paternal side.

Disclosures: E. Szelenyi: None. **Y. Kim:** None. **K.U. Venkataraju:** None. **P. Osten:** None.

Poster

515. Rett Syndrome

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Topic: C.06. Developmental Disorders

Support: R01-NS065027

R21-HD074418

Title: The BDNF val-66-met polymorphism impairs dendritic complexity and dendritic spine density and form in hippocampal neurons of Mecp2 knockout mice

Authors: *X. XU[1], J. GARCIA[2], R. EWALT[2], L. POZZO-MILLER[2]
[1]Neurobio., Univ. of Alabama At Birmingham, Birmingham, AL; [2]Neurobio., The Univ. of Alabama at Birmingham, Birmingham, AL

Abstract: Brain-derived neurotrophic factor (BDNF) has been highlighted in Rett syndrome (RTT), an X-linked neurodevelopmental disorder caused by loss-of-function mutations in MECP2. Bdnf mRNA and protein levels are lower in MeCP2-deficient models and RTT individuals, and its overexpression rescues cellular and behavioral deficits. The human BDNF gene has a single nucleotide polymorphism (SNP) - a methionine (met) substitution for valine (val) at codon 66 - that results in cognitive impairments because it affects BDNF trafficking and activity-dependent release; thus, it is highly relevant to know whether this BDNF SNP contributes to RTT pathophysiology. To examine the consequences of the BDNF SNP on dendritic and dendritic spine morphology, human val-BDNF or met-BDNF (tagged with GFP for their subcellular localization) were co-transfected with soluble GFP (to image neuronal morphology) in 6-8 DIV cultured hippocampal neurons prepared from P1 wildtype (wt) and Mecp2 knockout (ko) mice. After 48hs of expression, transfected neurons were fixed, processed for GFP immunocytochemistry, imaged in a laser-scanning confocal microscope, and analyzed with the Filament Tracing and Surface Rendering modules of Imaris software. We confirmed that met-BDNF fails to be properly transported in hippocampal neurons, being restricted to somata and proximal areas of primary dendrites, while val-BDNF is transported to more distal regions of primary and secondary dendrites. In wt neurons, met-BDNF fails to promote dendritic growth and branching, as val-BDNF does ($P<0.05$). In addition, met-BDNF does not increase dendritic spine density and the volume of individual spines in wt neurons, like val-BDNF does ($P<0.01$; K-S test). Of relevance to RTT, we found that Mecp2 ko neurons have impaired dendritic complexity, which is significantly improved by val-BDNF ($P<0.01$); on the other hand, met-BDNF reduces dendritic complexity in Mecp2 ko neurons. Lastly, Mecp2 ko neurons have lower spine density, while the volume of individual spines are larger than in wt neurons (see Li and Pozzo-Miller SfN 2014). Interestingly, val-BDNF increases dendritic spine density and individual spine volumes, while met-BDNF decreases dendritic spine volume ($P<0.001$; K-S test). These findings revealed a deleterious consequence of the human BDNF val-66-met SNP on

dendritic and dendritic spine morphology in Mecp2 ko neurons, suggesting that this BDNF variant contributes to RTT pathophysiology, and that BDNF-based therapies may not be equally effective in all RTT individuals.

Disclosures: X. Xu: None. **J. Garcia:** None. **R. Ewalt:** None. **L. Pozzo-Miller:** None.

Poster

515. Rett Syndrome

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 515.21/V25

Topic: C.06. Developmental Disorders

Support: AFM (Association Française contre les Myopathies)

Title: Phenotypic improvement in Mecp2-deficient mice after i.v. injection of a self-complementary AAV9 construct expressing a codon-optimized Mecp2 transgene

Authors: *V. MATAGNE, L. VILLARD, J.-C. ROUX
Aix Marseille Universite, UMRS 910, Marseille, France

Abstract: Rett syndrome (RTT) is an X-linked neurodevelopmental disorder that is primarily caused by a loss of function of methyl CpG binding protein 2 (MECP2) whose main function is that of a global transcriptional repressor. RTT is a disease affecting not only CNS functions (profound cognitive and motor deficits) but also peripheral functions (severe breathing abnormalities, autonomic dysfunction). The recent findings that reactivation of Mecp2 rescued adult diseased RTT mice not only indicates that MECP2 is needed for normal adult function (Robinson et al 2012) but also that gene therapy might be beneficial for RTT patients, even after the disease has started. Proof-of-principle that gene therapy was beneficial in a RTT mouse model (Mecp2 deficient or KO mice) has been recently reported by 2 different research teams (Gadalla et al 2013; Garg et al 2013). Although both studies reported an improvement in RTT symptoms, the therapeutic benefits seemed to depend on the age at which the virus was administered as well as the promoter driving the expression of Mecp2. In order to try and improve vector delivery and expression, we designed 2 plasmid constructs expressing GFP (control virus) or a codon-optimized version of Mecp2 (termed MCO) under the regulation of the mouse Mecp2 short promoter (pMe). These construct were used to generate self-complementary AAV9 (scAAV9) viruses. We first tested the scAAV9-pMe-GFP control vector after IV injection through the tail vein of 30-day-old male wild-type (WT) mice (5X10E12 vg/kg BW).

As reported by other groups (Foust et al 2009; Duque et al 2009), we found that this virus transduced CNS cells, although GFP was only expressed in neurons in the case of scAAV9-pMe-GFP. Given the low GFP expression (8.7±0.8 and 13.7±1.9 cells/0,6mm2 in frontal cortex (FC) and putamen-putamen area (CP), respectively, n=5), we elected to carry out a preliminary study injecting scAAV9-pMe-MCO at 5X10E13 vg/kg BW in Mecp2 KO mice. Twenty-five days after injection, mice were sacrificed and Mecp2 expression was quantified by IHC. Compared to WT mice (100% expression, n=3), we found that 8.6%±1% (FC) and 9.7%±1.5% (CP) cells expressed Mecp2 in Mecp2 KO mice (n=2). Despite this low percentage of Mecp2-expressing cells, we did find an improvement in spontaneous activity and sensorimotor coordination as measured in the open-field and rotarod tests. These encouraging preliminary data indicate that even a low level Mecp2 expression can improve RTT symptoms in Mecp2-deficient mice. Further studies will aim at confirming these data and investigating the potential therapeutic effect of scAAV9-pMe-MCO injection in female RTT mice (Mecp2 heterozygous females).

Disclosures: **V. matagne:** None. **L. Villard:** None. **J. Roux:** None.

Poster

515. Rett Syndrome

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 515.22/V26

Topic: C.06. Developmental Disorders

Support: 5R01 NS057398-07

Title: Mechanisms of prefrontal cortical dysfunction in mecp2 null mice

Authors: ***M. P. SCENIAK**[1,2], M. LANG[2], A. C. ENOMOTO[2], C. J. HOWELL[2], D. M. KATZ[2]
[2]Neurosci., [1]Case Western Reserve Univ., Cleveland, OH

Abstract: Limbic cortical dysfunction is thought to contribute to cognitive and behavioral features of diverse neurodevelopmental disorders, including those on the autism spectrum. However, underlying synaptic and circuit-level mechanisms remain undefined. We recently found that loss of *Mecp2*, the gene mutated in Rett syndrome (RTT) is associated with a marked decrease in neuronal expression of Fos protein, an index of neuronal activity, in midline limbic cortices, including the cingulate and retrosplenial and the pre- and infralimbic subdivisions of the medial prefrontal cortex (mPFC; Kron et al., 2012), suggesting that these regions are hypofunctional in *Mecp2* mutants. The present study was undertaken to test this hypothesis by

defining synaptic and circuit function in the mPFC of *Mecp2*$^{tm1.1Jae}$ null mice using acute brain slices. Compared to Wt, layer 5 excitatory neurons in the mutant mPFC exhibit significant reductions in 1) excitatory postsynaptic currents, 2) the duration of excitatory UP states, 3) the ratio of NMDA/AMPA currents and 4) evoked population activity in layers 2/3 and 5. These functional changes are associated with significant reductions in the density of excitatory dendritic spines, the ratio of vesicular glutamate (VGLUT1) to vesicular GABA (VGAT) transporters and the level of NMDA receptor subunit 1 expression. In addition we found circuit abnormalities that differ markedly from those previously described in other cortical regions of *Mecp2* mutants. Specifically, we saw no effect of *Mecp2* genotype on 1) inhibitory synaptic currents, which are increased in primary sensory cortices in *Mecp2* nulls (Dani *et al.* 2005; Dani and Nelson 2009) or 2) expression of the interneuron marker parvalbumin, which is increased in the visual cortex of *Mecp2* nulls (Durand et al., 2013). In addition, we observed a significant increase in the NR2B component of NMDA currents, which is decreased in visual cortex of *Mecp2* nulls (Durand et al., 2013). These data support the hypothesis that loss of *Mecp2* function is associated with reduced activation of excitatory neurons in the mPFC. Moreover, our data, together with previous reports from other laboratories highlight the fact that synaptic and circuit-level phenotypes associated with loss of MeCP2 differ among brain regions. Taking this heterogeneity into account will be critical for designing therapeutic interventions aimed at restoring excitatory/inhibitory synaptic balance in RTT and related disorders. Supported by NINDS (DMK).

Disclosures: **M.P. Sceniak:** None. **M. Lang:** None. **A.C. Enomoto:** None. **C.J. Howell:** None. **D.M. Katz:** 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.; AstraZeneca.

Poster

515. Rett Syndrome

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 515.23/V27

Topic: C.06. Developmental Disorders

Support: Rett Syndrome Research Trust

W.M. Keck Foundation

Intellectual and Developmental Disability Research Center

Howard Hughes Medical Institute

Title: Forniceal deep brain stimulation rescues the impairment of contextual fear memory in a mouse model of Rett syndrome

Authors: *S. HAO[1,2], Z. WU[1,2], B. TANG[1,2], Y. SUN[2,3], Y. GAO[2,3], R. C. SAMACO[2,3], H. Y. ZOGHBI[1,2,3,4,5], J. TANG[1,2]
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Abstract: Deep brain stimulation (DBS) is an established therapy for several neurological disorders. By stimulating disease-specific target regions of the brain, DBS in both human patients and animal models has been shown to improve symptoms in Parkinson's disease, obsessive-compulsive disorder, depression, schizophrenia as well as improve cognitive deficits in Alzheimer's disease (AD) and epilepsy. However, the mechanistic dissection of DBS is rare, especially in awake, freely moving transgenic mouse models. A recent study in AD patients suggested that DBS in the fornix, a fiber tract containing cholinergic input from the medial septum to the hippocampus, reduces memory decline and improves cognitive function. Accordingly, we proposed that forniceal DBS will improve cognitive function in female mice heterozygous for a Mecp2 null allele (Rett mouse model). Here we show that unilateral DBS in the fimbria-fornix enhances the retrieval of contextual fear memory in both wild type and MeCP+/- mice, a model of Rett syndrome. Therefore, forniceal DBS rescues the impairment of contextual fear memory retrieval in Rett mice. To examine the cholinergic involvement of forniceal DBS on memory, we bilaterally infused the muscarinic cholinergic blocker atropine into the dorsal hippocampus. DBS-induced memory enhancement was abolished by atropine, compared with saline control. These results suggest that forniceal DBS might improve hippocampus-dependent memory retrieval via modulation of the cholinergic system. Forniceal DBS may serve as a therapeutic intervention to rescue the cognitive deficits of developmental neurological diseases.

Disclosures: **S. Hao:** None. **Z. Wu:** None. **B. Tang:** None. **Y. Sun:** None. **Y. Gao:** None. **R.C. Samaco:** None. **H.Y. Zoghbi:** None. **J. Tang:** None.

Poster

515. Rett Syndrome

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 515.24/V28

Topic: C.06. Developmental Disorders

Support: IRSF Grant #2916

NIH R01 NS075062

Title: Astrocyte dysfunction in a mouse model of Rett syndrome

Authors: *M. L. OLSEN, V. A. CUDDAPAH, N. PACHECO, S. NWAOBI
Univ. of Alabama At Birmingham, Birmingham, AL

Abstract: Rett Syndrome is an X-linked neurodevelopmental disorder, caused by mutations in methyl-CpG-binding protein 2 (MeCP2) in > 95% of cases. RTT is typified by normal development until 6-18 months of age, when motor and communicative skills regress and hand stereotypies, autonomic symptoms, and seizures begin to present. Restoration of MeCP2 function selectively to astrocytes reversed several phenotypes in a murine model of RTT, but the mechanism of this rescue is unknown. Astrocytes serve to dampen neuronal excitability through maintenance of the extracellular concentration of K+, ([K+]o). In frontal brain regions dysregulation of [K+]o can lead to hyperexcitability and seizures, while in brain structures such as the brainstem, autonomic function may be disrupted. Given the high prevalence of seizures in girls with Rett and symptoms which originate from brain stem dysfunction, we hypothesized that MeCP2 loss may lead to disrupted [K+]o. Whole-cell patch clamp experiments of cortical astrocytes from symptomatic MeCP2-/y mice revealed a >50% deficiency in Ba2+-sensitive K+ currents attributable to Kir4.1, an inwardly-rectifying K+ channel implicated in the maintenance of [K+]o in the brain. K+-sensitive microelectrode recordings demonstrated that the baseline [K+]o was elevated by 1 mM in MeCP2-/y mice, and was associated with enhanced intrinsic excitability of layer II/III pyramidal neurons. Kir4.1 protein and mRNA were significantly downregulated in the cortex and brainstem. CHiP analysis revealed that Kir4.1 is a direct molecular target of MeCP2. Our data demonstrate that the loss of MeCP2 impairs astrocytic K+ homeostasis and provide novel mechanistic insight explaining how astrocytic dysfunction may contribute to RTT.

Disclosures: M.L. Olsen: None. **V.A. Cuddapah:** None. **N. Pacheco:** None. **S. Nwaobi:** None.

Poster

515. Rett Syndrome

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Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 515.25/V29

Topic: C.06. Developmental Disorders

Support: NIH 5R01NS057819

Title: A splicing-regulatory pathway controls neuronal excitation in Rett syndrome

Authors: *L. CHEN[1,2], P. YU[1,2], W. WANG[1,2], C. SHAW[1], H. Y. ZOGHBI[1,2,3]
[1]Baylor Col. of Med., Houston, TX; [2]Jan and Dan Duncan Neurolog. Res. Inst., Houston, TX; [3]Howard Hughes Med. Inst., Houston, TX

Abstract: Alternative splicing is prevalent in the mammalian nervous system, producing structurally and functionally distinct mRNA isoforms to modulate diverse neuronal processes. Although disruption in alternative splicing has been implicated in human brain disorders, the identity and the neuronal function of RNA variants involved in diseases are largely unknown, and the molecular basis for such aberrant alternative splicing remains poorly understood. Here we develop an algorism to reliably identify whole-genome alternative splicing events based on RNA sequencing (RNA-Seq) data. We apply this strategy and discover comprehensive alternative splicing events that are altered in the mouse models of Rett syndrome and MECP2 duplication syndrome. Notably, we identify numerous alternative splicing changes altering proteins involved in synaptic transmission and neuronal excitation. Molecular and genetic experiments reveal that the splicing regulator Rbfox1 functions downstream of MeCP2. In the absence of MeCP2, the expression of Rbfox1 is increased, whereas upon doubling MeCP2 levels the expression of Rbfox1 is decreased. To determine if some of the splicing alterations contribute to the phenotypes of MeCP2 null mice, we bred Mecp2+/- mice to Rbfox1+/- mice and found that heterozygous loss of Rbfox1 in the nervous system rescues alternative splicing and neuronal excitation abnormalities in MeCP2 null animals. We further provide evidence that MeCP2 directly represses the expression of Rbfox1 via remodeling its promoter to a closed chromatin state. Our study delineates a novel molecular pathway in mediating complex alternative splicing networks to control neuronal excitation in the brain.

Disclosures: L. Chen: None. **P. Yu:** None. **W. Wang:** None. **C. Shaw:** None. **H.Y. Zoghbi:** None.

Poster

515. Rett Syndrome

Location: Halls A-C

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Program#/Poster#: 515.26/V30

Topic: C.06. Developmental Disorders

Support: NIH R21HD073631

NIH R01HD064817

Title: Bioenergetic dysregulation in MeCP2-null microglia secondary to glutamine transporter SNAT1 overexpression

Authors: *I. MAEZAWA[1], M. HORIUCHI[1], H. WULFF[2], G. CORTOPASSI[3], J. D. ERICKSON[4], L.-W. JIN[1]
[1]M.I.N.D. Inst, UC Davis, Sacramento, CA; [2]Pharmacol., [3]Mol. Biosci., UC Davis, Davis, CA; [4]Louisiana State Univ., New Orleans, LA

Abstract: Rett syndrome is an autism spectrum disorder caused by loss-of-function mutations in the gene encoding MeCP2, an epigenetic modulator that binds the methyl CpG dinucleotide in target genes to regulate transcription. Previously we and others reported a role of microglia in the pathophysiology of RTT. Therefore, how epigenetic dysregulation due to MeCP2 deficiency causes functional impairment in microglia becomes a significant research topic. Our ChIP-seq data using a specific anti-MeCP2 identified a MeCP2 target gene *SLC38A1*, which encodes a major glutamine transporter SNAT1. To understand the possible role of SNAT1 in MeCP2-deficient microglia, we analyzed its regulation and impact on cellular functions using cultured primary microglia, microglia acutely isolated from juvenile *Mecp2*-null and wild-type mice, and BV-2 microglia cells with MeCP2 knockdown or SNAT1 overexpression. Using qPCR and Western blotting, we showed that MeCP2 acts as a transcriptional repressor for the *SNAT1* gene in microglia, but does not affect the expression of a related system A transporter gene *SNAT2*, supporting the SNAT1-targeted regulation by MeCP2. Because glutamine is mainly metabolized in the mitochondria, where it is used as an energy substrate and a precursor for glutamate production, we hypothesize that SNAT1 over-expression would impair the glutamine homeostasis in MeCP2-deficient microglia, resulting in mitochondrial dysfunction as well as microglial neurotoxicity due to glutamate over-production. Supporting this hypothesis, we found that MeCP2 downregulation or SNAT1 overexpression in microglia resulted in (1) glutamine-dependent death of BV-2 cells; (2) proliferation of mitochondria and enhanced mitochondrial production of reactive oxygen species; (3) increased oxygen consumption but decreased ATP production; and (4) over-production of glutamate that caused NMDA receptor-dependent neurotoxicity. Interestingly, the above abnormalities could be rectified by the general anti-oxidant vitamin E as well as mitochondria specific approaches such as the mitochondria-targeted expression of catalase (mCAT) and a cell permeable, mitochondria-targeted peptide antioxidant Szeto-Schiller 31 (SS-31). Our results reveal a novel mechanism via which MeCP2 regulates bioenergetic pathways in microglia.

Disclosures: **I. Maezawa:** None. **M. Horiuchi:** None. **H. Wulff:** None. **G. Cortopassi:** None. **J.D. Erickson:** None. **L. Jin:** None.

Poster

515. Rett Syndrome

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 515.27/V31

Topic: C.06. Developmental Disorders

Support: NIH (NS073875)

Title: Alterations of h-currents and voltage-gated Na+ currents in mesencephalic trigeminal propriosensory neurons increase excitability in a mouse model for Rett Syndrome

Authors: ***M. F. OGINSKY**, N. CUI, W. ZHONG, C. JIANG
Biol., Georgia State Univ., Atlanta, GA

Abstract: People with Rett syndrome (RTT) have motor defects in addition to autistic symptoms, which are also seen in *Mecp2*-null mice. It is known that normal motor function relies on motor neurons as well as propriosensory neurons. Whereas motor neurons have been studied for possible roles in the development of movement problems in RTT, much less attention has been given to the propriosensory neurons whose excitability changes can affect the essential feedback to motor neurons required for coordinated movement. To address this issue, we studied the mesencephalic trigeminal (Me5) neurons. In whole-cell current clamp, these propriosensory neurons responded to hyperpolarizing currents with a large sag and post-inhibitory rebound (PIR) suggesting the presence of the hyperpolarization–activated current (I_H). I_H plays a role in membrane excitability, pacemaking and bursting of action potentials. The sag and PIR were reduced in *Mecp2*-null mice by ~35% and ~20%, respectively. In voltage clamp, the I_H density was reduced by ~33%, the steady state activation was shifted by ~10mV toward more depolarization, and the activation time constant was slower in the *Mecp2*-null mice. Changes in the firing activity were studied by injecting depolarizing currents as the Me5 neurons did not fire spontaneously. We found that the Me5 neurons in *Mecp2*-null mice were more excitable than those in the WT despite the major deficiency in I_H. The density of the voltage-gated Na+ currents (I_{Na}) was unchanged but the I_{Na} steady state activation was shifted by ~5mV toward hyperpolarization in *Mecp2*-null mice. Consistently, the firing threshold measured from PIR was ~5mV less in the *Mecp2*-null neurons, while the latency to first spike was unchanged. These data suggest that the *Mecp2* knockout causes a major deficiency in I_H in Me5 neurons, and such

seemingly depressive effect was compensated by I_{Na} by unknown mechanisms making the cells more excitable instead.

Disclosures: **M.F. Oginsky:** None. **N. Cui:** None. **W. Zhong:** None. **C. Jiang:** None.

Poster

515. Rett Syndrome

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Program#/Poster#: 515.28/V32

Topic: C.06. Developmental Disorders

Support: NIH 1R01 HD064817

NIH 1R21 HD073631

International Rett Syndrome Foundation HeART Award

Title: Defective GABAergic neurotransmission in the nucleus tractus solitarius in Mecp2-null mice

Authors: ***L.-W. JIN**[1], Y.-C. LIN[1], M. A. ROGAWSKI[2], C.-C. LIEN[4], I. MAEZAWA[1], C.-Y. CHEN[3]
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Abstract: Respiratory dysfunction is one of the major clinical features of Rett syndrome (RTT), a devastating neurodevelopmental disorder caused by loss-of-function mutations in the X-linked methyl-CpG binding protein 2 (*Mecp2*) gene. GABAergic dysfunction has been implicated contributing to the respiratory dysfunction. The NTS is the first central site receives and integrates respiratory sensory inputs. Plasticity in the NTS can change the nature of the reflex output. We test the hypothesis that deficiency in *Mecp2* gene reduces GABAergic neurotransmission in NTS. Using whole cell patch clamp technique, we recorded spontaneous inhibitory postsynaptic currents (sIPSCs), miniature IPSCs (mIPSCs), evoked IPSCs (eIPSCs), and agonist-induced whole cell currents from NTS neurons in brainstem slices acutely prepared from age-matched male *Mecp2*-null mice and wild-type littermates. Using qPCR, we determined GABA-A receptor subunit gene expression from NTS punches. Compared to those from wild-type mice (n=11), NTS neurons from *Mecp2*-null mice (n=12) had significantly ($P<0.05$) smaller sIPSC and mIPSC amplitudes without significant changes in frequencies. There was no

significant difference in eIPSC amplitude and pair pulse ratio (PPR) between WT and *Mecp2*-null mice (n=4-6) albeit a greater PPR variability was observed in *Mecp2*-null mice. *Mecp2*-null mice had enhanced response to exogenous GABA-A agonist application (n=4-6) and elevated GABRA1 gene expression (n=3) in the NTS. The data suggest that reduced GABAergic signaling via a post-synaptic mechanism(s) in the NTS contributes to respiratory dysfunction in RTT.

Disclosures: L. Jin: None. **Y. Lin:** None. **M.A. Rogawski:** None. **C. Lien:** None. **I. Maezawa:** None. **C. Chen:** None.

Poster

516. Down Syndrome Anatomical and Behavioral Correlations

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

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Topic: C.06. Developmental Disorders

Support: National Institute of Child Health and Human Development (KJG)

Jerome Lejeune Foundation (KJG)

Linda Crnic Institute for Down Syndrome (KJG and CAL)

Philanthropic Funds (CAL)

Title: Age-related changes and gender differences in the Down syndrome Dp(10)1Yey mouse brain

Authors: *K. X. LE[1], B. LIU[1,2], S. J. CHOWDHURY[1], J. L. FROST[1], J. FOK[1], A. BLOCK[3], M. M. AHMED[3], K. J. GARDINER[3,4], C. A. LEMERE[1,2]
[1]Ctr. for Neurologic Dis., Brigham and Women's Hosp., Boston, MA; [2]Harvard Med. Sch., Boston, MA; [3]Pediatrics, Linda Crnic Inst. for Down Syndrome, Aurora, CO; [4]Biochem. and Mol. Genetics, Neurosci. and Human Med. Genet. and Genomics Programs, Univ. of Colorado Sch. of Med., Aurora, CO

Abstract: Down syndrome (DS) is the most common genetic chromosomal disorder and is associated with intellectual disability and the early onset of Alzheimer's disease. DS is caused by an extra copy of chromosome 21 (Hsa21) and the increased expression of the encoded genes. Hsa21 contains approximately 160 protein-coding genes, the orthologs of which map to

segments of mouse chromosomes 16, 17 and 10 (Mmu16, 17, 10). While several mouse models trisomic for segments of the Mmu16 segment have been studied in detail, little is known about the Dp(10)1Yey (Dp10) mice that are trisomic for the 40 protein coding genes mapping to Mmu10. Here, we compare protein levels and related pathologies in the hippocampus, cortex and cerebellum of 5.5, 8 and 16 month old Dp10 mice, to their age-matched wildtype (WT) littermate controls. In 16 month, but not 5.5. month-old Dp10 mice, by Western blot (WB), we observed significantly elevated astrocytic S100β and pre-synaptic synaptophysin protein levels and significantly less post-synaptic PSD95; GFAP levels were significantly lower in Dp10 vs. WT mice at both ages. By reverse phase protein arrays, we measured levels of ~100 proteins/protein modifications in 8 month old male and female mice. Approximately 40% of proteins showed abnormal levels, including components of MAP kinase and MTOR pathways, glutamate receptors and immediate early gene proteins. We observed both brain region- and gender-specific abnormalities: males showed more perturbations in hippocampus and females more in cerebellum. By immunohistochemistry, in 5.5 and 16 month old mice, no Aβ deposition was observed, however, by stereological analysis of NeuN- positive neurons at 16 months, Dp10 mice showed increased numbers of hippocampal CA1, but not dentate gyrus, neurons. Together these data show that trisomy of Hsa21 orthologs of the Mmu10 region causes age-related cell and molecular changes in brain. These observations are important because male Dp10 mice have shown no learning/memory impairment at 2-4 months of age. Therefore, it is critical to analyze these mice at older ages to determine if age and/or gender-specific abnormalities exist and potentially contribute to the DS phenotype and/or to drug responses.

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Poster

516. Down Syndrome Anatomical and Behavioral Correlations

Location: Halls A-C

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Topic: C.06. Developmental Disorders

Support: Fondation Jérôme Lejeune

Fondo de Investigaciones Sanitarias-ISCIII PI11/00744

Spanish Ministry of Economy (SAF2010-16427)

CIBEROBN

CIBERER

Title: Fluency semantic measures in young Down syndrome adults correlate with amyloidosis plasma biomarkers

Authors: L. XICOTA[1,2,3], L. DEL HOYO[1,4], G. SÁNCHEZ-BENAVIDES[1], S. DE SOLA[1,2], A. CUENCA[1], J. RODRÍGUEZ[1], M. FARRÉ[1], M. DIERSSEN[2,3], *R. DE LA TORRE[5,3]
[1]Human Pharmacol. and Clin. Neurosciences Res. Group, IMIM (Hospital del Mar Res. Institute), Barcelona, Spain; [2]Cell. & Systems Neurobio., CRG, Barcelona, Spain; [3]Univ. Pompeu Fabra, Barcelona, Spain; [4]Univ. Autònoma de Barcelona, Barcelona, Spain; [5]Imim-Hospital Del Mar Med. Res. Inst., Barcelona, Spain

Abstract: Down syndrome (DS) patients are known to develop early onset of Alzheimer-like disease (AD). The overexpression of the APP gene combined with the modulation of DYRK1A, that phosphorylates APP, results in an increased Aβ peptides production. Aβ plasma concentrations are increased in young DS adults; nevertheless their correlation with the cognitive performance remains unexplored. Previous studies performed in elderly DS population have successfully linked Aβ plasma concentrations and cognitive decline. Semantic verbal fluency task, developed as a measure of semantic memory and executive function, requires the generation of as many animal names as possible in one minute. Total number of words and its clustering provide an indirect measure of the organization of semantic representations, while word retrieval strategies, such as switching, yield information about set shifting ability. The objective of this study is to understand whether amyloidosis plasma biomarkers correlate with semantic fluency measures. A DS population comprised of 86 (44 males and 42 females) DS subjects aged 17-34 y (mean 23.4 y) were evaluated according to standard scoring rules. Blood samples were obtained from DS patients to evaluate Aβ plasma concentrations (Inno-bia plasma Abeta forms,Innogenetics). An inverse correlation was found between Aβ42 concentrations, the total number of correct words ($r=-0.448, p=0.001$), the number of switchings ($r=-0.337$, $p=0.017$), and the total number of words produced in the last 45s ($r=-0.378$, $p=0.006$) in DS. The last variable is the period in which prototypical items are exhausted and additional search strategies should be engaged. To our knowledge this is the first time Aβ plasma concentrations have been correlated with fluency outcomes in DS patients. Because higher Aβ concentrations translate into worse cognitive performance, not only in the semantic organisation, but also in the set- shifting ability, and these patients still do not have any sign of AD, the scores obtained in the fluency tasks could be considered a first sign of the decline. However, more work is needed to confirm this hypothesis.

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Poster

516. Down Syndrome Anatomical and Behavioral Correlations

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 516.03/W3

Topic: C.06. Developmental Disorders

Title: Physical exercise rescues adult neurogenesis, synaptic plasticity and memory in Down syndrome mice

Authors: *M. PARRINI[1], D. GHEZZI[2], G. DEIDDA[2], L. MEDRIHAN[2], F. BENFENATI[2], P. BALDELLI[2], L. CANCEDDA[2], A. CONTESTABILE[2]
[1]NEUROSCIENCE AND BRAIN TECHNOLOGIES, [2]FONDAZIONE ISTITUTO ITALIANO DI TECNOLOGIA, GENOVA, Italy

Abstract: Down syndrome (DS), caused by the triplication of human chromosome 21, is the most frequent genetic cause of mental retardation. The Ts65Dn mouse model of DS show many neurological similarities to the human syndrome, including decreased hippocampal neurogenesis and cognitive impairment. In this study, we have investigated the effect of aerobic physical exercise on adult neurogenesis, synaptic plasticity and memory in Ts65Dn mice. Exposure of adult Ts65Dn mice to running wheels for one month increased the proliferation of neuronal precursor cell and stimulated adult neurogenesis in the hippocampal dentate gyrus. Moreover, physical exercise promoted the recovery of hippocampal synaptic plasticity and, most importantly, fully restored learning and memory in different behavioral task in trisomic mice. These findings demonstrate that Ts65Dn mice benefit from voluntary wheel running and provide evidence that physical exercise could represent a valuable complementary therapy for pharmacological interventions aimed at rescuing cognitive disabilities in DS patients.

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Poster

516. Down Syndrome Anatomical and Behavioral Correlations

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Topic: C.06. Developmental Disorders

Support: NICHD/NIH Grant R01HD67731

Title: Wiring the Brain of Down Syndrome

Authors: *J. R. KORENBERG[1], L. DAI[2], C. VACHET[3], E.-K. JEONG[4], J. S. ANDERSON[5], G. GERIG[3]
[1]Brain Institute, Pediatrics, Univ. of Utah, SALT LAKE CTY, UT; [2]Brain Institute/ Dept. of Pediatrics, [3]Scientific Computing and Imaging Inst., [4]Radiology Res., [5]Neuroradiology, Interdepart Prog Neurosci, Brain Inst, Bioengineering, Univ. of Utah, Salt Lake City, UT

Abstract: Down Syndrome and fcMRI Violence Abstract

Disclosures: J.R. Korenberg: None. **L. Dai:** None. **C. Vachet:** None. **E. Jeong:** None. **J.S. Anderson:** None. **G. Gerig:** None.

Poster

516. Down Syndrome Anatomical and Behavioral Correlations

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Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 516.05/W5

Topic: C.06. Developmental Disorders

Support: DSADIIP-13-284845

Title: Diffusion MRI signature of Down syndrome brain abnormal trajectories

Authors: *E. D. HAMLETT[1], X. NIE[2], A.-C. GRANHOLM[3], H. A. BOGER[3], M. F. FALANGOLA[2]
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Abstract: The Ts65Dn mouse model (TS) of Down syndrome (DS) is widely studied, developing neuropathology and cognitive impairment with age similar to that seen in the brain of humans with DS. These mice exhibit abnormal development and maturation of the brain with changes in dendritic structure and abnormal synaptic plasticity. Although TS mice have been well characterized cognitively and morphologically, little has been published using *in vivo* neuroimaging. Diffusional kurtosis imaging (DKI) is a diffusion MRI technique that extends

diffusion tensor imaging (DTI) by quantifying the non-Gaussian behavior of water diffusion, improving the characterization of the neurite cytoarchitecture. This study investigates the ability of DKI to detect the progressive abnormal developmental brain changes that have been well documented in TS mice. Mice (TS, n=8) and normosomic mice (NS, littermates, n=8) were studied at 2, 5 and 8 months of age. DKI experiments were performed on a 7T Bruker MR system. From DKI, mean kurtosis (MK), axial ($K_{//}$) and radial ($K_{\perp}$) kurtosis metrics were extracted. Regions of interest at the level of the frontal cortex (FC), striatum and hippocampus (HC) were manually drawn using NIH-ImageJ. One-way ANOVA corrected for multiple comparisons using Bonferroni was performed to assess group differences in DKI metrics (statistically significant at $p < 0.05$). The slopes of two group trajectories were compared, with a probability value of less than 0.05 indicating significant differences. Mouse behavior was measured at 8 months of age and the TS group exhibited alterations consistent with previous findings from our group, indicating hyperactivity and behavioral perseverance in a working memory task (novel object recognition). Our DKI results detected significant changes in the FC of the TS mice compared to NS already at 2 months of age (increased MK and $K_{//}$ ($p< 0.03$). At 5 months old, TS mice showed decrease in $K_{\perp}$ in the striatum ($p< 0.03$) and $K_{//}$ in the dorsal hippocampus (DH) ($p< 0.02$), which persisted in the 8 months old mice. Particularly in the DH, the slopes of two group trajectories were significantly different for MK ($p< 0.04$) and $K_{//}$ ($p< 0.03$). Although preliminary, these results validate that DKI metrics can detect TS brain developmental abnormalities; FC diffusion changes may represent less coherence in neurite orientations and abnormal cortical lamination, which may be affecting the myelination process, represented by the decrease in $K_{\perp}$ in the striatum, and triggering defects in axonal spread, represented by the decrease of $K_{//}$ in the dorsal hippocampus. Future studies will correlate DKI findings with specific morphological assessments.

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Poster

516. Down Syndrome Anatomical and Behavioral Correlations

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 516.06/W6

Topic: C.06. Developmental Disorders

Support: NIH Grant HD065160

Title: Peripheral biomarkers in Down syndrome follow the same pattern as in Alzheimer ’s disease

Authors: V. VASILEVKO[1], N. MOVSESYAN[2], E. DORAN[2], *I. T. LOTT[2,3]
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Abstract: Deposition of amyloid and neuritic plaques in brain parenchyma and cerebral vasculature is a characteristic feature of Alzheimer’s disease (AD). The amyloid-beta protein is a main component of this pathological marker. The process of amyloid brain deposition is paralleled to a significant decrease of amyloid in the periphery which is an eminent biomarker of AD progression. In individuals with Down syndrome (DS) the amyloid precursor protein gene, the source of amyloid-beta, is triplicated. There is strong evidence that in individuals with DS there is an excessive production of amyloid-beta which results in accelerated amyloid plaque deposition and neurotoxicity and development of AD symptoms as early as 40 years of age. In the current study we tested the hypothesis that AD progression in DS is following the same pattern as in the general population and could be detected with traditional biomarker panel. We analyzed CSF and plasma from patients with DS with or without AD as well as MCI, AD and normal controls from the general population. We used MSD multiplex platform for amyloid detection and Millipore Milliplex kit for cytokine/chemokines. We found that amyloid-beta in CSF and plasma of individuals with DS is significantly elevated compared to DS+AD and general population samples. During the disease progression in DS amyloid-beta CSF levels significantly dropped while plasma levels remained stable. Three major groups of cytokines/chemokines in CSF were affected with AD progression in DS individuals. The most significant increase in immune system related factors was observed in IL-6 cytokine, as well as sCD40L and RANTES. Trophic factors IL-7, G-CSF and PDGF-BB were increased also. Angiogenic factor FGF-2 was increased in the CSF of individuals with DS+AD. In plasma changes in cytokine/chemokines levels were not as dramatic as detected in CSF, however, immune system related factors were increased in IL-6 cytokine, IL-8 and MCP-1. Trophic factors G-CSF and PDGF-BB were also increased in DS+AD vs DS individuals. The most remarkable increase in angiogenic factors in plasma was observed in IL-8 cytokine with disease progression. These dramatic changes in immune, trophic and angiogenic factors are, probably, associated with the pathological processes of neurodegeneration, cerebrovascular impairments, apoptosis and misbalanced reaction of the organism to slow down/reverse these changes. Remarkably, cytokine/chemokine changes in CSF are more revealing about the disease progression than plasma levels. Our data suggest that AD is a systemic disorder and the disease progression in individuals with DS follow the same pattern as in general population.

Disclosures: V. Vasilevko: None. **I.T. Lott:** None. **N. Movsesyan:** None. **E. Doran:** None.

Poster

516. Down Syndrome Anatomical and Behavioral Correlations

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

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Topic: C.06. Developmental Disorders

Support: Instituto ALANA

Ohio Department of Developmental Disabilities

Awakening Angels Foundation

Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES)

Title: Delayed development of visual acuity in the mouse model of Down syndrome Ts65Dn

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Abstract: Down syndrome (DS), which is caused by the trisomy of chromosome 21, is the most common genetically defined cause of intellectual disability. This genetic disorder also affects the visual system in a variety of ways, which include high incidence of refractive errors, accommodative inaccuracy, amblyopia, strabismus, nystagmus, abnormal oculomotor and vestibular functions, decreased visual acuity, and decreased color and contrast sensitivities. In a previous study (Scott-McKean et al., IOVS 51: 3300- 3308, 2010), we demonstrated that adult mice Ts65Dn (a murine model of DS) exhibit deficits in luminance threshold, spatial resolution, and contrast threshold, compared with euploid control mice, as assessed electrophysiologically by pattern visual evoked potentials. Here, we investigated visual thresholds of optokinetic tracking (OKT), a fundamental visual behavior that facilitates the relative stabilization of retinal images. Using the methods originally described in detail by Prusky el al. (IOVS 45:4611-4616, 2004), we were able to quantify OKT thresholds in untrained and freely moving Ts65Dn and control euploid mice, daily from eye opening (postnatal day 14) to 35 days of age, and then in longer intervals (5-10 days), until the animals were 60-day old. We found that Ts65Dn mice show a significant delay in the maturation of the visual system. Whereas the mean spatial frequency sensitivity to a 100% contrast grating projected on a virtual cylinder for 14-day old euploid control mice was 0.21 c/deg, the mean value of this measure was 0.08 c/deg for Ts65Dn

mice. Ts65Dn mice were only able to achieve a mean spatial frequency sensitivity of 0.21 c/deg at 16 days of age. At age 45 days, the measured values of mean spatial frequency sensitivity were 0.45 and 0.43 c/deg for control euploid and Ts65Dn mice, respectively. The observed delay in the maturation of the visual system in a mouse model of DS mimics the qualitative features of the same phenomenon seen in young persons with DS. This study provides us with a new surrogate endpoint for the emerging field of translational research of potential pharmacotherapies designed to enhance the cognitive and adaptive skills of persons with DS.

Disclosures: M.R. Stasko: None. **A.C. Costa:** None. **J.J. Scott-McKean:** None. **D. Herrero:** None.

Poster

516. Down Syndrome Anatomical and Behavioral Correlations

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 516.08/W8

Topic: C.06. Developmental Disorders

Support: IUPUI OVCR RSFG

Title: Effects of epigallocatechin-3-gallate treatment on cognitive deficits in an adolescent Down Syndrome mouse model

Authors: M. E. STRINGER[1], I. S. ABEYSEKERA[2], R. J. ROPER[2], *C. R. GOODLETT[3]
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Abstract: Down syndrome (DS) is caused by three copies of human chromosome 21 (Hsa21) and results in a constellation of phenotypes that include intellectual disability (ID) and skeletal abnormalities. Ts65Dn mice, the most extensively studied model of DS, have three copies of approximately half the genes homologous to Hsa21 and display many of the phenotypes including skeletal and ID deficits. DYRK1A is found in three copies in humans with Trisomy 21 and has increased expression in a number of tissues. Dyrk1a is also found in three copies in Ts65Dn mice, shows similar increased expression, and has been shown to be involved in a number of critical pathways including CNS development and osteoclastogenesis. Epigallocatechin-3-gallate (EGCG), the main polyphenol compound found in green tea, is an inhibitor of Dyrk1a activity. We have previously shown that a three week treatment with EGCG during adolescence normalizes skeletal abnormalities in Ts65Dn mice. We hypothesize that a similar EGCG treatment will also rescue cognitive deficits observed in Ts65Dn mice. Trisomic

mice and euploid littermates were given ~10mg/kg/day EGCG or water (control) beginning on postnatal day 24, and the treatments continued either for three weeks or throughout subsequent behavioral testing. Beginning at six weeks of age, the mice were tested on locomotor activity (LMA, two daily 30-min sessions in an activity chamber), novel object recognition (NOR), acquisition of delayed non-matching to place in a T-maze (DNMP) and Morris water maze spatial memory task. Results to date indicate that the Ts65Dn mice were not different from controls on LMA, but showed deficits on NOR (reduced discrimination index for the novel object), the DNMP task (more trials to acquisition criterion), and the MWM task (longer acquisition latencies and reduced time in the target location on probe trials). The EGCG treatment did not significantly improve performance of the Ts65Dn mice on these cognitive tasks. These preliminary results indicate that neither a three-week nor continuous treatment with a low dose of EGCG during adolescence was sufficient to alleviate learning and memory deficits in the Ts65Dn mouse. Supported by Research Support Grant Funds from the IUPUI Office of the Vice Chancellor of Research.

Disclosures: M.E. Stringer: None. **R.J. Roper:** None. **C.R. Goodlett:** None. **I.S. Abeysekera:** None.

Poster

516. Down Syndrome Anatomical and Behavioral Correlations

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 516.09/W9

Topic: C.06. Developmental Disorders

Support: Fondation Jerome lejeune

Title: Aging brain in Down syndrome mouse models: Lessons from the cerebellum?

Authors: *N. CREAU[1], S. BENNAI[1], B. SOUCHET[1], E. CABET[1], A. DUCHON[2], J. DELABAR[1], Y. HERAULT[2], F. DAUBIGNEY[1]
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Abstract: In Down syndrome, the different organization/ connections of the brain, due to 3 copy genes, may impact the aging processes and induce vulnerability to Alzheimer disease. In the aging mouse, the neurons in the cerebellum are altered earlier than those in the hippocampus (Woodruff-Pak et al., 2010), suggesting that analysis of the cerebellum may bring information on

the aging processes at an earlier age. To highlight pathways involved in DS aging, we studied the Ts1Cje model which has a trisomy of MMU16 containing 80 genes, orthologs of HSA21 genes, and in a range of ages that may correspond from young (25y) to mature (55y) adults in humans. We have analyzed the cerebellum of the Ts1Cje at 3 ages: adult (4months), middle-aged (12months) and aged (18months), in parallel to control of the same ages. Neuronal and glial molecular markers were quantified by the slot-blot method to evaluate the effect of age and genotype. Already at middle-age, changes in the levels of several molecular markers (up or down) were identified in the controls that were amplified further in aging. In the Ts1Cje, some of them were altered in the same direction; but, some appeared to be specifically altered. The level of the glial marker, GFAP, was found to increase with age in the controls while less in the Ts1Cje (age and genotype effects). These age and genotype effects were also observed at the transcript level. Interestingly, at the same age, in the cerebellum of TgPCP4 (Mouton-Liger et al., 2014) no increase of GFAP was found suggesting that the overexpression of the neuronal calpacitin Pcp4 (in 3 copies in the Ts1Cje) may counteract the age-related glial alteration. Moreover, changes in neuronal (ex:inositol 1,4,5-trisphosphate receptor, type 1 ITPR1) and glial (ex : S100 calcium binding protein B, S100B) markers during aging highlighted calcium signaling pathways as important modifiers of cerebellum function with age, particularly in the DS model. Additionally, the kinase DYRK1A (in 3 copies in the Ts1Cje) a potential player in Alzheimer disease, was found increased in the controls and trisomics already at middle-age though still following the gene dosage ; this increase was also observed in the forebrain of the same mice at the same age. Current analysis by immunohistochemistry, also in the Ts65Dn, is performed to precise the localization of these molecular markers and of their changes and defined the cell populations and the mechanisms involved. This work was supported by Univ. Paris Diderot, CNRS and grants from the « Fondation Jérôme Lejeune » and the « Association Française de la Recherche sur la Trisomie 21 ».

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Poster

516. Down Syndrome Anatomical and Behavioral Correlations

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Program#/Poster#: 516.10/W10

Topic: C.06. Developmental Disorders

Support: PAPIIT IN-217211-3 Patología y plasticidad de las espinas dendríticas en el síndrome de Down. Contribución de la Trombospondina 1 (TSP-1)

PAPIIT RG300313 Desarrollo de lenguaje en niños con síndrome de Down: la comprensión temprana

Title: Cognitive flexibility and receptive vocabulary in Down syndrome

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Abstract: Down syndrome (DS) is the most prevalent genetic cause of intellectual disability. Lower performance, in comparison to children with typical development or other disabilities, in tasks measuring cognitive flexibility is typically reported. However, preserved language comprehension skills can be found. Although a significant relationship between cognitive flexibility and receptive vocabulary has been reported, little is known regarding the factors involved in such a relationship. The present research aimed to investigate the association between the receptive vocabulary of children with DS and their cognitive flexibility. Fourteen participants (9M, 5F; mean chronological age = 11.66 years; SD = 2.41) performed two tasks: the Card Sorting Task, extracted from the Frontal Lobes and Executive Functions Battery (BANFE), and the Peabody Picture Vocabulary Test (PPVT-III). The results from PPVT-III indicated a receptive vocabulary score of 3.10 years (SD= 0.66). Results from the Card Sorting Task yielded a mean standard score of correct responses of 6.71 (SD = 1.858), and 3.57 (SD= 2.848) in perseverations. According to the BANFE scale of the Card Sorting Task, 71.4% of the participants reached normal performance, 21.4% showed a mild-moderate deficit level and 7.1% a severe detriment level; while for perseverations 7.1% reached normal performance, 35.7% mild-moderate deficit level and 57.1% a severe detriment level. The mean performance time in the Card Sorting Task was 736.9 seconds; according to the BANFE scale, 14.3% of the participants reached normal performance, 14.3% showed a mild-moderate deficit level and 71.4% of the participants could not be classified since their performance mean time exceeded the 600 seconds permitted to respond. Correlations between receptive vocabulary from PPVT-III, mean correct responses and performance time in the Sort Card Task revealed that the receptive vocabulary score was positively correlated with the standard score of correct answers ($r=.639$, $p<0.05$) and negatively with performance time ($r=-.541$, $p<0.05$). Moreover, chronological age only correlated with receptive vocabulary ($r=.535$, $p<0.05$). The current results suggest a preserved ability, by DS children, to generate in a spontaneous manner a classification criterion (by color, shape); however, they showed deficits to change in a flexible manner the classification criteria. Also, in most of the cases the time to solve the task of cognitive flexibility was longer than expected. Finally, cognitive flexibility in DS children is not linked to receptive vocabulary, thus intervention strategies centered in executive functions improvements are deem prudent.

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Poster

516. Down Syndrome Anatomical and Behavioral Correlations

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Topic: C.06. Developmental Disorders

Support: NIH Grant NS066072

Down Syndrome Research and Treatment Center, University of California, San Diego, USA

Lumind Foundation, USA

Title: Behavioral, physiological and morphological studies in the Dp(16)1Yey mouse model of Down syndrome

Authors: *P. V. BELICHENKO[1], A. M. KLESCHEVNIKOV[1], V. A. AKULININ[2], E. G. WAGNER[1], A. BECKER[1], L. V. LYSENKO[1], S. S. STEPANOV[2], M. MICHALKO[1], I. MAHAPARN[1], M. A. PITTMAN[1], N. Y. KLESCHEVNIKOVA[1], E. MASLIAH[1], E. Y. YU[3], W. C. MOBLEY[1]
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Abstract: Down syndrome (DS), human trisomy 21, is the most common genetic cause of intellectual disability in children and young adults. The genomic regions on human chromosome 21 (Hsa21) are syntenic to the three regions in the mouse genome, located on mouse chromosome 10 (Mmu10), Mmu16, and Mmu17. We examined Dp(16)1Yey/+ (Dp16) mice, a recently created genetic model of DS which harbors a third copy of ~113 genes located on 22.9 Mb segment syntenic to Hsa21 on Mmu16. In comparison to Ts65Dn, Dp16 mice carry three copies of 16 additional Hsa21 genes and do not have triplication of the subcentromeric Mmu17 region. Previously, we reported that Dp16 mouse model exhibits DS-related neurological defects, including impaired hippocampal-mediated learning and memory (Morris water maze and contextual fear conditioning tests) and reduced hippocampal long-term potentiation (Yu et al.,

2010). In this study, we explored DS-related phenotypes in Dp16 mice with the age ranging from 21 days to 17 months. We assessed locomotor and cognitive behaviors, hippocampal electrophysiology and brain morphology, as well as biochemistry. Compared to wild type (WT) mice, there was a reduction in the body weight starting at 10 months old ($p = 0.02$) that continued to 17 months old ($p = 0.01$). Brain weight was at first reduced at 10 months ($p = 0.02$) in Dp16 mice. Abnormalities in the Y-maze test were evident from 21 days of age ($p = 0.007$) and were still detected at 8 months ($p = 0.002$). Novel object recognition with a 24 hours delay just showed deficits in 17 months Dp16 mice ($p = 0.005$). No changes were found in locomotor activity at 10 or 17 months of age (total distance traveled: $p = 0.42$, $p = 0.33$, respectively). CA1 hippocampal LTP was reduced at 3 months of age in Dp16 mice ($p = 0.013$). Hippocampal volume was significantly reduced at 10 months ($p = 0.028$) and the cholinergic phenotype was evident at the same age with a reduction in the area of ChAT+ cell ($p = 0.048$) and the number of ChAT+ neurons ($p = 0.066$) in medial septum. In conclusion, Dp16 mice conservate many of the phenotypes of the Ts65Dn mice but demonstrate changes in the onset of time deficits. Dp16 mice thus provide an excellent model for the future studies of neurobiology of DS.

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Poster

516. Down Syndrome Anatomical and Behavioral Correlations

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 516.12/W12

Topic: C.06. Developmental Disorders

Support: MRC Grant

NIHR Cambridge Biomedical Centre Grant

Down's Syndrome Association Grant

Title: The cortical landscape of the Down’s syndrome brain with and without Alzheimer’s disease neuropathology

Authors: *T. ANNUS[1], L. R. WILSON[1], J. ACOSTA-CABRONERO[2], A. CARDENAS-BLANCO[2], S. ZAMAN[1], A. J. HOLLAND[1], P. J. NESTOR[2]
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Abstract: Post mortem and morphometric studies have shown distinctive gross anatomical differences between the brains of people with Down's syndrome (DS) and typically developing individuals. Furthermore, studies have investigated the effects of early development of Alzheimer's disease (AD) neuropathology on DS brain morphometry and volume, which resembles the neuropathological pattern seen in sporadic AD. However to our knowledge, there are no studies that have reported the differences in the cortical landscape of the DS brain with respect to the typically developing brain and the subsequent changes that emerge with the development of AD neuropathology in this population. The aim of this study is to provide a detailed account of the pattern of cortical thickness in the DS brain at baseline (without amyloidosis) and to investigate how this is affected by the development of AD neuropathology. We measured cortical thickness using a set of automated tools (Freesurfer) to reconstruct the brain's cortical surface from T1-weighted structural MRI data (MPRAGE) in 34 subjects with DS, of which 10 were positive for fibrillar β-amyloid on PiB-PET scans, and 30 age-matched healthy controls. Regression analysis with age was performed using SPM8. Compared to cognitively normal subjects, adults with DS without amyloidosis had thinner cortex primarily in bilateral motor cortex and right retrosplenial cortex. Cortical thickening was visible in bilateral prefrontal, primary sensory, posterior cingulate and occipito-temporal cortex in those subjects with DS. In comparison to healthy controls, the 10 oldest subjects with DS without amyloidosis showed cortical thinning in bilateral, motor cortex, left superior temporal gyrus and right retrosplenial cortex, whereas cortical thickening was localised to bilateral prefrontal, primary sensory, posterior cingulate and occipital cortex. Adults with DS with amyloidosis had thinner cortex in bilateral retrosplenial, motor, lateral parieto-temporal and left subcollosal cortex and thicker cortex in prefrontal cortex, when compared to healthy controls. Age had no effect on the cortical thickness in subjects with DS without amyloidosis (single group regression corrected for TIV, FWE=0.05). The results of this study provide evidence of the detailed pattern of cortical thickness in DS brain at baseline and how it deviates from the cortical anatomy of the healthy control brain. With the development of AD neuropathology and amyloid deposition, a further change of cortical thickness from the baseline is visible, providing insights into the effects of AD neuropathology and amyloid deposition on cortical thickness in adults with DS.

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Poster

516. Down Syndrome Anatomical and Behavioral Correlations

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 516.13/W13

Topic: F.01. Human Cognition and Behavior

Support: Hobbs Discovery Grant VKC 4-04-218-9745

Vanderbilt CTSA ULI TR000445

Title: Nicotine treatment improves neural and cognitive measures of memory in adults with down syndrome: An open-label pilot study

Authors: *A. R. KAMKWALALA[1], A. KEY[2], E. DYKENS[2], D. JONES[2], V. C. GAU[1], A. POTTER[3], P. A. NEWHOUSE[1]
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Abstract: Adults with Down Syndrome (DS) are at higher risk for age-related cognitive changes and dementias, and experience age-related cognitive changes up to 20 years earlier than normally developed individuals. The heightened risk of Alzheimer's disease (AD) in DS is conferred by a partial or full duplication of chromosome 21, which includes the amyloid precursor protein, APP. Cognitive symptoms of AD are associated with progressive damage to the cholinergic receptor system, thus cholinergic receptors and projections decrease as cognitive status worsens. Prior studies in our lab have shown that activation of remaining nicotinic receptors with chronic transdermal nicotine has positive cognitive effects on adults with mild-cognitive impairment (MCI), a prodromal disorder to AD (Newhouse et al, Neurology 78: 91-101). We hypothesized that treatment with nicotine may have similar benefits in middle-aged adults with DS, by improving memory and attention with chronic treatment. We administered 4 weeks of a low-to-moderate dose (7-14mg) of nicotine via transdermal patch to adults (25 years+) with DS. Participants were tested on a cognitive battery to assess working memory, attention, and visual event-related potentials (ERP) at Baseline, Day 14, Day 28, and a post-treatment follow-up at Day 42. Safety and tolerability measures were assessed at all visits, to monitor side-effects of the drug. Modified Buschke Selective Reminding task results show an increase in 'consistency', a measure of ability to hold words in working memory, at Day 28 vs. Baseline. On the Choice Reaction Time task, one individual's performance showed a promising decrease in reaction time from baseline to Day 28 that persisted to Day 42. Preliminary ERP analyses show that nicotine treatment caused increased amplitude of visual ERP P600 wave in response to a repeated stimulus during a passive memory task at Day 28 and Day 42 vs. Baseline, an indication of visual recognition/memory. Vital signs responded as expected, with decreases in weight by Day 28. Blood pressure remained stable across study duration, within normal ranges. Nicotine

treatment was well tolerated, safety results suggest that slower titration of dose is ideal in this population. This study provides evidence that chronic administration of nicotine may have the potential for beneficial CNS effects on attention and memory processes in adults with DS.

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Poster

517. Developmental Disorders II

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 517.01/W14

Topic: F.01. Human Cognition and Behavior

Support: NICHD (P50 HD052117)

Title: Neural correlates of reading comprehension pre- and post-intervention in struggling readers

Authors: ***M. A. ROE**[1], J. E. MARTINEZ[1], J. A. MUMFORD[1], J. J. JURANEK[3], L. A. OLMEDO[1], R. A. POLDRACK[1], S. R. VAUGHN[2], J. M. FLETCHER[4], J. A. CHURCH[1]
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Abstract: One way to assess a reading intervention is to look for functional brain changes between pre- and post-intervention. We are part of a large, multi-city, in-school 4th grade reading intervention project that focuses on reading comprehension and its interactions with attention skills. We thus investigated the neural processes of reading comprehension and inhibition as part of an evaluation of the intervention. Children (ages 8-11 years), identified as struggling or typical readers, participated in a dual-site pilot neuroimaging study: the University of Texas at Austin and the University of Texas Medical School at Houston. At the UT Austin site, 20 struggling readers completed an fMRI session before intervention, 16 struggling readers completed an fMRI session after the first year of intervention - 11 subjects completed both fMRI sessions - and 23 typical readers completed one fMRI session. From these data, a subset of subjects (who performed sufficiently, and had low enough movement) was included in each task analysis. Participants performed a sentence comprehension task (SC) and a stop-signal task (SST). The SC task (3 runs, 32 sentences/run) included four sentence categories: active sensible,

active non-sensible, passive sensible, and passive non-sensible. Subjects indicated if the sentence was sensible or non-sensible. The SST (2 runs; 96 “go” trials and 32 “stop” trials each run) included a visual stop signal staircased to maintain stop accuracy performance at 50%. Subjects were excluded from the SC fMRI analyses if accuracy on the task was <60%. SST exclusion criteria and calculations were based on Congdon et al. 2012. fMRI analyses of the SC task suggest struggling readers show more similar brain activity to typical readers after one year of reading intervention relative to pre-intervention measurements in numerous reading-related regions. Looking within just the struggling readers from whom we had repeated measures, the greatest changes during sentence comprehension were in putative task control regions (including anterior cingulate and bilateral insula). During the SST “stop” trials, we observe sub-threshold differences in right temporoparietal junction (TPJ) and right superior temporal sulcus (STS) in struggling readers relative to typical readers. During SST “go” trials we observe differences between pre-intervention and post year-1 intervention scans in struggling readers in putative task control regions. Our results are interpreted in the context of both reading and task control literatures, and suggest that changes in task control may precede behavioral and reading-related changes.

Disclosures: **M.A. Roe:** None. **J.E. Martinez:** None. **J.A. Mumford:** None. **J.J. Juranek:** None. **L.A. Olmedo:** None. **R.A. Poldrack:** None. **S.R. Vaughn:** None. **J.M. Fletcher:** None. **J.A. Church:** None.

Poster

517. Developmental Disorders II

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 517.02/W15

Topic: F.01. Human Cognition and Behavior

Title: Manipulation of cue switching variables in children and adults

Authors: *J.-R. BAUER, J. E. MARTINEZ, M. A. ROE, J. A. CHURCH
Univ. of Texas at Austin, Austin, TX

Abstract: Cognitive flexibility, the ability to adjust to new tasks and demands, is one of the last executive functions to develop and is also one of the most taxing (Diamond, 2013). We examined the relative impact of different manipulations on task switching across development with two goals in mind. First, we aimed to reduce differences in adult and child performance, thus reducing behavioral confounds observed in previous imaging studies. To that end, we

created a computer-based cue-target task consisting of nine runs of increasing difficulty via manipulations of response mapping consistency, stimulus congruency, number of tasks, and number of possible responses. Second, we wanted to measure short-term learning within the task paradigm as a function of age. To quantify learning, we added a tenth run identical in difficulty to the easiest first run. We collected behavioral data from 60 children aged 6-16 years and 60 adults aged 18-27 years in Study 1 and 33 children aged 7-15 years and 46 adults aged 18-25 years for Study 2. In Study 1, response mappings remained on the screen when the target appeared to potentially reduce difficulty for children. Study 2 induced greater cognitive load by having the onscreen reminders of response mappings disappear when the target arrived. With respect to our first goal, attempts to reduce differences in adult and child performance were largely unsuccessful; children were slower, less accurate, and more affected by the task-level manipulations than adults. However, in Study 1 we found a critical transition where children 12 years and up displayed more adult-like responses relative to younger children. In Study 2, we found a similar transition, but later, occurring around 15 years. We also found an interaction between switch costs and response-mapping manipulations where runs with mixed mappings had equal switch and repeat costs. Further, in runs with more than two response choices, repeat trials were more costly than switch trials for participants across age, while the opposite held true when there were only two response choices. Relevant to our second goal, we found similar amounts of behavioral improvement for both age groups, despite high starting levels of performance in adults. As a result of the higher cognitive load in Study 2, there was greater relative improvement in both age groups compared to Study 1. These results support the idea that more challenging tasks can promote higher degrees of short-term learning. Both studies show evidence of the ability to temporarily train and improve task-switching abilities in children within a single session, and have relevance for the study of atypical developmental populations in the future.

Disclosures: **J. Bauer:** None. **J.E. Martinez:** None. **M.A. Roe:** None. **J.A. Church:** None.

Poster

517. Developmental Disorders II

Location: Halls A-C

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Program#/Poster#: 517.03/W16

Topic: F.01. Human Cognition and Behavior

Support: Tourette Syndrome Association fellowships (DJG, JAC)

NIH K24 MH087913 (KJB)

NIH R21 MH091512 (BLS)

NIH R01 HD057076 (BLS)

NIH F32NS065649 (JAC)

Title: Support vector machine classification of pediatric Tourette syndrome using resting state functional connectivity

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Abstract: Tourette syndrome (TS) is a childhood-onset neuropsychiatric disorder characterized by motor and vocal tics. While tics constitute the major diagnostic symptom, TS is quite heterogeneous. Not only does tic severity vary widely across individuals, but TS also commonly involves other psychiatric and cognitive symptoms, has a waxing and waning profile of symptoms, and has a long-term prognosis that is difficult to accurately estimate. Current understanding of the brain mechanisms underlying TS is based on research that often treats individuals with TS as essentially homogenous. Thus, approaches that can capture the underlying features of an individual's particular presentation would be immensely beneficial to the field. In the present study, we aimed to take a first step toward this goal and make diagnostic predictions about individuals using resting state functional connectivity (RSFC) MRI. We applied support vector machine (SVM) classification to test whether patterns in whole-brain RSFC could predict diagnostic group membership. SVM classification takes a multivariate approach that can be sensitive to detecting group differences in patterns of data that may go unseen by traditional univariate analyses. RSFC data from 42 children with TS (8-15 yrs) and 42 tic-free controls matched for age, IQ, and in-scanner movement were included in the study. Data underwent strict volume censoring and preprocessing procedures to minimize motion-related effects. Correlations between a large set of RSFC-derived ROIs that covers the whole brain were submitted to both traditional univariate t-tests (corrected for multiple comparisons) and to the SVM classification algorithm. While univariate tests revealed no significant group differences, SVM was able to classify group membership with 74% accuracy ($p < .001$). Interrogation of the RSFC features driving the classification revealed an aggregation of functional connections that were both within and between RSFC-derived brain networks. These results support the contention that multivariate methods may be necessary to capture the complexity of some brain disorders, and hold promise for predicting prognosis and treatment outcome for individuals with TS.

Disclosures: **D.J. Greene:** None. **J.A. Church:** None. **K.J. Black:** None. **B.L. Schlaggar:** None. **B. Adeyemo:** None. **B. Nardos:** None.

Poster

517. Developmental Disorders II

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 517.04/W17

Topic: C.06. Developmental Disorders

Title: Altered synaptic plasticity in Tourette's syndrome and its relationship to motor skill learning

Authors: *V. C. BRANDT[1], E. NIESSEN[2], C. GANOS[3], T. BÄUMER[1], A. MÜNCHAU[1]
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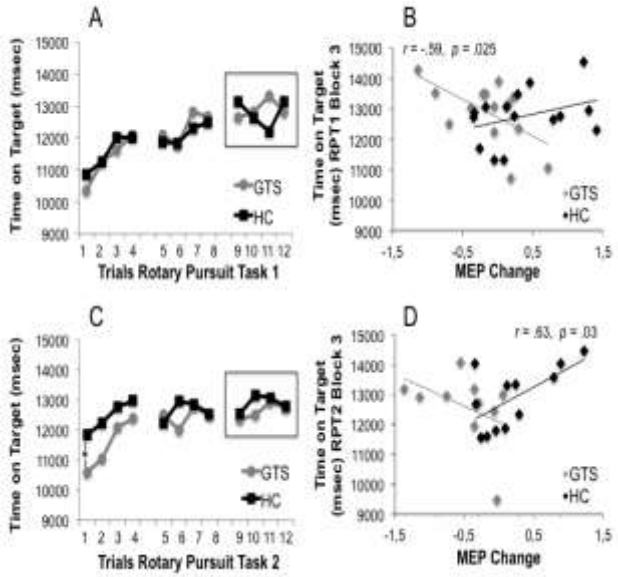
Abstract: Gilles de la Tourette syndrome (GTS) is a neuropsychiatric disorder characterized by motor and phonic tics that can be considered motor responses to preceding inner urges. It has been shown that GTS patients have inferior performance in some motor learning tasks and reduced synaptic plasticity induced by transcranial magnetic stimulation. However, it has not been investigated whether altered synaptic plasticity is directly linked to impaired motor skill acquisition in GTS patients. In this study, cortical plasticity was assessed by measuring motor-evoked potentials before and after paired associative stimulation (PAS) in 14 GTS patients (13 male; age 18 - 39) and 15 healthy controls (12 male; age 18 - 33). Tic and urge severity were assessed using the Yale Global Tic Severity Scale and the Premonitory Urges for Tic Disorders Scale. Motor learning was assessed 45 mins after inducing synaptic plasticity and 9 months later, using the rotary pursuit task. On average, long-term potentiation-like effects in response to the paired associative stimulation were present in healthy controls but not in patients. In GTS patients, long-term potentiation-like effects were associated with more and long-term depression-like effects with less severe urges and tics. While motor learning did not differ between patients and healthy controls 45 mins after PAS (figure 1a), the learning curve of the healthy controls started at a significantly higher level than the Tourette patients' 9 months later (figure 1c). Induced synaptic plasticity correlated positively with motor skills in healthy controls 9 months later (figure 4d). The present study confirms previously found long-term improvement in motor performance after PAS in healthy controls but not in GTS patients. Patients did not show long-term potentiation in response to PAS and showed reduced levels of motor skill consolidation after 9 months compared to healthy controls. Moreover, long-term depression-like effects in GTS patients were related to superior motor skill learning (figure 1b) and to fewer symptoms, suggesting a compensatory mechanism.



Disclosures: **V.C. Brandt:** A. Employment/Salary (full or part-time):; University clinic Schleswig-Holstein Lübeck, Institute of neurogenetics. **T. Bäumer:** A. Employment/Salary (full or part-time):; University clinic Schleswig-Holstein Lübeck, Institute of neurogenetics. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Commercial research **Support:** Honoraria for lectures from Pharm Allergan, Ipsen, Merz Pharmaceuticals. **A. Münchau:** A. Employment/Salary (full or part-time):; University of Lübeck, Institute of neurogenetics. 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.; Commercial research support Grants by Pharm Allergan, Ipsen, Merz Pharmaceuticals. Honoraria for lectures from Pharm Allergan, Ipsen, Merz Pharmaceuticals, Actelion, GlaxoSmithKline and Desitin. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Possehl-Stiftung, Lübeck Dystonia Coalition (USA) Tourette Syndrome Association (Germany) European Huntington Disease Network N.E.MO. Charity supporting the research of paediatric movement disorders. **E. Niessen:** A. Employment/Salary (full or part-time):; Institute of Neuroscience and Medicine, Research Centre Jülich. **C. Ganos:** A. Employment/Salary (full or part-time):; Deutsche Forschungsgemeinschaft (DFG) Deutsche Forschungsgemeinschaft (MU1692/2-1) European Science Foundation. 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.; Grants by Actelion, Ipsen, Pharm Allergan and Merz Pharmaceuticals. Deutsche Forschungsgemeinschaft (DFG) Deutsche Forschungsgemeinschaft (MU1692/2-1) European Science Foundation..

Poster

517. Developmental Disorders II

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 517.05/W18

Topic: C.06. Developmental Disorders

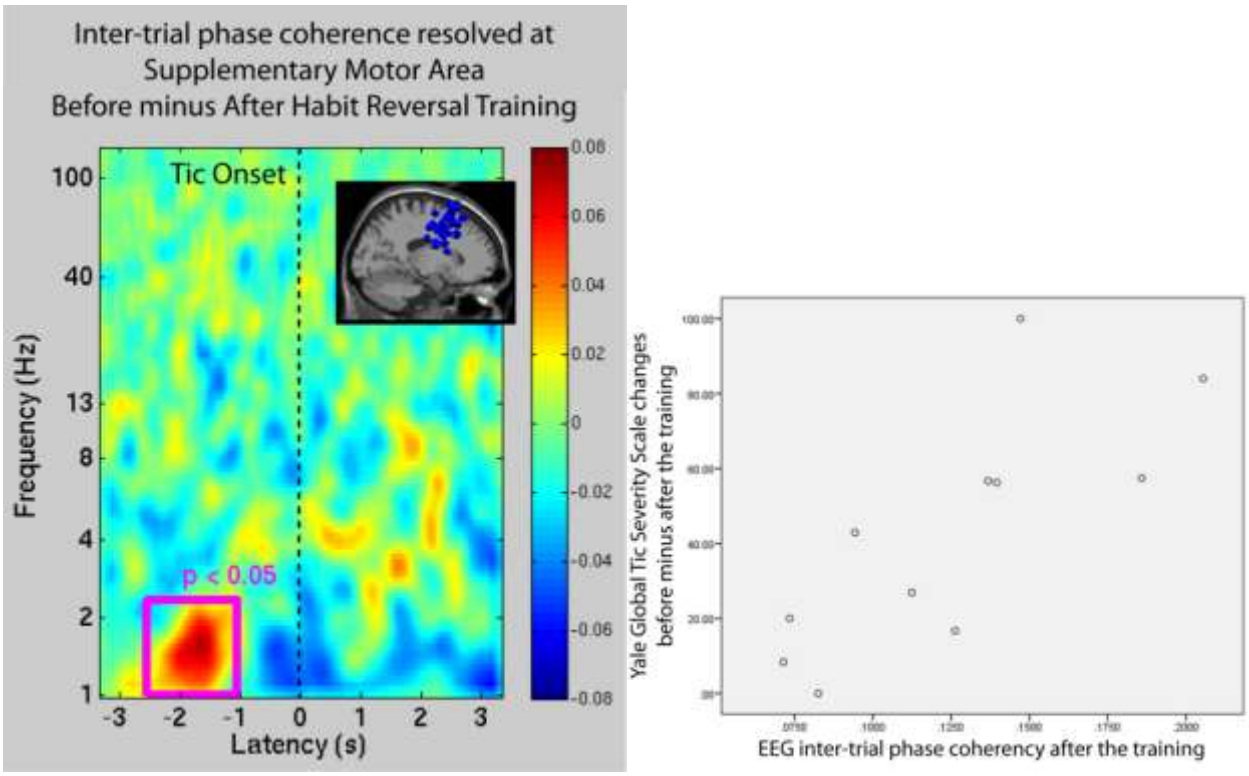
Support: NS80160

MH077248

Title: Treatment-related changes in EEG activation in Tourette's syndrome

Authors: *M. MIYAKOSHI[1], S. MAKEIG[1], J. PIACENTINI[2], P. WALSHAW[2], S. CHANG[2], J. MCCRACKEN[2], S. K. LOO[2]
[1]Swartz Ctr. For Computat. Neuroscience, INC, UCSD, La Jolla, CA; [2]Dept. of Psychiatry and Biobehavioral Sci., Univ. of California Los Angeles, Los Angeles, CA

Abstract: Background The challenge in studying cortical activity in Tourette's Syndrome is involuntary movements that prohibit the use of MRI due to movement artifacts. We propose the use of EEG measurement with computational data mining as a promising alternative. Materials and Methods 21 patients of chronic tic disorder participated, and 17 of them underwent two sessions before and after Habit Reversal Training. We administered three 5-minute sessions: tic freely; verbal instruction to suppress tic; the same but with $10 reward. Tic severity was evaluated using Yale Global Tic Severity Scale. EEG was recorded from 40 scalp channels. EEG data were annotated with tic onset markers and analyzed with EEGLAB (SCCN, UCSD). Results Tic counts during tic freely condition was average 29 (SD 15); verbal suppression 14 (8); reward suppression 13 (10). Independent component clustering analysis found a cluster centered at [0 13 43] in Talairach coordinates, which we identified to be supplementary motor area, to which 12/17 participants contributed. The inter-trial phase coherence of this cluster after training was significantly associated with treatment-related behavioral improvement in tic severity and frequency ($r = 0.78$, $p < 0.005$). Conclusion We found treatment-related changes in EEG activation in Tourette's Syndrome. We demonstrated the usefulness of EEG measurement with this neurological disorder, and revealed the electrophysiological representation of pre-tic brain dynamics in the supplementary motor area.



Disclosures: **M. Miyakoshi:** None. **S. Makeig:** None. **S.K. Loo:** None. **J. Piacentini:** None. **P. Walshaw:** None. **S. Chang:** None. **J. McCracken:** None.

Poster

517. Developmental Disorders II

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Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

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Topic: C.06. Developmental Disorders

Support: KAKENHI 25830010

Tourette Syndrome Association -USA

Title: Distinct cortical and subcortical networks drive myoclonic and vocal tics in the nonhuman primate model of Tourette syndrome: A PET and electrophysiological study

Authors: ***K. W. MCCAIRN**[1], Y. NAGAI[2], Y. HORI[2], A. IRIKI[3], M. TAKADA[1], T. MINAMIMOTO[2], M. ISODA[4], M. MATSUMOTO[5]

[1]Kyoto University, Primate Res. Inst., Inuyama, Aichi, Japan; [2]Natl. Inst. of Radiological Sci., Inage, Japan; [3]RIKEN Brain Sci. Inst., Wako-Shi, Japan; [4]Kansai Med. Univ., Kansai, Japan; [5]Tsukuba Univ., Tsukuba, Japan

Abstract: Myoclonic and vocal tics, believed to arise from abnormalities in the cortico-basal ganglia (CBG) system, are primary symptoms associated with Tourette syndrome (TS). We used a nonhuman primate model of TS to investigate the neurophysiological and behavioral dynamics of myoclonic and vocal tics. Tourettisms were induced through bicuculline (GABA antagonist) disinhibition of different functional territories in the CBG system. Specifically, bicuculline targeted to the sensorimotor territory of the striatum (putamen) led to repetitive myoclonic tics, while placement in the limbic territory (nucleus accumbens) induced persistent vocalizations. Using video, electromyographic (EMG), local field potential (LFP) recordings, and PET scanning, we demonstrate that simple myoclonic and vocal tics exhibit partly similar but largely distinct properties with respect to the emergence of pathological behavior and brain states. Critically, myoclonic tics showed a faster expression rate and had a longer duration period than vocal tics. At the cortical level, the amplitude of LFP spikes in the primary motor cortex (M1) and the anterior cingulate cortex (ACC), major origins of the CBG system, characterized the two behavioral conditions. Tic-associated cortical LFP spikes were larger for simple myoclonic than vocal tics. In addition, simple myoclonic tics were associated with a strong 1:1 relationship between the LFP spikes in the M1 and EMG activity, while the relationship to vocal tics and the LFP spikes in the ACC was more complex. Although there was a strong correlation between the emergence of LFP spikes in the ACC and vocalizaion, it was also observed that the LFP spikes during vocal tics could occur at variable latencies prior to and after vocalization. However, reduction of regional cerebral blood flow was seen in both the M1 and the ACC during the peak effects of bicuculline-induced tourettisms, possibly reflecting silencing of cortical activity during inter-tic intervals. The present findings indicate that different CBG network alterations underlie the major symptom subtypes of TS (myoclonic vs. vocal tics), and this segregation opens the possibility of discrete therapeutic targeting to suppress individual symptom subtypes in TS.

Disclosures: K.W. McCairn: None. **Y. Nagai:** None. **Y. Hori:** None. **A. Iriki:** None. **T. Minamimoto:** None. **M. Isoda:** None. **M. Matsumoto:** None. **M. Takada:** None.

Poster

517. Developmental Disorders II

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Topic: C.06. Developmental Disorders

Support: NIH NINDS R01-NS054994

Brain and Behavior Research Foundation (NARSAD) 18590

NIMH K08 MH099424-01

Simons Foundation

Allison Foundation

Shire

Title: Integrative analysis of gene expression and rare single nucleotide variations in RNAseq data of the striatum in Tourette syndrome

Authors: *G. COPPOLA[1], J. B. LENNINGTON[1], Y. KATAOKA-SASAKI[1], T. FERNANDEZ[1], D. PALEJEV[1], Y. LI[1], A. HUTTNER[2], M. PLETIKOS[3], N. ŠESTAN[3], J. F. LECKMAN[1], F. M. VACCARINO[1]
[1]Child Study Ctr., [2]Dept. of Pathology, [3]Dept. of Neurobio., Yale Univ., New Haven, CT

Abstract: The etiology of Tourette syndrome (TS) is unknown. Despite a high population prevalence and substantial genetic contribution, attempts to identify common gene variants through genome wide association studies have not revealed candidates above threshold significance. Here we focused on evaluating transcriptional differences in the striatum (caudate and putamen), a brain region highly implicated in TS. We sequenced RNA isolated from post-mortem tissue of 9 individuals that had severe persistent TS symptoms in adulthood compared to 9 matched controls. Differential gene expression analysis revealed 309 down-regulated genes related primarily to neuronal signaling, specifically of striatal interneurons, and 822 up-regulated genes, mainly encoding for inflammatory and immune-related proteins. Weighted gene co-expression network analysis and gene set enrichment analysis revealed 17 gene co-expression modules associated with TS. The top-scoring down- and up- regulated modules were enriched in striatal interneuron-related genes and immune-related genes, respectively. Immunohistochemical studies of fixed tissue series further confirmed that three classes of striatal interneurons are significantly reduced in TS: (1) cholinergic and (2) parvalbumin^{+}-GABAergic interneurons, which had been previously reported; and (3) nitric oxide synthase1(NOS)$^{+}$/neuropeptide Y(NPY)$^{+}$/somatostatin(SST)$^{+}$-GABAergic interneurons. Reductions in these classes of interneurons were also indicated by significant decreased striatal expression of CHAT, CHRM2, SLC5A7 and several other cholinergic-related genes, and several GABAergic-related genes including GAD1, NOS1, NPY, and SST. These data strongly implicate disrupted interneuron signaling in the pathophysiology of severe TS. However, we found a lack of correlation between the expression of interneuron-related and immune system-related classes of transcripts, suggesting that these two signatures may independently contribute to TS pathophysiology. We

also evaluated the intersection of the network modules with genomic variants. Intersection of our network modules with genes implicated in a recent study of TS-associated copy number variants revealed enrichment in interneuron- and protocadherin- related gene modules. Analysis of rare single nucleotide variants (SNVs) from the RNA seq data revealed significant association of UTR SNVs within the immune-related gene module with TS, and marginal association of non-synonymous SNVs within the protocadherin-related gene module. Together these genomic alterations implicate cell adhesion and immune function loci in TS.

Disclosures: G. Coppola: None. **J.B. Lennington:** None. **Y. Kataoka-Sasaki:** None. **T. Fernandez:** None. **D. Palejev:** None. **Y. Li:** None. **A. Huttner:** None. **M. Pletikos:** None. **N. Šestan:** None. **J.F. Leckman:** None. **F.M. Vaccarino:** None.

Poster

517. Developmental Disorders II

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 517.08/W21

Topic: C.06. Developmental Disorders

Title: Oscillations and plasticity in Tourette Syndrome

Authors: *R. HASHEMIYOON
Univ. of Miami, Coral Gables, FL

Abstract: Aberrations of synchronous neuronal activity have been correlated with a multitude of neurological disorders, including Parkinson's disease (PD), tinnitus, schizophrenia, autism spectrum disorder, and Tourette syndrome (TS). While the pathophysiology of these and other brain disorders is both distinct and unresolved, pathological hypersynchronization of neuronal groups has been consistently reported. Human, animal, and theoretical studies have further demonstrated a shift back towards physiological state concomitant with desynchronization of the abnormal activity. Additionally, models of plasticity have been derived to simulate and explain network dynamics with high levels of synchronization. One type of plasticity in particular, spike time dependent plasticity (STDP), has been explored and extrapolated to disorders of hypersynchrony such as PD and tinnitus. Circuit function with respect to disorders of synchrony, however, must also include hyposynchrony such as described in TS; yet, there are virtually no reports addressing this regime. Our recent study using deep brain stimulation (DBS) electrodes to record from the human thalamus demonstrated a correlation between low synchrony in the gamma band and positive symptoms of the disorder (tics). Despite being persistent, tic

expression waxes and wanes, however the mechanism by which this occurs is unclear. I propose here that the governing dynamic is one of alternation between strongly and weakly synchronized states, representing the waning and the waxing modes of tic expression, respectively. Furthermore, by considering STDP in this regime, the two states coexist in the network with synaptic strength dictating the level of synchrony. A model for this bistability has been described as the Sisyphus Effect, in which a network is characterized by spontaneous fluctuations between the two levels of synchrony. Mechanisms of STDP may also be useful for producing therapeutic changes in TS pathophysiology. Under conditions of STDP, a network can be moved into either a synchronized, desynchronized, or bistable state. Additionally, by means of feed forward inhibition, such as occurs in the recurrent loop of TS, GABA can control STDP by reversing its polarity. Either or both of these processes can be used or manipulated to increase neuronal coordination from the hyposynchronized pathological state seen in TS to a normal physiologic one.

Disclosures: R. Hashemiyoon: None.

Poster

517. Developmental Disorders II

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 517.09/W22

Topic: F.01. Human Cognition and Behavior

Support: Autism Speaks 7608

Department of Defense AR130106

Title: Individual differences in implicit learning abilities in young children: Implications for autism treatment predictors

Authors: *R. M. JONES[1,2], C. LORD[2]
[1]Rebecca Jones, NEW YORK, NY; [2]Psychiatry Dept., Weill Cornell Med. Col., New York, NY

Abstract: Implicit associative learning is an individual's ability to form connections between objects or stimuli without conscious awareness. There is inconsistency in the literature as to whether individuals with Autism Spectrum Disorder (ASD) have difficulties with such learning. Some suggest that there is impairment, while others report no differences compared to typically developing (TD) individuals. We hypothesize that the discrepancy is not simply explained by

methodological differences across studies, but reflects variability within ASD for implicit learning. Building upon the implicit learning literature, we designed a child-friendly task that measures differences in reaction time (RT) behavior and accuracy to a target stimulus predicted by two cues at differing probabilities. In each trial, children were instructed to touch a target image presented on an iPad but to refrain from touching the cues and distractor image. Unbeknownst to the participants, one of the cues predicted the target image at a high probability (75%), while the other cue preceded the target at a low probability (25%); the distractor appeared when the target was not presented. Trials were divided into thirds (early, middle and late) in order to assess learning differences. Preliminary results (N = 8; 2M; 3-6 years of age; Mean IQ = 113) are reported in TD children. All children demonstrated an increase in false alarms (incorrectly pressing the distractor) when presented with the high probability cue versus the low probability cue. All children show speeding in reaction times by the late trials to the target when preceded by the 75% cue compared to the 25% cue. Some children, regardless of age, demonstrate this pattern during middle trials, suggesting individual differences in implicit learning. Interestingly, after the task, only 2 of 8 children correctly matched the target image with the 75% cue image, suggesting learning occurred without conscious awareness. Preliminary data suggest, all TD children regardless of age, form associations during an implicit probabilistic learning task. Individual differences in how quickly children learn the patterns may be important for predicting intervention success in young children with ASD. Ongoing research in TD children, TD children who have a sibling with ASD and children with ASD will categorize individual variability in implicit learning abilities both within and across groups of children. The behavioral findings will be the foundation for future research with this task during functional Magnetic Resonance Imaging to understand the neural underpinnings of individual variability in implicit learning abilities in young children with ASD.

Disclosures: R.M. Jones: None. **C. Lord:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Western Psychological Services.

Poster

517. Developmental Disorders II

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 517.10/W23

Topic: F.01. Human Cognition and Behavior

Title: Cognitive Profiles in adults with autism spectrum disorder and attention deficit hyperactivity disorder in Japan

Authors: *C. KANAI[1,2], M. TANI[2], R. HASHIMOTO[2], T. YAMADA[2], H. OTA[2], A. IWANAMI[2], N. KATO[2]
[1]Sagami Women's Univ., Kanagawa, Japan; [2]Showa Univ., 6-11-11 Kitakarasuyama Setagaya-ku, Tokyo, 157-851, Japan

Abstract: Background: Little is known about the cognitive profiles of high-functioning autism spectrum disorder (ASD) and attention deficit hyperactivity disorder (ADHD) in adults based on the Wechsler Intelligence Scale III (WAIS-III). Objectives: To examine cognitive profiles of adults with no intellectual disability (IQ > 70), and in adults with ASD (Asperger's disorder (AD), High-Functioning Autism (HFA)) and ADHD using the WAIS-III. Methods: The clinical group of this study comprised outpatients at Showa University Hospital attending a diagnostic outpatient clinic for adults 18 years of age and older with suspected ASD. All patients were referred by physicians from other clinics. Inclusion criteria were WAIS-III FIQ ≥ 70; age of 18 to 60 years; no current use of anti-psychotics; and formal diagnosis of ASD, including autistic disorder, AS, and ADHD based on the DSM-IV-TR. To assess the presence of autistic traits, the Japanese version of the Autism-Spectrum Quotient (AQ) and Pervasive Developmental Disorders Autism Society Japan Rating Scale (PARS). Results: Verbal Intelligence (VIQ) - Performance Intelligence (PIQ) differences were detected between the two groups. Some subtest scores were significantly higher in ASD than in ADHD. Conclusions: The findings demonstrated cognitive profiles characteristic of adults with high-functioning ASD and ADHD.

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Poster

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Topic: C.06. Developmental Disorders

Support: NINDS/NIH R01 NS049096

NICHD/NIH P30 P30HD015052

NCATS/NIH UL1 TR000445

NCRR, NIH, under P41 RR15241

Johns Hopkins School of Medicine General Clinical Research Center

Title: Functional connectivity of the visual cortex in children with neurofibromatosis type 1 and reading disability during single word reading

Authors: *L. A. BARQUERO, K. SWETT, S. K. BAILEY, S. S. BURNS, L. E. CUTTING
Vanderbilt Univ., Nashville, TN

Abstract: Neurofibromatosis Type 1 (NF1) is a genetic disorder with a prevalence of about 1:3500. Reading disabilities (RD) and visuospatial deficits are commonly associated with NF1, yet few investigations have characterized differences between RD in NF1 and idiopathic RD. A previous behavioral study indicated that reading interventions in populations with NF1 helped remediate both reading and visuospatial processes, raising questions about the contribution of visual systems in NF1 reading deficits. The purpose of this study was to explore functional connectivity of the visual cortex during reading, comparing children with NF1 and RD (NF+RD) to children with idiopathic RD. Participants were 39 children and adolescents (8-14 yrs) comprising three groups: NF+RD (n = 11), RD only (n = 13), and typically developing readers (TD; n = 15). During fMRI, participants performed a single word reading task, reading words that varied along dimensions of concreteness ("door" vs. "need") and spelling regularity ("came" vs. "clothes"). Connectivity analyses revealed significant differences in functional connectivity in readers with NF1. When reading abstract words, the NF+RD group alone showed decreased connectivity of the visual cortex (BA 17, 18, 19) with the left dorsolateral prefrontal cortex (p<0.001, uncorrected; k> 90), an area that has been implicated in visuospatial attention. Further analyses comparing conditions of regularly spelled vs. irregularly spelled abstract words revealed that the irregular condition appears to drive the connectivity difference observed for NF+RD in the left dorsolateral prefrontal cortex. In addition, when reading irregular abstract words, the NF+RD group exhibited decreased functional connectivity between visual cortex and left angular gyrus (p<0.001, uncorrected; k> 90), a semantic association area, and this difference was not observed in RD. Results suggest that reading deficits associated with NF1 may have underlying neurobiological differences as compared to idiopathic RD, and these differences are potentially related to visuospatial deficits commonly associated with NF1.

Disclosures: L.A. Barquero: None. **K. Swett:** None. **S.K. Bailey:** None. **S.S. Burns:** None. **L.E. Cutting:** None.

Poster

517. Developmental Disorders II

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 517.12/W25

Topic: C.06. Developmental Disorders

Title: Rhythm in music: Improving auditory processing in an animal model of developmental dyslexia

Authors: A. D. GRIFFIN, K. A. MCGINNIS, *A. H. BRADY
Psychology and Neurosci., St. Mary's Col. of Maryland, St Marys City, MD

Abstract: Language processing abilities are believed to be driven by the capacity to efficiently process rapidly presented auditory stimuli. Deficits in rapid auditory processing have been associated with language learning disorders such as developmental dyslexia. The induction of cortical microgyria via freeze lesion surgery leads to rapid auditory processing deficits as well as neuroanatomical abnormalities similar to those observed in post-mortem dyslexic brains, and has been proposed as an animal model of developmental dyslexia. Music exposure has previously been found to be effective in alleviation of auditory processing deficits in humans. In the present study, we investigated the degree to which passive music exposure with manipulated rhythms was effective in alleviation of these auditory processing deficits in rats with induced microgyria. Rats received either freeze lesion surgery or sham surgery on postnatal day 1, and began passive music exposure for three hours a day at postnatal day 11. We compared the effectiveness of music with simple, regular rhythms against music with complex, irregular rhythms. Beginning at postnatal day 26, a series of startle response paradigms was used to test juvenile rats' ability to process auditory stimuli, namely the ability of a subthreshold cue or gap to suppress the acoustic startle response. The first day of behavioral testing consisted of a Normal Single Tone procedure which measured a baseline ability to process auditory stimuli. These startle response scores were used to account for individual differences in startle testing in later procedures. The following four days of testing consisted of a Long Silent Gap procedure and the last four days of testing consisted of a Short Silent Gap procedure which measured the rats' ability to detect varying gap durations. The results of this study supported previous findings that while all rats were able to process auditory stimuli presented at slower speeds, the microgyric juvenile rats were significantly impaired at processing rapidly auditory stimuli in comparison to sham-treated animals. Furthermore, although sham-treated animals were relatively unaffected by music exposure, rapid auditory processing deficits in microgyric rats were alleviated by exposure to either music condition, with slightly stronger effects in the simple rhythm condition. These results may suggest that passive music exposure starting as early as infancy may benefit rapid auditory processing skills in humans at risk for developmental dyslexia.

Disclosures: **A.D. Griffin:** None. **K.A. McGinnis:** None. **A.H. Brady:** None.

Poster

517. Developmental Disorders II

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 517.13/W26

Topic: C.06. Developmental Disorders

Support: MB is supported by the Rhodes Trust

Project is supported by the Wellcome Trust Program Grant (092071/Z/10/Z)

Title: What is the role of dyslexia susceptibility candidate gene KIAA0319-Like in mouse cortical development?

Authors: *M. BAILEY[1], L. GUIDI[1,2], I. MARTINEZ GARAY[1], Z. HOLLOWAY[2], A. VELAYOS-BAEZA[2], A. P. MONACO[2,3], Z. MOLNÁR[1]
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Abstract: Developmental dyslexia (DD) is a common childhood learning disorder characterised by significant impairments in reading abilities, despite adequate intelligence and educational opportunity [1]. The biological mechanisms underlying DD still remain poorly understood, although different pieces of evidence suggest that impaired neuronal migration may be a cellular antecedent [2]. Genetic studies have identified 9 *loci* (*DYX1-DYX9*) and up to 14 candidate genes [3]. Among these, *KIAA0319-Like* (*KIAA0319L*) has been proposed as a candidate gene for *DYX8 locus* supported by its homology to the *DYX6 locus* candidate *KIAA0319* [4]. The expression pattern of *KIAA0319L* suggests its role is not restricted to the nervous system. What this role is in brain is important to understand its putative involvement in dyslexia. Little is known about it except the KIAA0319L protein has been reported to interact with the axon guidance protein Nogo Receptor 1 [5], the mouse homologue is expressed in adult brain [6] and, similarly to other dyslexia candidates, rat *in utero* knockdown suggests its involvement in neuronal migration [7]. To investigate the putative function of this gene in cortical development, we generated mice where exon 3 of *Kiaa0319L* was targeted. Homozygous KO mice are viable, fertile and with no apparent phenotype. Analysis of the distribution of mitotic profiles, early transcription factor expression in the germinal zone and layer-specific marker expression, as well as overall morphological structure, showed no important differences between KO and wild-type mice. We have performed qRT-PCR at several developmental stages (E15, E18, P2, P10) to have a clear picture of *Kiaa0319* and *Kiaa0319L* brain expression pattern. This analysis will help

identifying regions of interest for development and analysis of specific conditional KO mice. We are also investigating if *Kiaa0319L* is involved in later events during development, such as dendritogenesis and spine formation, after acute elimination by *in utero* Cre electroporation into floxed mice. Our results suggest that, contrary to the shRNAi-mediated knockdown findings in rat, neuronal migration is not affected by disruption of the *Kiaa0319L* gene. This is a similar result to those found in KO mice for other dyslexia candidate homologous genes such as *Kiaa0319* (unpublished) and *Dcdc2* [8], and contributes to the mounting evidence that neuronal migration is neither an essential nor an exclusive function of these genes in the mouse cerebral cortex. 1. Peterson & Pennington 2012 2. Gabel et al. 2010 3. Carrion-Castillo et al. 2013 4. Couto et al. 2008 5. Poon et al. 2011 6. Poon et al. 2011 7. Platt et al. 2013 8. Wang et al. 2011

Disclosures: **M. Bailey:** None. **L. Guidi:** None. **I. Martinez garay:** None. **Z. Holloway:** None. **A. Velayos-baeza:** None. **A.P. Monaco:** None. **Z. Molnár:** None.

Poster

517. Developmental Disorders II

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 517.14/W27

Topic: C.06. Developmental Disorders

Support: CIHR

Title: Phenotyping the dihydropyrimidine dehydrogenase knockout mouse

Authors: ***S. SPRING**[1], L. NUTTER[2], A. M. FLENNIKEN[3], I. VUKOBRADOVIC[3], C. MCKERLIE[3,2], J. P. LERCH[1]
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Abstract: Dihydropyrimidine dehydrogenase (Dpyd) deficiency is an autosomal recessive disease in humans characterized by excess quantities of uracil and thymine in the blood, urine and cerebrospinal fluid. Homozygous deficiency in patients has been associated with a variable clinical phenotype with the most common symptoms including convulsive disorders, motor retardation and mental retardation. Less frequent symptoms include autism, microcephaly and ocular abnormalities. To investigate the relationship between genotype and expressed phenotype, a Dpyd knockout mouse (*Mus musculus*) was produced on an isogenic C57BL/6NCrl

background. To study the phenotype associated with homozygous deficiency of Dpyd in the mouse, assays were conducted as per the standardized phenotyping protocols implemented by the International Mouse Phenotyping Resource of Standardised Screens (IMPReSS, www.mousephenotype.org/impress/). Some of these results are discussed here. A "knockout-first, conditional-ready" approach (1) was used to produce the cre-excised deletion of a critical exon in *Dpyd* resulting in a tm1b null allele. The open field test was used on male and female homozygous mutant mice at 9 weeks to assay general locomoter activity levels and anxiety compared to co-raised wild-type counterparts. A minimum of 7 mice per experimental group were used. Each mouse was placed in the middle of a peripheral zone of an arena facing the wall and allowed to explore the apparatus freely for 20 minutes. The activity of each mouse was measured in each of the arena zones in 5 minute bins. Clinical blood chemistry analysis was also conducted on test groups at 16 weeks of age with at least 7 mice per group. The open field test showed the Dpyd mutant mice had higher whole arena average speed, higher number of rears, higher latency to center entry, higher periphery average speed and higher distance traveled measurements. These results are indicative of hyperactivity, increased vertical activity and abnormal behaviours. Preliminary results also indicate significant differences in blood creatinine levels between Dpyd null mice and wild-type counterparts. Magnetic resonance imaging of fixed brain samples of Dpyd null mice is ongoing. The results of this work will assess brain morphometry changes associated with Dpyd deficiency and combined with the IMPReSS results will provide a thorough characterization of the Dpyd deficiency phenotype. (1) Skarnes WC, et al. Nature. 2011;474:337–342. We thank the Canadian Mouse Mutant Repository, the Toronto Centre for Phenogenomics, and NorCOMM2 (www.norcomm2.org) for providing C57BL/6N-Dpyd<tm1b(KOMP)Wtsi>/Tcp mice and baseline phenotyping data.

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Poster

517. Developmental Disorders II

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Program#/Poster#: 517.15/W28

Topic: C.06. Developmental Disorders

Title: Monoaminergic interactions between lisdexamfetamine and duloxetine determined by dual-probe intracerebral microdialysis in freely-moving rats

Authors: *D. J. HEAL[1], H. L. ROWLEY[1], R. S. KULKARNI[1], P. H. HUTSON[2]
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Abstract: Lisdexamfetamine dimesylate (LDX) is a prodrug of *d*-amphetamine that is approved to treat ADHD. In this study, we have investigated the effects of combining LDX with the SNRI, duloxetine, on monoaminergic changes in several regions of the rat brain. Male Sprague-Dawley rats were stereotaxically implanted with two concentric microdialysis probes into hippocampus [HIPP] (4 mm tip, AP: -4.8 mm; L: +/-4.8 mm relative to bregma; V: -7.8 mm relative to the skull surface) and nucleus accumbens [ACC] (2 mm tip, AP: +2.2 mm; L: +/-1.5 mm; V: -8.0 mm) or prefrontal cortex [PFC] (2 mm tip, AP: +3.2 mm; L: +/-2.5 mm; V: -4.0 mm) and striatum [STR] (4 mm tip, AP: +0.2 mm; L: +/-3.0 mm; V: -7.8 mm (2). The following day after collection of 3 basal samples, rats (n = 7-8) were given vehicle (po)/vehicle (ip), LDX (1.5 mg/kg *d*-amphetamine base po)/vehicle (ip); vehicle (po)/duloxetine (5 mg/kg ip) or LDX (1.5 mg/kg po)/duloxetine (5 mg/kg ip). Noradrenaline, dopamine and 5-HT were quantified by reverse-phase, ion-pair, HPLC-ECD. Duloxetine increased the efflux of noradrenaline and 5-HT in PFC (AUC[0-3.0hr] = 293%, $p<0.01$; 168%, $p<0.05$, respectively) and HIPP (274%, $p<0.05$; 650%, $p<0.001$, respectively) and 5-HT in STR (479%; $p<0.01$). Duloxetine also increased extracellular dopamine in PFC (218%, $p<0.05$) and HIPP (220%, $p<0.001$). LDX increased extracellular noradrenaline and dopamine in PFC (449%; $p<0.001$; 264%, $p<0.01$, respectively). It increased dopamine, but not noradrenaline, efflux in HIPP (263%; $p<0.001$), ACC (282%; $p<0.001$) and STR (197%; $p<0.001$). Combining LDX with duloxetine increased extracellular noradrenaline and dopamine in PFC, all three monoamines in HIPP, dopamine and 5-HT in STR and dopamine in ACC. Thus, when administered in combination, the complementary actions of LDX and duloxetine on monoaminergic neurotransmission were realised in all regions. The possible exception was the PFC where the findings were equivocal. There was a synergistic augmentation of LDX-induced dopamine efflux in ACC (377%; $p<0.05$) and STR (315%; $p<0.001$). LDX contributes increased dopaminergic neurotransmission in PFC, HIPP, ACC and STR to complement the increases of noradrenaline and 5-HT in PFC and HIPP, and 5-HT in STR evoked by duloxetine. Combining duloxetine with LDX synergistically augmented the effect of LDX on dopamine efflux in ACC and STR. Together, these findings suggest that the combination of duloxetine and LDX may enhance monoaminegic transmission more extensively in the CNS than duloxetine alone. This study was funded by Shire Pharmaceuticals, UK

Disclosures: **D.J. Heal:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; This study was funded by Shire Pharmaceuticals, UK. **H.L. Rowley:** 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 study was funded by Shire Pharmaceuticals, UK. **R.S. Kulkarni:** 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 study was funded by Shire Pharmaceuticals, UK. **P.H. Hutson:** A. Employment/Salary (full or part-time):; I am employed by Shire Developments Inc.

Poster

517. Developmental Disorders II

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Topic: C.06. Developmental Disorders

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National Research Foundation of South Africa

NIH Fogarty International Center Grant R01 TW008040

Title: The effect of maternal separation on striatal protein levels is strain dependent: A proteomic study

Authors: *J. S. WOMERSLEY[1], L. A. KELLAWAY[2], D. J. STEIN[3], M. VLOK[4], V. A. RUSSELL[2]
[2]Human Biol., [3]Psychiatry and Mental Hlth., [4]Ctr. for Proteomic and Genomic Res., [1]Univ. of Cape Town, Cape Town, South Africa

Abstract: Introduction: The spontaneously hypertensive rat (SHR) is a well-validated model of attention-deficit/hyperactivity disorder (ADHD), a developmental disorder characterised by poor sustained attention, hyperactivity and impulsivity. Previous research has indicated that the effects of maternal separation, a model of mild chronic developmental stress, are strain dependent with SHR appearing to be behaviourally resilient to the anxiogenic effects of MS when compared to the Wistar-Kyoto (WKY) and Sprague-Dawley comparator strains. Therefore we sought to investigate the underlying differences in protein expression in the striatum, an area of the brain implicated in ADHD and involved in motor activity, orientation to salient stimuli and reward processing. Methods: Maternally separated (MS) SHR, WKY and Sprague-Dawley (SD) litters were removed from the dam for 3 hours per day between postnatal days 2 and 14, as opposed to non-maternally separated (nMS) litters, which stayed with the dam at all times. On postnatal day 60, rats were decapitated and the striata rapidly removed and snap frozen in liquid nitrogen.

Supernatant from striatal samples (n=4 per group) were analysed by isobaric Tag for Relative and Absolute Quantification (iTraq) of the proteome with matrix-assisted laser desorption/ ionization (MALDI) tandem mass spectrometry analysis of peptides. Mass spectrometry data were analysed using ProteinPilot 4.0 and the Uniprot Rattus rattus database. Significant differences in protein expression were calculated using Student's unpaired t test. Results: Proteomic analysis of striatal tissue from MS and nMS SHR, WKY and SD revealed multiple differences in proteins relating primarily to energy metabolism, signalling and cell structure. Strain differences in protein expression were found primarily between SHR and the control WKY and SD strains, suggesting that these differences contribute to the ADHD-like phenotype of SHR. The minimal number of differences found between WKY and SD suggest that both of these strains are suitable controls for SHR. Comparison of proteins affected by MS in more than one strain revealed that developmental stress had opposite effects on SHR and WKY, consistent with their different behavioural responses to developmental stress. Conclusion: WKY and SD rats are suitable controls for SHR. MS produced opposite effects on protein expression in SHR and the comparator strains, WKY and SD, consistent with previous research that suggests that the effect of developmental stress is strain-dependent.

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Poster

517. Developmental Disorders II

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Topic: C.06. Developmental Disorders

Support: INHS Italian National Health Service GR-2010-2319328

Title: Modulation of cognitive performance in dyslexic children and adolescents with transcranial direct current stimulation

Authors: *D. MENGHINI[1], F. COSTANZO[1], S. ROSSI[1], C. VARUZZA[1], S. SDOIA[1], M. OLIVERI[2], G. KOCH[2], S. VICARI[1]
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Abstract: Traditional remedial treatment for dyslexics increases their reading ability and modifies brain activation patterns in critical cortical areas, such as the left inferior parietal lobule.

One open question is whether a direct intervention on the same areas of the brain can affect reading ability. Non-invasive brain stimulation offers the possibility to investigate this topic. It induces excitability alterations in cortical areas and it may result in positive modulation of cognitive performance (an increase of accuracy or decrease of response time). The effect of transcranial direct current stimulation (tDCS) on cognitive performance and reading of dyslexic children and adolescents is still under-investigated. To test the effect of tDCS on dyslexic performance, reading (word, non-word and text reading) and cognitive measures (lexical decision, working memory, verbal fluency) have been assessed in 14 dyslexic children and adolescents (age range 12-18) in different conditions of stimulation. The polarities of tDCS over the left inferior parietal lobule were manipulated according to the following conditions: 1) left anodal/right cathodal tDCS, 2) right anodal/left cathodal tDCS, 3) sham tDCS and 4) in a baseline condition without tDCS. Stimulation was delivered for 20 minutes in each condition and set at 1 mA. tDCS was well tolerated from all participants. Preliminary results in dyslexics showed a better performance in text reading ($p < 0.05$) and in lexical decision ($p < 0.05$) after left anodal/ right cathodal tDCS compared to the baseline. In conclusion, our result document that tDCS transitorily improves the performance of dyslexic children and adolescents. Although preliminary, these findings suggest new brain-based perspectives in the treatment of dyslexia during childhood and adolescence.

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Poster

517. Developmental Disorders II

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Topic: C.06. Developmental Disorders

Support: Clarendon Fund Fellowship

Stammer Trust Grant

Title: Activity in putamen an indicator of dysfluent state in stuttering

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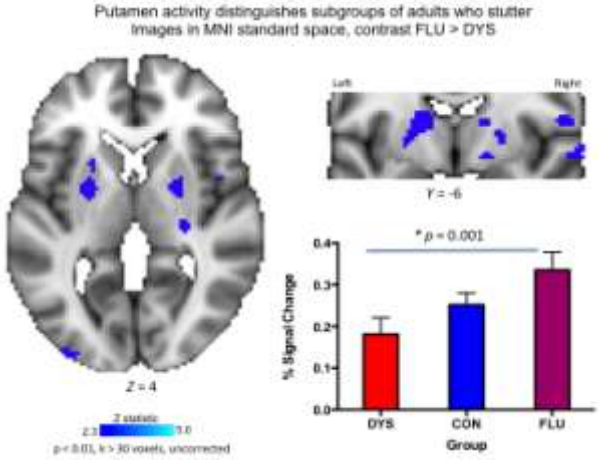
Oxford, United Kingdom; [2]Clin. Language Sci., [3]Ctr. for Integrative Neurosci. and Nuerodynamics, Univ. of Reading, Reading, United Kingdom

Abstract: Several brain regions are implicated in developmental stuttering, these include the basal ganglia, frontal opercular and auditory cortex and cerebellum. The extent to which the dysfluent state relates to abnormal function in these brain regions is unknown. We used functional MRI to address this question by (i) examining differences between groups of adults who stutter who were either fluent or dysfluent during scanning and (ii) comparing percent signal change in regions showing group differences to percent signal change in fluent controls (CON). We collected overt speech samples and related BOLD activity under two conditions (picture description and sentence reading) using sparse-sampling functional MRI (3T, 32 axial slices 4 mm3, TR=9s, TA=2s, TE=30ms) in 17 adults who stutter (aged 19 - 54 yrs, 4 females) and 17 CON (aged 19-53 years, 3 females). Each condition was contrasted with a silent baseline and average percent signal change was extracted for three regions of interest based on group results. For the between-subjects analysis, we divided our sample into individuals who were mostly fluent (FLU, n=8) or dysfluent (DYS, n=9) based on an arbitrary cutoff of 10 dysfluent utterances per scan session. Strikingly, during overt speech production (i.e. both conditions), the DYS group showed no regions with greater activity than the FLU group. The DYS group had less activity than FLU in the putamen bilaterally, left posterior cerebellum and right central operculum during picture description and sentence reading. We probed for differences between adults who stutter and CON in those regions, collapsed across condition. Only the putamen showed a significant main effect of group (after accounting for age and structure volume), with the general pattern of DYS < CON < FLU. These data support the putamen as a candidate structure that shows abnormal function during both fluency and dysfluency in people who stutter. The pattern of activation and deactivation during different fluency states might explain variability in findings across studies with respect to abnormal basal ganglia activations.



Disclosures: **E.L. Connally:** None. **D. Ward:** None. **C. Pliatsikas:** None. **K.E. Watkins:** None.

Poster

518. Developmental Disorders: Animal Models I

Location: Halls A-C

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Topic: C.06. Developmental Disorders

Support: NIH Grant R01MH082893

Title: Effect of physical exercise on attentional function and social behavior: Comparison with psychostimulants and implications for ADHD

Authors: *D. J. BUCCI, A. M. ROBINSON
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Abstract: Psychostimulants continue to be the primary treatment for the overt behavioral symptoms associated with Attention-Deficit/Hyperactivity Disorder (ADHD). However, growing research has identified important limitations of their use, such as concerns over the negative consequences of long-term use on the developing brain. Moreover, psychostimulants are often ineffective in treating the cognitive impairments that are now thought to be central to ADHD (e.g., behavioral inhibition, inattention). In addition, it is unclear how these therapies affect often-observed, yet rarely researched, hyper-social behavior associated with ADHD. Thus, there is currently great research interest in developing adjunctive or replacement therapies for psychostimulants. Our prior research using an animal model of ADHD, the Spontaneously Hypertensive Rat strain (SHR), demonstrated that exercise reduces distractibility and hyper-social behavior in SHRs compared to a normo-active control strain. Here we determined the duration of exercise that is needed to affect attentional function and social behavior and compared the effects of exercise to the standard of care pharmacotherapy, methylphenidate. Attentional function was assessed by measuring the amount of orienting behavior (i.e., rearing up on the hind legs) during the repeated presentation of a non-reinforced visual cue (a light). Normo-active rats initially exhibit a high level of orienting behavior that habituates over the course of several non-reinforced presentations of the light. Social behavior was assessed using a procedure adapted from File and colleagues in which a rat is allowed to freely explore a large arena containing an unfamiliar conspecific rat located in a restrainer in the center of the arena. Consistent with their hyper-responsive phenotypes, non-exercising SHRs exhibited a high level of orienting to the light, little habituation of the orienting response, and excessive social interaction compared to normoactive rats. Exercise and methylphenidate decreased orienting behavior and social behavior in SHRs in a dose-dependent fashion. In addition, a group of rats received a combination of both exercise and methylphenidate, using the doses of each that alone

were ineffective in altering orienting behavior. We found that these does of exercise and methylphenidate, when combined, now had a significant effect on orienting behavior. These data indicate that physical exercise is at least as effective as methylphenidate in reducing distractibility and hyper-social behavior in SHRs, and that smaller doses of drug can be used in combination with moderate amounts of exercise to reduce distractibility.

Disclosures: D.J. Bucci: None. **A.M. Robinson:** None.

Poster

518. Developmental Disorders: Animal Models I

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Support: MH091230 (A.K.)

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Brain & Behavior Research Foundation (A.K)

Brain Science Foundation (A.K.)

Title: S-Nitrosylation of NDEL1 mediates activity-dependent dendritic development in the prefrontal cortex

Authors: *A. SAITO[1,3], Y. TANIGUCHI[1], S.-H. KIM[1], B. SELVAKUMAR[2], M. D. BALLINGER[1], J. SABRA[1], M. JALLOW[1], P. YAN[1], K. ITO[1], S. HIROTSUNE[4], A. WYNSHAW-BORIS[5], S. H. SNYDER[2], A. SAWA[1,2], A. KAMIYA[1]
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Abstract: S-Nitrosylation is a critical mechanism mediated by neuronal nitric oxide synthase (nNOS) which governs diverse signaling cascades that regulate neuronal functions. Although it has been studied extensively *in vitro* and in invertebrate animals, effects on mammalian brain development and underlying mechanisms remain poorly understood. Here we report that genetic deletion of nNOS disrupts dendritic development in the prefrontal cortex (PFC). S-nitrosylation

of nuclear distribution element-like 1 (NDEL1), a protein involved in multiple developmental processes, accelerates dendritic arborization in the PFC. This posttranslational modification is enhanced by NMDA receptor-mediated neuronal activity, a main regulator of dendritic formation. These findings highlight S-nitrosylation as a key activity-dependent mechanism underlying brain maturation.

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Poster

518. Developmental Disorders: Animal Models I

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Topic: C.06. Developmental Disorders

Support: Allision Family Foundation (CP)

Tourette Syndrome Association (MX, CP)

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K08MH081190 (CP)

Title: Targeted ablation of cholinergic interneurons in the dorsolateral striatum produces behavioral manifestations of Tourette syndrome

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Abstract: Tourette syndrome (TS) represents the most severe end of a spectrum of tic disorders that, in aggregate, affect 5% of the population and produce great morbidity. Existing somatic treatments are of limited efficacy. Convergent evidence implicates the cortico-basal ganglia

circuitry in the genesis of tics, but pathophysiological details are not well understood. Recent post-mortem work has revealed that specific interneuronal populations are abnormal in patients with severe, refractory TS. Cholinergic interneurons (ChAT), also known as tonically active neurons (TANs), constitute only about 1% of the neurons in the striatum, but they are critical regulators of striatal function. Abnormalities in ChAT interneurons in the ventral striatum have been reported in schizophrenia, but deficits in the dorsal striatum (the caudate and putamen) have only been described in TS. We tested the hypothesis that ChAT interneuron disruption in the dorsolateral striatum is sufficient to produce tics, using a novel approach for targeted ablation of these cells in mice. We exposed ChAT-ablated animals to acute mild stress. ChAT-ablated mice exhibited no behavioral abnormalities prior to the stressor, but increased grooming during the startle block. There was also a nominal increase in grooming in the 30 minutes after the block of startle stimuli, though it did not reach statistical significance. In contrast, mice with ChAT ablation in the DMS showed no alteration in acute stress-induced repetitive behaviors. Following amphetamine administration, the ChAT-ablated animals showed repetitive movements that last longer and reaching higher levels, while DMS-ChAT ablated mice showed enhanced locomotor activation but no change in stereotypy. We tested ChAT-ablated mice on the rotorod, which assays both baseline motor performance and improvement over time. Mice with a DLS ChAT lesion showed a marked deficit in baseline rotorod performance but rapidly improved with practice to a level of performance indistinguishable from controls. We tested ChAT-ablated mice and found no PPI deficit. These results support the importance of cholinergic dysregulation to the pathophysiology of TS and establish a new model system in which the consequences of this dysregulation can be examined.

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Poster

518. Developmental Disorders: Animal Models I

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Topic: C.06. Developmental Disorders

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Medical Research Institute, TMDU

Title: Deficiency of glutamate transporter in astrocyte increases brain excitability and induces Tourette's Syndrome-like excessive repetitive behaviours in mice

Authors: T. AIDA[1], J. YOSHIDA[1], M. NOMURA[2], A. TANIMURA[3], Y. IINO[1], M. SOMA[1], N. BAI[1], Y. ITO[1], W. CUI[1], H. AIZAWA[1], T. NAGAI[4], N. TAKATA[4], R. TAKAYANAGI[2], M. KANO[3], M. GÖTZ[5], H. HIRASE[4], *K. TANAKA[1,6]
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Abstract: Brain hyperexcitability has been suggested to cause neuropsychiatric disorders including autism, obsessive-compulsive disorder (OCD), and Tourette's syndrome. Brain excitability is controlled by both neurons and astrocytes. In astrocytes, the glutamate transporters GLT1 and GLAST are critical for regulating brain excitability. However, the role of glial glutamate transporters in the pathogenesis of neuropsychiatric disorders remains unknown. Here, we show that GLT1 inducible knockout [GLAST(CreERT2/+)&GLT1(flox/flox), iKO] mice developed severe cervicofacial skin lesions and exhibited pathologic repetitive behaviours including excessive self-grooming and motor tics that resemble Tourette's syndrome. In iKO mice, seizure sensitivity and excitatory transmission at the corticostriatal synapses after repetitive stimulation were increased. Furthermore, treatment with an N-methyl-D-aspartate (NMDA) receptor antagonist memantine ameliorated the pathologic repetitive behaviours in iKO mice. These results suggest that iKO mice as a novel animal model for Tourette's syndrome and suppression of brain hyperexcitability by NMDA receptor blockade or glial glutamate transporter activation may be novel therapeutics for Tourette's syndrome. Further, because the repetitive behaviours are common core symptoms of autism, OCD and Tourette's syndrome, iKO mouse is a useful tool for understanding a common mechanism underlying these psychiatric disorders.

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Poster

518. Developmental Disorders: Animal Models I

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Support: NIH Grant R21HD079095

California National Primate Research Center Pilot Award through NIH base grant OD011107

Simons Foundation Autism Research Initiative

NIH Grant OD010962

Title: Identifying novel biomarkers of naturally occurring social impairments in male rhesus monkeys

Authors: *O. OZTAN[1], J. P. GARNER[1], K. CHUN[2], S. A. HYDE[1], E. H. SHERR[3], J. P. CAPITANIO[2], K. J. PARKER[1]
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Abstract: Autism spectrum disorder (ASD) is characterized by core social impairments, but its basic biology remains poorly understood. Progress has been impeded by the difficulty of obtaining relevant tissue samples from patients and matched controls. In mouse models, while tissue is available, there is frequently discordance between complex human behavior and laboratory-based mouse behavior, even with shared genetic etiologies. These limitations underscore the tremendous value in developing a monkey model of social impairments with more reliable behavioral and biological correlates to the human disease. Like humans, rhesus monkeys (Macaca mulatta) are highly social, and both species display stable and pronounced individual differences in social functioning. At the behavioral extremes, low social compared to high social male monkeys initiate fewer affiliative interactions and display more inappropriate social behavior, suggesting both lower social motivation and poorer social skills. Study subjects were male rhesus monkeys aged 2-5 years (N=42). Subjects were selected on the basis of archived behavioral data thought to predict later low (N=21) or high (N=21) social functioning. Quantitative social behavior data and personality assessments were collected by blinded observers using established protocols. After completion of behavioral phenotyping, monkeys with the most extreme scores (N=15 low social monkeys and N=15 high social monkeys) were

selected for biological sample collection. Cerebrospinal fluid (CSF) and blood were drawn, respectively, from the cisterna magna and femoral vein during the same session to test whether candidate biomarkers [e.g., arginine-vasopressin (AVP) and oxytocin (OXT) peptide and receptor mRNA levels] were associated with social functioning, and whether the degree of biomarker dysregulation co-varied with the degree of social deficits. Low social monkeys were judged to be less affiliative, less contact seeking, more isolated/withdrawn, and more socially avoidant than high social monkeys. Preliminary biomarker data indicate that CSF AVP concentrations predict group classification, such that low social monkeys had diminished CSF AVP concentrations compared to high social monkeys. CSF AVP concentrations also positively predicted initiation but not receipt of social grooming for all monkeys. With continued investigation this research program may lead to the discovery of novel "drugable" targets and the streamlined development of the first effective therapeutics to treat social impairments in people with ASD. Research supported by NIH and the Simons Foundation.

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Poster

518. Developmental Disorders: Animal Models I

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Topic: C.06. Developmental Disorders

Support: NRF

Title: Changes in hippocampal proteins involved in glutamate and GABA transmission following early life stress in a rat model of ADHD, the spontaneously hypertensive rat

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Abstract: Background: Experiencing stress early in life increases the risk of developing a psychiatric disorder. Maternal separation is an animal model used in research to mimic early life stress in humans. The spontaneously hypertensive rat (SHR) is the most widely used animal model of ADHD, exhibiting the three major characteristics of ADHD. We have previously shown opposite effects of maternal separation on $GABA_A$ receptor-mediated inhibition of glutamate-stimulated release of norepinephrine in hippocampus of SHR compared to Wistar-

Kyoto rats (WKY). The present study investigated the hippocampal protein profile of WKY, SHR and Sprague-Dawley rats (SD), and how these profiles were affected by maternal separation. Methods: Rat litters were subjected to maternal separation or non-maternal separation from postnatal day 2 to 14. On postnatal day 31-35, rats were decapitated and the hippocampus dissected. Proteomic analysis (iTRAQ) of hippocampal proteins was followed by Western blot analysis to confirm differential expression of 2 key proteins: glutamate transporter GLT1 and solute carrier family 12 member 5 (KCC2). Results: Main proteomics findings were that energy-related proteins were increased in SHR hippocampus compared to WKY and SD, and reduced in WKY hippocampus compared to SD. GLT1b (splice variant of GLT1) and KCC2 levels were increased in SHR hippocampus compared to WKY, and reduced in WKY hippocampus compared to SD. Maternal separation increased energy-related proteins in WKY hippocampus and reduced them in SHR hippocampus. Maternal separation reduced GLT1b in SHR hippocampus. Western blot analysis of GLT1 and KCC2 showed that GLT1 was reduced, while KCC2 was increased, in SHR hippocampus compared to WKY and SD. Maternal separation increased GLT1 in WKY, SD and SHR right hippocampus. Conclusion: Reduced GLT1 in SHR hippocampus possibly led to increased extracellular glutamate, inducing a compensatory increase in GLT1b. The increase in GLT1 in SHR hippocampus caused by maternal separation possibly reduced extracellular levels of glutamate, and thereby removed the induction of GLT1b, reducing GLT1b in SHR hippocampus only. KCC2 is responsible for establishing the Cl^- balance across the neuronal membrane and by so doing, determines the functioning of $GABA_A$ receptors. Increased KCC2 in SHR hippocampus was possibly a compensatory attempt to increase inhibitory GABAergic signalling.

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Poster

518. Developmental Disorders: Animal Models I

Location: Halls A-C

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Program#/Poster#: 518.07/X2

Topic: C.06. Developmental Disorders

Support: JSPS KAKENHI 23500427

Title: Functional analysis of FAM107B, a newly identified stress hormone responsive molecule

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Abstract: Numbers of studies indicate exposure to stress hormone, glucocorticoid (GC) during prenatal period affects neural development and causes abnormal behaviors in animals. The effects of GC may be also critical for prenatal brain development of human and cause psychiatric disorders such as depression (Neurosci Biobehav Rev. 43: 137-162) and autism (Neuroscience and Biobehavioral Reviews 32: 1519-1532, 2008). Here, we show a new GC responsive molecule, FAM107B. Treatment with GC agonist, DEX, or restraint stress on pregnant mice resulted in reduction of FAM107B in the brain of their embryos. To figure out its function, we study effects of RNAi or over expression of FAM107B in neuron-like PC12 cells and mouse neural cells. Transwell assay indicates reduction of FAM107B by RNAi accelerated PC12 cells migration induced by NGF while the over expression did opposite effect. Neurite outgrowth study in both PC12 and primary neurons showed FAM107B inhibits neurite outgrowth by over expression. Co-localization of F-actin and FAM107B was observed in outer membrane ruffles after NGF induction. FAM107B may have some roles in actin assembly and affect neural migration and neurite outgrowth. To study *in vivo* function, we also tried gene transfer of FAM107B *in utero* and found defects of migration in the brain cortex. Interestingly, exposure to DEX also showed similar effects on PC12 cells migration and neurite outgrowth. Possibly, FAM107B is one of the key molecules that responds to GC and gives critical effects on neural development in the embryonic brain that cause psychiatric disorders after birth.

Disclosures: K. Koizumi: None. **M. Ito:** None. **K. Nakao:** None. **H. Nakajima:** None.

Poster

518. Developmental Disorders: Animal Models I

Location: Halls A-C

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Program#/Poster#: 518.08/X3

Topic: C.06. Developmental Disorders

Support: SFI Grant SFI/12/RC/2273

Title: The microbiota modulates transcription in brain areas necessary for social behaviour

Authors: *R. M. STILLING[1], F. RYAN[1], A. E. HOBAN[1,2], M. CLAESSON[1,3], T. G. DINAN[1,4], J. F. CRYAN[1,2]

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Abstract: Increasing evidence implicates host-microbe interactions at virtually all system levels within the body including the central nervous system (CNS) and new data implicates the microbiota in neurodevelopmental and stress-related disorders such as autism and anxiety. Our lab could recently show that, mice that have been raised in a sterile environment (germ-free, GF), demonstrate a marked lack of pro-social behaviour in the well-established three-chamber social interaction test (3SIT). Alterations in brain function and cognition are suggested to be mediated via the microbiota-gut-brain axis, the bidirectional communication pathways between the CNS and intestinal organs. However, the underlying mechanisms of this interaction and the nature of the pathways are only insufficiently understood. We recently proposed a likely role for dynamic transcriptional regulation in those brain regions involved in social behaviour, including the amygdala, and by using the 3SIT, we have identified key response genes to social interaction exposure in the amygdala and compared these to data derived from analyses in the hippocampus. Furthermore, exploiting state-of the art transcriptional profiling combined with new bioinformatic techniques, we have determined modifications of the amygdala transcriptomes of conventionally raised and GF mice and GF mice that have been colonised after weaning to elucidate the effect of the microbiota on gene expression. Finally, plastic chromatin modifications have recently been identified to play an important role in cognitive processes during health and disease by regulating gene expression in the brain. However, the role of epigenetics in mediating host-microbe interactions, has received little attention. We therefore investigated the potential of the microbiota to influence histone acetylation in the brain. Together, data from these experiments have identified a novel mechanism by which the microbiota can affect social behaviour, which may have far reaching implications for human neurodevelopmental disorders such as autism.

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Poster

518. Developmental Disorders: Animal Models I

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Topic: C.06. Developmental Disorders

Support: University of Florida, startup funds

McKnight Brain Institute startups

Title: Brain MR relaxation times in an animal model recapitulating features of autism spectrum disorders

Authors: *L. M. COLON-PEREZ[1,2], M. KHARITON[3], L. VON ZABERN[4], P. CHAKRABARTY[5], M. FEBO[2]
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Abstract: Valproic acid (VPA) has been used clinically as an anticonvulsant medication during pregnancy; however, it poses a neurodevelopmental risk of fetal malformations and autism spectrum disorders (ASD). VPA treatment in pregnant rats results in birth defects similar to human conditions and thus provides a useful tool to model ASD. It has been shown that VPA treated rats show greater increases in BOLD signal response to social stimulus, lower social interaction and greater locomotor activity compared to controls. In humans, voxel based relaxometry has shown increased values in transverse relaxation times in patients with autism. Diffusion tensor imaging (DTI) studies report conflicting results of DTI metrics and its correlations to ASD. On one hand, a study comparing sedated autistic children to naturally asleep typically developing children reported decreased FA values in autistic children. On the other hand, a study of a control group compared to autistic children and their typically developing siblings found no difference between autistic children and their typically developing siblings but there was a considerable difference between control kids to autistic children and their siblings. Barnea-Goraly et. al. suggests that reduced FA values is a potential biomarker for genetic risk for autism. A factor not accounted for in these DTI studies is the increase in transverse relaxation times observed in autistic children; this will alter the MRI images SNR and it is a possible confound when comparing autistic children to its control counterparts. In order to create a proper comparison between autistic and typically developing children, MRI experiments must account for these changes. In this study, rats treated with VPA (n = 5) during development were imaged and their MR relaxation rates were compared to controls rats (n = 4). Excised rat brains were scanned at the Advanced Magnetic Resonance Imaging and Spectroscopy (AMRIS) facility of the McKnight Brain Institute at the University of Florida in a 17.6T magnet. T1 values were estimated from a RARE sequence with 8 TR's and T2 values from a multi echo sequence with 8 echos. An image resolution of 90 x 90 x 280 μm3 was obtained for both scans. Mean T1 and T2 values were obtained over masks covering: entire brain, genu and splenium of corpus callosum, inferior colliculus, visual cortex, fimbria and internal capsule. VPA brains displayed significantly increased transverse relaxation times over the entire brain (T2 = 54.9 ± 3.6 ms) compared to controls (T2 = 41.9 ± 4.3 ms), as well in all other brain regions. However, no differences were obtained for longitudinal relaxation times. Our results agree with previous autism human studies.

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Poster

518. Developmental Disorders: Animal Models I

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 518.10/X5

Topic: C.06. Developmental Disorders

Title: The role of Promoter IV-driven BDNF expression in fear extinction

Authors: *J. L. HILL[1], D. V. JIMENEZ[1], K. R. MAYNARD[1], R. J. SCHLOESSER[1,2], K. MARTINOWICH[1,3,4]
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Abstract: The neural substrates of fear-related behavior have been well-studied. However, the molecular and cellular signaling cascades that are responsible for fear expression and its extinction are still not completely understood. The ability to extinguish, or decrease fear expression to a no longer aversive signal, has been consistently linked to expression of Brain-Derived Neurotrophic Factor (BDNF). The *Bdnf* gene is highly regulated, with different promoters driving expression in both a spatially and temporally unique pattern. The gene has nine promoters, each driving transcription from an alternative 5′ untranslated exon, which is spliced to a common 3′ coding exon. *Bdnf* promoter IV is highly responsive to induction of neural activity, and alterations in *Bdnf* expression from promoter IV have been observed in key regions of the fear circuitry during auditory fear conditioning and extinction. Previous data established that mice genetically altered to attenuate activity-dependent expression or activity-dependent secretion of BDNF show deficits in the ability to extinguish conditioned fear. We conducted a series of experiments to further investigate how disruption of BDNF expression specifically from promoter IV (BDNF-e4 mice) can affect fear extinction. First, we developed an extinction paradigm in which mice were extinguished 24h following conditioning, and found that homozygous BDNF-e4 mice show highly increased levels of freezing relative to wild-type animals after extinction trials while heterozygous BDNF-e4 animals show an intermediate extinction deficit. Mice with a genetic alteration that leads to a reduction in activity-dependent secretion of BDNF show deficits in extinction when extinction trials are conducted immediately

following conditioning suggesting that local release of BDNF is required to facilitate immediate extinction. In BDNF-e4 mice, activity-dependent production of BDNF from promoter IV rather than activity-dependent extinction is impaired. Thus, we hypothesized that immediate extinction should be intact in our animals. Mice underwent extinction training 5min post-conditioning; this time point was chosen because it is prior to conditioning-related increases in *Bdnf* transcription from promoter IV. We found no difference in levels of freezing between wild-type and BDNF-e4 mice. In summary, mice in which BDNF expression is disrupted from promoter IV possess a delayed, but not an immediate extinction deficit, contributing to the hypothesis that local secretion of BDNF is required to mediate successful fear extinction.

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Poster

518. Developmental Disorders: Animal Models I

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Topic: C.06. Developmental Disorders

Support: NSERC Grant 40076

Title: Prenatal exposure to a 'double-dose' of valproic acid alters lifelong behavior and global methylation patterns: Modeling differing severities of autism spectrum disorder

Authors: *S. RAZA, A. HARKER, C. NIEHAUS, B. KOLB, R. GIBB
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Abstract: Autism spectrum disorder (ASD) is an umbrella term used to designate a broad spectrum of neurodevelopmental disorders ranging from mild, including Asperger's and high-functioning autism, to severe symptomology. Due to heightened awareness and shifting diagnosis, the prevalence of ASD has significantly risen, now affecting 1 in every 55 children. While current animal research models appear to parallel the anatomical, functional, and behavioral pathology as reported in human cases of high-functioning autism, translation to the entire spectrum of autistic disorders is less well explored. That is, current animal models have been unable to entirely simulate the human condition, specifically cases of severe or low-functioning autism. One viable animal model of ASD is the valproic acid (VPA) rodent model, whereby prenatal exposure of VPA produces striking similarities to ASD symptomology in

humans. As such, modifications to such an animal model of ASD to better simulate severe, or low-functioning, autism may allow for greater understanding of the functional pathology that is representative of autistic individuals on an extreme end of the spectrum. The current study utilizes the widely used VPA rodent model of autism. While a single dose of VPA, *in utero,* produces autistic-like anomalies in rodents, the symptoms are relatively mild. We hypothesized that two does of VPA, *in utero*, would better simulate characteristics of low-functioning autistics. Using a within-litter design, pregnant Long-Evans rats were exposed to VPA on gestational days 10 and 12.5 (800mg/kg on each day, respectively), a period of fetal embryogenesis. Subsets of the offspring prenatally exposed to VPA (a single or 'double dose' of VPA) were sacrificed at weaning, and DNA from the medial prefrontal cortex (mPFC), amygdala, hippocampus, and cerebellum were extracted for global DNA methylation analysis. The remaining offspring underwent behavioral testing at three developmental milestones: pre-weaning (P7-P15), juvenile (P30+), and in adulthood (P95+). Several behavioral tasks designed to assess motor, emotional, and cognitive domains were administered. The results will not only serve as a plausible translational rodent model for low-functioning autism, but also shed light on the etiology of the multifaceted diagnoses of ASD.

Disclosures: S. Raza: None. **A. Harker:** None. **C. Niehaus:** None. **B. Kolb:** None. **R. Gibb:** None.

Poster

518. Developmental Disorders: Animal Models I

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Topic: C.06. Developmental Disorders

Support: Simons Foundation Autism Research Initiative

Title: Cognitive disability in the Cntnap2 mouse model of ASD

Authors: H. L. H. RUTZ, *L. A. ROTHBLAT
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Abstract: Cortical dysplasia-focal epilepsy syndrome (CDFE), a syndromic form of autism spectrum disorder (ASD), is caused by recessive mutations in *contactin associated protein-like 2* (*CNTNAP2*). Along with abnormalities in neuronal migration and early-onset focal epilepsy, children with CDFE present with language regression, intellectual disability, and impaired social

abilities. Single nucleotide polymorphisms in the *CNTNAP2* gene have been identified as risk alleles for language deficiencies and ASD. Moreover, recent brain imaging studies report significantly altered cortical connections in children with predisposing alleles, whether autistic or neurotypical. As with other syndromic forms of ASD, there now is a mouse model that replicates many features of the human disorder. *Cntnap2* knockout (KO) mice show migration abnormalities in cortical projection neurons and a reduction in the number of GABAergic interneurons (Penagarikano et al., 2011). Along with these morphological anomalies, these mice demonstrate reduced cortical synchrony and develop epileptic seizures at around 6 months of age. In addition, *Cntnap2* KO mice display some behavioral traits seen in ASD including repetitive grooming behavior, impaired communication (ultrasonic vocalizations), and decreased social interaction. To assess the role of *Cntnap2* deletion on cognitive ability, we tested 3-month old *Cntnap2* KO, heterozygous (HET), and wildtype (WT) mice (The Jackson Laboratory) on a series of tasks using computer automated touchscreen procedures. Compared to WT and HET littermates, most--though not all--KO mice exhibit significant deficits learning and reversing a visual discrimination. The striking cognitive impairment seen in KO mice occurred despite the fact that we saw no evidence of seizure activity even in mice over 12 months old.

Disclosures: H.L.H. Rutz: None. **L.A. Rothblat:** None.

Poster

518. Developmental Disorders: Animal Models I

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Topic: C.06. Developmental Disorders

Support: NIH Grant R01MH082893

Title: Noradrenergic mechanisms mediate the effects of physical exercise on attentional function in a rat model of ADHD

Authors: *A. M. ROBINSON[1], J. T. GREEN[2], T. R. BUTTOLPH[3], D. J. BUCCI[1]
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Abstract: Recent studies in our laboratory indicate that physical exercise (access to a running wheel) can ameliorate attentional dysfunction in an animal model of ADHD, the Spontaneously Hypertensive Rat strain (SHR). Attentional function was assessed by measuring the amount of

orienting behavior (i.e., rearing up on the hind legs) during the repeated presentation of a non-reinforced visual cue (a light). Normo-active rats initially exhibit a high level of orienting behavior that habituates over the course of several non-reinforced presentations of the light. Consistent with their hyper-responsive phenotypes, control SHRs exhibited a high level of orienting to the light and no habituation of orienting behavior, suggesting that they continue to be distracted by the irrelevant visual stimulus. However, 5, 10, or 21 days of access to a running wheel significantly reduced hyper-orienting behavior to the level of normo-active control rats while 2 days of wheel access had no effect. Here, we tested the hypothesis that the effects of exercise were mediated by changes in noradrenergic neurotransmission. First, we measured the levels of norepinephrine transporter (NET) in the prefrontal cortex of SHRs that had access to a running wheel or remained sedentary. We found that exercise produced a dose-dependent reduction in NET, reflecting an increase in NE levels in exercising rats. In the second experiment, we tested the causal nature of this relationship by implanting propranolol pellets subcutaneously in exercising and non-exercising SHRs. Rats then had access to a running wheel for 10 days, or remained in the home cage with no wheel. Other groups received a sham surgery in which no pellet was implanted. We found that blocking noradrenergic receptors with propranolol had no effect on orienting behavior in the sedentary rats. In contrast, propranolol significantly attenuated the effects of exercise on orienting behavior compared to the sham-surgery exercising group. These data indicate that physical exercise alters NE levels in the prefrontal cortex and that changes in NE mediate the effects of exercise on distractibility in the SHR model of ADHD.

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Poster

518. Developmental Disorders: Animal Models I

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Topic: C.06. Developmental Disorders

Support: NIH Grant MH086050

NARSAD Young Investigator Award

Title: The schizophrenia and autism associated gene, transcription factor 4 (TCF4), regulates the intrinsic excitability of prefrontal cortical neurons

Authors: M. D. RANNALS, S. CERCEO-PAGE, M. N. CAMPBELL, A. BRILEY, A. E. JAFFE, R. TAO, T. M. HYDE, J. E. KLEINMAN, D. R. WEINBERGER, *B. J. MAHER
Lieber Inst. For Brain Develop., Baltimore, MD

Abstract: Schizophrenia is a neurodevelopmental disorder with unknown pathophysiology. Genome-wide association studies (GWAS) have identified a number of loci associated with increase risk for SZ and several of these risk variants are located within introns of TCF4. In addition, autosomal dominant mutations in TCF4 result in Pitt Hopkins Syndrome (PTHS), a rare neurodevelopmental disorder characterized by a spectrum of symptoms including hyperventilation, seizures, autistic behaviors, intellectual disability, and brain malformations. Currently, the molecular mechanisms and underlying pathophysiology responsible for these two disorders are not understood. Our goal is to determine the function of TCF4 during cortical development and to understand the molecular mechanism of risk that is associated with genetic variants of TCF4. To test the function of TCF4 in the developing neocortex we altered its expression by transfecting layer 2/3 pyramidal cells in the rat prefrontal cortex by *in utero* electroporation. We knockdown TCF4 expression using two shRNA constructs that target independent sequences within the TCF4 transcript. Embryonic knockdown of TCF4 decreased action potential output and resulted in the ectopic appearance of spike-frequency adaptation in layer 2/3 pyramidal neurons ($p<0.002$; TCF4 shRNA n=24, Con shRNA n=32). These phenotypes were associated with an increase in the afterhyperpolarization (AHP) amplitude ($p<0.01$; TCF4 shRNA n=29, Con shRNA n=26) and suggested knockdown of TCF4 may increase the activity of Ca^{2+}-activated K^{+} channels. We tested this hypothesis by first rescuing action potential output by manipulating intra- and extracellular Ca^{2+} levels. We demonstrate that intracellular application of the Ca^{2+} chelator BAPTA ($p<0.002$; BAPTA n= 23; TCF4 shRNA n=18) or application of the L-type Ca^{2+} channel blocker nimodipine was effective at rescuing these phenotypes ($p<0.05$; n=8). To identify specific Ca^{2+}-activated conductances responsible for these phenotypes we performed voltage-clamp experiments and found that TCF4 knockdown results in a significant enhancement of the slow AHP (sAHP; $p<0.006$; TCF4 shRNA n=14; Con shRNA n=15). We further validated this result by rescuing the TCF4-dependent decrease in action potential output via application of carbachol, a M1/M3 agonist that is known to activate signaling pathways that inhibit the sAHP. Overall, these results suggest the dosage of TCF4 expression is critical for modulating neuronal excitability by regulating the expression of genes associated with the sAHP, and therefore could hypothetically explain cognitive deficits observed in PTHS and schizophrenia.

Disclosures: M.D. Rannals: None. **S. Cerceo-Page:** None. **M.N. Campbell:** None. **A. Briley:** None. **A.E. Jaffe:** None. **R. Tao:** None. **T.M. Hyde:** None. **J.E. Kleinman:** None. **D.R. Weinberger:** None. **B.J. Maher:** None.

Poster

518. Developmental Disorders: Animal Models I

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 518.15/X10

Topic: C.06. Developmental Disorders

Support: Japan Foundation for Neuroscience and Mental Health

Title: Effect of docosahexaenoic acid (DHA) on a gene/prenatal stress autistic mouse model

Authors: *F. MATSUI, P. HECHT, E. JASAREVIC, K. FRITSCHE, D. BEVERSDORF
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Abstract: Autism Spectrum Disorder (ASD) is characterized by impairments in social interaction and social communication, and repetitive and stereotyped behaviors. While genetics is thought to play a large role in the disorder, environmental factors, such as stress are also thought to contribute to it. We previously reported that prenatal stress exposure in stress-susceptible heterozygous serotonin transporter (SERT) KO pregnant dams in a mouse model altered social interaction in the offspring. The present study additionally found impaired social recognition as measured by the olfactory habituation/dishabituation test but not repetitive behavior as measured by the time spent self-grooming in a novel environment and the marble burying test in the mice. Moreover, the maternal docosahexaenoic acid (DHA) level on postpartum day 21 (P21) in frontal cortex in the mice was significantly reduced, indicating a decrease during gestation and lactation periods. Interestingly, DHA level in only prenatal stress mice (wild type) at P21 was also significantly reduced, while the level in SERT mice (no prenatal stress) was not significantly reduced. DHA, one of the major omega-3 polyunsaturated fatty acids (PUFAs), plays an important role in the functioning and development of the brain and central nervous system and a deficiency or an insufficient intake of omega-3 PUFAs can cause developmental abnormalities. We administrated a DHA rich diet to the offspring just after weaning and assessed their social behavior and fatty acid levels during adulthood. DHA improved their social interaction but not social recognition. The omega-3 PUFAs level in frontal cortex was significantly increased, while the omega-6 PUFAs level was significantly reduced. The results indicate that some behavioral aspects of autism-associated behavior can be reversed with DHA in the stress-susceptible genotype/prenatal stress mouse model. Future studies will need to further study the potential role of DHA in autism.

Disclosures: F. Matsui: None. **P. Hecht:** None. **E. Jasarevic:** None. **K. Fritsche:** None. **D. Beversdorf:** None.

Poster

518. Developmental Disorders: Animal Models I

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 518.16/X11

Topic: C.06. Developmental Disorders

Title: Maternal immune activation and juvenile social motivation in mice

Authors: *J. J. SCHWARTZER, K. O. SUEN, K. A. GILL, P. NANDA, L. A. ALTOMARE, R. D. SANCHEZ
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Abstract: Activation of the mother's immune system during pregnancy is associated with neurodevelopmental disorders including Autism and Schizophrenia. These disorders are characterized by a broad range of impairments in social-emotional behaviors that manifest from alterations in one of several social brain networks, including disruptions in social cognitive and/or social motivational processes. Mouse models have repeatedly demonstrated that maternal immune activation results in deficits in social approach behavior in the three-chambered social approach task. However, it remains unknown which neurobehavioral processes are contributing to the social approach deficits. Specifically, it is hypothesized that reductions in social approach behavior in offspring of MIA dams are due to deficits in social motivation (i.e. the rewarding value associated with social interactions). To test this, pregnant mice were treated with the viral mimic polyinosinic:polycytidylic acid (polyI:C) or vehicle on gestational day 12.5 and juvenile offspring were measured for changes in social motivation using a social conditioned place preference task. Additionally, to determine whether MIA alters species-typical play behaviors in juvenile mice, offspring of polyI:C-treated and control dams were tested in the reciprocal social interactions task. Data provide insight into which neurobehavioral processes within the social brain network are disrupted by maternal immune activation.

Disclosures: J.J. Schwartzer: None. **K.O. Suen:** None. **K.A. Gill:** None. **P. Nanda:** None. **L.A. Altomare:** None. **R.D. Sanchez:** None.

Poster

518. Developmental Disorders: Animal Models I

Location: Halls A-C

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Topic: C.06. Developmental Disorders

Support: SAF2011-22855

Prometeo/2012/005

BFU2011-27326

CSD2008-00005

POIG 01.01.02-00-008/08

Title: Loss of neuronal 3D chromatin organization causes transcriptional and behavioral deficits related to serotonergic dysfunction

Authors: *A. BARCO[1], S. ITO[1], A. MAGALSKA[2], M. ALCARAZ-IBORRA[1], J. P. LOPEZ-ATALAYA[1], V. ROVIRA[1], M. LIPINSKI[1], R. LUJAN[3], E. GEIJO-BARRIENTOS[1], G. WILCZYNSKI[2]
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Abstract: The interior of the neuronal cell nucleus is a highly organized 3-dimensional (3D) structure in which regions of the genome that are millions of bases apart participate in specialized sub-structures with dedicated functions. To investigate neuronal chromatin organization and dynamics *in vivo*, we generated bitransgenic mice that express histone GFP-tagged H2B in principal neurons of the forebrain. Surprisingly, the expression of this chimeric histone in mature neurons causes chromocenter declustering and disrupts the association of heterochromatin with the nuclear lamina. The loss of these structures does not affect neuronal viability but is associated with specific transcriptional and behavioral deficits related to serotonergic dysfunction. Overall, our results demonstrate that the 3D-organization of chromatin in the neuronal nucleus supports an additional level of epigenetic regulation of gene expression that critically influences neuronal function and indicate that some loci associated with neuropsychiatric disorders may be particularly sensitive to changes in chromatin architecture.

Disclosures: **A. Barco:** None. **S. Ito:** None. **M. Alcaraz-Iborra:** None. **A. Magalska:** None. **J.P. Lopez-Atalaya:** None. **V. Rovira:** None. **M. Lipinski:** None. **R. Lujan:** None. **E. Geijo-Barrientos:** None. **G. Wilczynski:** None.

Poster

518. Developmental Disorders: Animal Models I

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Topic: C.06. Developmental Disorders

Support: Autism Speaks [Postdoctoral Fellowship] (#8679)

Intramural Research Program National Institute of Mental Health

Title: Decreased protein synthesis in a mouse model of Tuberous Sclerosis Complex: unexpected consequences of mTORC1 activation

Authors: *R. M. REITH, T. HUANG, M. QIN, C. BEEBE SMITH
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Abstract: Tuberous Sclerosis Complex (TSC) is an autosomal dominant neurogenetic disorder affecting about 1 in 6,000 people, leading to benign growths throughout the brain and other vital organs. TSC usually has effects on the central nervous system manifested by a high incidence of seizures, intellectual disability, and autism. TSC is caused by mutations in either TSC1 or TSC2, which encode for proteins that form a complex and interact with a small GTP-binding protein, RHEB, to inhibit mammalian target of rapamycin complex 1 (mTORC1). mTORC1 is a central regulator of ribosomal biogenesis and translation initiation, and loss of TSC1/2 function results in increased activity of mTORC1. Therefore, we hypothesized that haploinsufficiency of Tsc2 (Tsc2+/-) in mice would lead to increased regional rates of cerebral protein synthesis (rCPS). We measured rCPS in freely-moving awake male Tsc2+/- mice (age 3 months) with the quantitative autoradiographic L-[1-14C]leucine method. We compared rCPS in 16 brain regions in 11 Tsc2+/- mice with 10 control mice. Unexpectedly, we found statistically significant decreased rates of protein synthesis in many brain regions, including hippocampus (-17%), cortex (-16.5%), thalamus (-16%), and hypothalamus (-20%). These results suggest a possible novel role/ regulation of protein synthesis in the brain.

Disclosures: **R.M. Reith:** None. **T. Huang:** None. **M. Qin:** None. **C. Beebe Smith:** None.

Poster

518. Developmental Disorders: Animal Models I

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Program#/Poster#: 518.19/X14

Topic: C.06. Developmental Disorders

Support: FAPESP 11/21509-3

FAPESP 12/11307-7

FAPESP 12/13433-0

Title: Transient changes in the number of parvalbumin-immunoreactive neurons in the prefrontal cortex of malnourished rats

Authors: *R. J. CRUZ-RIZZOLO[1], L. L. LIMIERI[1], I. R. PAIVA[1], J. O. BARBOSA-RIBEIRO[1], E. ERVOLINO[1], L. PINATO[2], E. A. LIBERTI[3]
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Abstract: Perinatal protein malnutrition (PPM) is considered a serious public health concern, affecting a significant portion of the world's population. Epidemiological studies indicate that protein perinatal deprivation may represent risk factors for the late onset of some mental and psychiatric disorders, fundamentally schizophrenia, condition where the prefrontal cortex plays a fundamental role. In view of the correlation between these neuropsychiatric conditions and PPM, it remains to be determined if some of the structural changes found mainly in the prefrontal cortex of patients in these situations may be reproduced in PPM experimental models. Between the structural changes observed in schizophrenic patients, it has been reported alterations in the GABAergic system, and particularly in the number of parvalbumin positive neurons, a GABAergic subpopulation of the cerebral cortex. Thus, in the present study, using a model of rat PPM, immunohistochemical techniques, and unbiased stereological analysis we quantified the neuronal population of parvalbumin-immunoreactive neurons (Pv+) and the total neuronal population revealed by NeuN immunohistochemistry in the rat medial prefrontal cortex (mPFC). Twenty-four young males rats (Rattus norvegicus; Wistar) were divided in control (C) and malnourished (M) groups. The two groups were fed with proteic or hypoproteic diets (20% and 5% casein, respectively) from one week before conception to the end of the experiment. At postnatal ages day 21 and 60 (P21, P60) twelve animals of each group were anesthetized and transcardially perfused with paraformaldehyde. After perfusion, brains were removed, cryoprotected, frozen and sectioned at 50 μm on the coronal plane. Free-floating sections were

processed for NeuN and Pv immunohistochemistry. The total neuron numbers was estimated using the optical fractionator, with the region of interest (ROI) including all layers and areas of the mPFC. Our stereological analysis showed that PPM did not significantly alter the number of NeuN+ neurons in the rat mPFC in any of the ages studied (P21: C, 527,200±61,290; M, 499,800±62,180; p>0.05. P60: C, 488,600±50,990; M, 493,500±100,500; p>0.05). However, we observed a significant decrease in the number of Pv+ neurons in the mPFC at P21 (C: 29,530±2,650; M 24,780±4,478; p0.05). Considering that there are no differences in the total number of neurons in the mPFC of control and malnourished rats, the changes found in the number of Pv+ neurons indicate a decrease in the synthesis of this protein in the early stages of development.

Disclosures: R.J. Cruz-Rizzolo: None. **L.L. Limieri:** None. **I.R. Paiva:** None. **J.O. Barbosa-Ribeiro:** None. **E. Ervolino:** None. **L. Pinato:** None. **E.A. Liberti:** None.

Poster

518. Developmental Disorders: Animal Models I

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Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 518.20/X15

Topic: C.06. Developmental Disorders

Support: NIH MH086050

NARSAD Young Investigator Award

Title: The schizophrenia and autism associated gene, Transcription Factor 4 (TCF4), regulates cortical column formation in the prefrontal cortex

Authors: *S. C. PAGE, M. D. RANNALS, M. N. CAMPBELL, A. BRILEY, R. A. GALLO, B. MAYFIELD, B. N. PHAN, A. E. JAFFE, R. TAO, J. SHIN, T. M. HYDE, J. KLEINMAN, D. R. WEINBERGER, B. J. MAHER
Lieber Inst. For Brain Develop., BALTIMORE, MD

Abstract: Schizophrenia is a debilitating disease with a complex genetic pathophysiology. Genome-wide association studies (GWAS) have identified a number of loci associated with increased risk for SZ and several of these risk variants are located within introns of TCF4 (E2-2, SEF2, ITF2). In addition, autosomal dominant mutations in TCF4 result in Pitt Hopkins Syndrome (PTHS), a rare neurodevelopmental disorder characterized by a spectrum of

symptoms including hyperventilation, seizures, autistic behaviors, intellectual disability, and brain malformations. TCF4 is a basic helix-loop-helix (bHLH) transcription factor that is critical for brain development but whose function is largely unknown. Therefore, we are altering TCF4 expression in the developing rat prefrontal cortex using *in utero* electroporation to transfect neuroprogenitor cells that are giving rise to layer 2/3 pyramidal neurons. We observe that overexpression of the full-length human isoform TCF4B significantly accelerates neuronal differentiation and migration. In addition, expression of either TCF4B or a shorter isoform TCF4A, which lacks a nuclear localization sequence, results in the formation of aberrant cortical microcolumns. These abnormal cortical structures are not observed from expression of a PTHS variant that contains a single point mutation in the bHLH domain that does not permit DNA binding. TCF4B-dependent microcolumn formation is rescued by co-expressing calmodulin (CaM) with TCF4B, and this rescue appears to be dependent on Ca^{2+}, because rescue is prevented when a mutant calmodulin (CaM1,2,3,4) that is incapable of binding Ca^{2+} is co-expressed with TCF4B. Furthermore, analysis of human RNAseq data from postmortem dorsal lateral prefrontal cortex (DLPFC) identified a single TCF4 5' exon that showed significantly decreased expression in schizophrenia patients compared to controls. This differentially expressed exon is unique to TCF4H and *in utero* transfection of this isoform does not produce abnormal cortical microcolumns. We are currently investigating the molecular mechanisms responsible for these phenotypes. Overall, these results suggest the dosage of TCF4 expression is critical for the proper formation of the proposed cortical processing unit, the cortical column, and therefore could hypothetically explain the cognitive deficits observed in Pitt-Hopkins syndrome and schizophrenia patients.

Disclosures: **S.C. Page:** None. **M.D. Rannals:** None. **M.N. Campbell:** None. **R.A. Gallo:** None. **B. Mayfield:** None. **B.N. Phan:** None. **A.E. Jaffe:** None. **R. Tao:** None. **J. Shin:** None. **T.M. Hyde:** None. **J. Kleinman:** None. **D.R. Weinberger:** None. **B.J. Maher:** None. **A. Briley:** None.

Poster

518. Developmental Disorders: Animal Models I

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

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Topic: C.06. Developmental Disorders

Support: NIH/NINDS grant R21NS077163

Title: Analysis of a novel 17p13 duplication locus causing human cerebellar malformation

Authors: *V. V. CHIZHIKOV, E. STESHINA
Univ. of Tennessee Hlth. Sci. Ctr., Memphis, TN

Abstract: Malformations of the cerebellum affect 1 in every 4,000 births and are an important cause of ataxia, mental retardation, autism and other handicaps. The most common human cerebellar malformation is Dandy-Walker malformation, which is characterized by a small and upwardly rotated cerebellar vermis and enlarged posterior fossa. Currently the genetic basis of most Dandy-Walker cases is unknown, and the molecular mechanisms contributing to Dandy-Walker malformation are poorly understood. Recently we identified a novel locus on human chromosome 17p13, duplication of which is associated with Dandy-Walker malformation. By mapping 17p13 duplications in patients, we defined "most likely" Dandy-Walker critical region, which contains only nine genes, none of which have been previously implicated in cerebellar development or Dandy-Walker malformation. To model Dandy-Walker malformation of individuals with 17p13 duplication, we created BAC transgenic mice, carrying three of the most promising genes located within our most likely 17p13 Dandy-Walker critical region. These BAC transgenic mice recapitulated several features of the human Dandy-Walker brain phenotype, suggesting that at least one of the three human genes present in their genome is a Dandy-Walker causative gene. By analyzing these BAC transgenic mice, we will determine which of our candidate genes is a Dandy Walker causative gene and will identify new molecular mechanisms of cerebellar development and Dandy-Walker malformation.

Disclosures: V.V. Chizhikov: None. **E. Steshina:** None.

Poster

518. Developmental Disorders: Animal Models I

Location: Halls A-C

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Topic: C.06. Developmental Disorders

Support: Lieber Institute

Title: Disruption of Brain-Derived Neurotrophic Factor (BDNF) from promoters I and II, but not IV and VI, leads to increased aggression and altered serotonin signaling in male mice

Authors: *K. R. MAYNARD[1], B. LU[2], L. TESSAROLLO[3], R. J. SCHLOESSER[4,1], K. MARTINOWICH[1,5]
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Abstract: Transcription of *Bdnf* is controlled by several promoters, which drive expression of multiple transcripts encoding an identical protein. Promoters I and IV contribute significantly to activity-dependent *Bdnf* transcription. The existence of unique *Bdnf* transcripts allows for precise temporal, spatial, and stimulus-specific regulation of BDNF production, but the functional consequences of multiple transcripts encoding the same protein remains to be elucidated. We studied mice in which the production of BDNF from promoters I (BDNF-e1), II (BDNF-e2), IV (BDNF-e4), and VI (BDNF-e6) was specifically disrupted. We tested male mice for deficits in social interaction and used quantitative RT-PCR to analyze serotonin transporter and receptor expression in multiple brain regions associated with mood regulation. In a cagemate paradigm of aggression, BDNF-e1 and BDNF-e2 animals displayed decreased latency to attack, increased number of fights, and increased number of tail rattles compared to wild-type controls. Increases in aggression in BDNF-e1 and BDNF-e2 mice were also observed in the home cage requiring separation of mutant animals by 5-6 weeks of age. These behavioral changes were accompanied by increased mRNA expression of the serotonin transporter and $5HT_{2A}$, $5HT_{2B}$, and $5HT_{2C}$ receptors in the hypothalamus, medial prefrontal cortex, and hippocampus of BDNF-e1 and BDNF-e2 mice. In comparison, BDNF-e4 and BDNF-e6 animals did not show signs of enhanced aggressiveness in their home cage or a cagemate paradigm of aggression. Furthermore, BDNF-e4 and BDNF-e6 mice displayed divergent impairments in the 5HT system. These results indicate that *Bdnf* promoters I and II play a critical role in regulating aggressive behavior in male mice, likely through interaction with the serotonin system. Furthermore, our data suggest that BDNF produced from different activity-regulated promoters may have unique functions in neurodevelopment, plasticity, and behavior.

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Poster

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Brain & Behavior Research Foundation

Brain Science Foundation

Title: Postnatal $GABA_A$ receptor signaling is mediated by DISC1 to allow proper prefrontal cortex development and regulation of adult behavior

Authors: Y. TANIGUCHI[1,2], A. SAITO[1,3], M. D. RANNALS[4], M. D. BALLINGER[1], M. KOGA[1], Y. OHTANI[1], T. W. SEDLAK[1], A. CROSS[5], S. J. MOSS[6,7], N. J. BRANDON[5], B. J. MAHER[4], *A. KAMIYA[1]
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Abstract: Brain maturation is a dynamic and complex process in which many genetic factors for a wide range of neuropsychiatric disorders play diverse roles. Thus, elucidation of the precise molecular mechanisms of how genetic insults provoke aberrant brain development, resulting in subsequent disease-related functional deficits, is critical. We report that postnatal suppression of Disrupted-in-Schizophrenia-1 (DISC1) expression in the prefrontal cortex, drives disturbances of synaptic GABA function and dendritic development in pyramidal neurons, as well as abnormalities in sensorimotor gating, albeit without profound memory deficits. DISC1 regulates $GABA_A$ receptors function specifically in immature developing neurons, but not after full maturation. Notably, transient pharmacological intervention with subtype-selective $GABA_A$ receptor positive allosteric modulators during the postnatal period ameliorates dendritic deficits induced by suppression of DISC1. These findings highlight a critical role of DISC1-mediated postnatal GABA signaling for proper prefrontal cortex maturation and function, and suggest that the postnatal developmental phase may ultimately be considered an important strategic target of treatment for neurodevelopmental conditions.

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Poster

518. Developmental Disorders: Animal Models I

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Title: Differences in responsiveness between methamphetamine, nisoxetine and methylphenidate may reflect specific developmental characteristics in juvenile DAT KO mice

Authors: *Y. KUBO[1,2], Y. KASAHARA[1,2], Y. ARIME[2,3], F. S. HALL[4], Y. TAKAMATSU[5], K. IKEDA[5], G. R. UHL[4], I. SORA[2,6], H. TOMITA[1,2]
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Abstract: Attention deficit hyperactivity disorder (ADHD) is a heterogeneous syndrome manifesting symptoms including hyperactivity, impulsivity and inattention. The incidence of these symptoms typically changes with age, and often persists into adulthood. Dopamine transporter (DAT) knockout (KO) mice exhibit remarkable hyperactivity, and therefore have been widely considered as an animal model of ADHD. Although developmental processes may be involved in the pathogenesis of ADHD, there have been few studies evaluating developmental processes in this mouse model. We have thus tested locomotor activity of juvenile (4 weeks old) vs adult (12 to 20 weeks old) DAT KO and wildtype (WT) littermate mice in a novel environment after pretreatment with methylphenidate (a non-selective dopamine/norepinephrine transporter inhibitor), nisoxetine (a selective norepinephrine transporter inhibitor), methamphetamine (a relatively non-selective monoamine releaser) or saline. The results showed that 3, 10, 30, or 60 mg/kg methylphenidate failed to reduce locomotion in young DAT KO mice, whereas 10, 30, or 60 mg/kg nisoxetine and 2 mg/kg methamphetamine significantly

decreased hyperactivity in young DAT KO mice. By contrast, both methylphenidate (3, 10, 30, or 60 mg/kg) and methamphetamine (2.0mg/kg) were able to significantly decrease hyperactivity of adult DAT KO mice. The effects of methylphenidate or methamphetamine did not differ in WT mice regardless of age and in all cases produced locomotor stimulation, while nisoxetine (10, 30, or 60 mg/kg), exerted no significant effects on locomotor activity in WT mice. Methylphenidate differs from methamphetamine in its primary mechanism of action, which seems to affect the sensitivity of young DAT KO mice to these drugs. Curiously, the selective NET blocker nisoxetine was effective in both young and adult DAT KO mice, while methylphenidate was not, suggesting that they might have different mechanisms of action. These data indicate that pharmacological effects on locomotor activity in DAT KO mice have a specific neurodevelopmental basis that influences their response to ADHD medications that act through different mechanisms. Considering that methylphenidate was ineffective in juvenile mice, this may indicate that certain ADHD medications may have differential efficacy depending on age.

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Poster

518. Developmental Disorders: Animal Models I

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Topic: C.06. Developmental Disorders

Support: Michael J. Fox Foundation for Parkinson's Disease Research

Hussman Institute for Autism

Title: Striatal Gad67 knockout induces spatial learning and social behavior deficits

Authors: K. ZHANG[1], S. LABAK[1], K. HILL[1], G. BLATT[2], *J.-J. SOGHOMONIAN[1]
[1]Anat. and Neurobio., Boston Univ. Sch. of Med., Boston, MA; [2]Hussman Insitute for Autism, Catonsville, MD

Abstract: GABA is the neurotransmitter of striatal projection neurons, however the contribution of the striatal GABAergic output to behavior is not well understood. We assessed motor function, spatial learning, social and olfactory preference in a conditional mouse model lacking the GABA-synthesizing enzyme glutamic acid decarboxylase, Gad67, in striatal neurons. Gad67-

deficient mice show no impairments in motor coordination and balance on the rotarod and the pole test, but exhibit enhanced locomotor activity, lack of anxious phenotype and stereotypic grooming behavior on the elevated maze and the open-field. Furthermore, Gad67-deficient mice show impairments in spatial learning and social behavior as assessed in the tri-chambers test and impairments in preference for social odors. These findings provide original evidence that striatal Gad67 expression is involved in the modulation of learning and social behavior. Some of the behavioral abnormalities observed in Gad67-deficient mice are reminiscent of Autism-Spectrum-Disorders (ASD) deficits, suggesting that abnormal striatal GABAergic output may contribute to behavioral deficits in ASD

Disclosures: K. Zhang: None. **S. Labak:** None. **K. Hill:** None. **G. Blatt:** None. **J. Soghomonian:** None.

Poster

518. Developmental Disorders: Animal Models I

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 518.26/X21

Topic: C.06. Developmental Disorders

Title: Ketogenic diet attenuates behavioral abnormalities and alters brain mitochondrial respiration in the BTBR mouse model of autism

Authors: *Y. AHN, N. CHENG, R. MYCHASIUK, J. SMITH, R. TOBIAS, J. M. RHO
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Abstract: Autism spectrum disorder (ASD) is an increasingly prevalent neurodevelopmental disorder characterized by three core symptoms: abnormal social interactions, communication deficits, and repetitive/stereotyped behaviors. Further, ASD is a chronic condition that can impair gastrointestinal, immune, hepatic, and endocrine systems. In the U.S., the prevalence of ASD is now as high as 1 in 68 children, a 30% increase over two years (CDC 2014). The BTBR T+tf/J (BTBR) mouse is a robust model of ASD and displays all three core behavioral features compared to C57 mice. Earlier, it was shown that the ketogenic diet (KD) can enhance mitochondrial bioenergetics in normal rats (PMID: 16807920) and reduces autistic behaviors in BTBR mice (PMID: 23755170). Here, we asked whether the behavioral improvements induced by the KD are associated with changes in mitochondrial metabolism. Mice were administered either the KD or a standard diet (SD) for 10-14 days from weaning at P21 (C57/SD, C57/KD, BTBR/SD, and BTBR/KD; N>8/group), at which point mice underwent the 3-chamber

sociability test and the inchworming test (P35) (Smith et al, 2014). In the 3-chamber test, BTBR mice spent less time in the chamber with the stranger mouse (340±18 vs. 230±51 sec; p=0.072), but more time in the empty chamber, compared to C57 mice (180±15 vs. 310±54 sec; p=0.039). After KD treatment, BTBR mice spent increased time in the stranger chamber (SD vs. KD in BTBR mice = 230±51 vs. 400±14 sec; p=0.003) and decreased time in the empty chamber (310±54 vs. 160±15 sec; p=0.01). To assess inchworming, we placed an intra-strain pair of mice in a 30x30 cm Plexiglas chamber containing sawdust bedding (~1 inch deep) in a quiet darkened room. Activity was recorded for 10 min sessions. Compared to C57 controls, BTBR mice exhibited longer and more frequent inchworming activity, and a shorter latency to initiate this behavior (13 vs. 300 sec for duration, 2 vs. 33 for frequency, 250 vs. 3 sec for latency to inchworming; $p<0.01$). After KD treatment, inchworming in BTBR mice was significantly decreased (SD vs. KD in BTBR mice = 190 vs. 110 for duration, 35 vs. 21 for frequency, and 3.5 vs. 30 for latency to inchworming; $p<0.01$). Next, we studied mitochondrial respiration with the Seahorse XF24 extracellular analyzer. BTBR mice showed higher oxygen consumption rates (OCRs) than C57 controls (94±1.1 vs. 120±1.3; $p<0.01$), whereas the KD decreased OCR levels in BTBR mice (120±1.3 vs. 88±1.0; $p<0.01$; N=7/group). While these results are clearly correlative, we conclude that the KD may trigger alterations in brain mitochondrial bioenergetics in BTBR mice and that these changes may underlie in part the beneficial behavioral effects of this diet.

Disclosures: Y. Ahn: None. **N. Cheng:** None. **R. Mychasiuk:** None. **J. Smith:** None. **R. Tobias:** None. **J.M. Rho:** None.

Poster

518. Developmental Disorders: Animal Models I

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 518.27/X22

Topic: C.06. Developmental Disorders

Support: NIH Grant NS060765

Baby Alex Foundation

Title: *In utero* hypoxia-ischemia and inflammation results in complex white matter abnormalities and gait deficits in young adult rats

Authors: *L. L. JANTZIE[1], S. ROBINSON[2]
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Abstract: Infants born preterm commonly suffer from a combination of hypoxic-ischemic (HI) and infectious perinatal inflammatory insults that lead to cerebral palsy, cognitive delay, behavioural issues and epilepsy. Using a novel rat model of combined late gestation HI and lipopolysaccharide (LPS)-induced inflammation, we tested our hypothesis that inflammation from HI and LPS differentially impact white matter development and motor impairment during the first postnatal month. Pregnant rats underwent laparotomy on embryonic day 18 and transient systemic HI (TSHI) and/or intra-amniotic LPS injection. Shams had laparotomy and anesthesia, but no TSHI or LPS. Pups were born at term (n=11-18/group). Immunohistochemistry and Western blots were performed for myelin basic protein (MBP) and neurofilament (NF) expression. White matter erythropoietin (EPO) ligand and receptor levels were quantified using qPCR. Digigait analysis detected gait deficits. Statistical analysis was performed with one-way ANOVA and post-hoc Bonferonni correction. At P15, MBP expression is reduced by 31% in TSHI+LPS pups compared to shams ($p< 0.05$), whereas TSHI alone leads to only a 13% reduction in MBP. By P28, white matter injury shifts from the acute injury pattern to a chronic injury pattern in TSHI pups only. Both MBP expression ($p<0.01$) and the pNF/NF ratio, a marker of axonal dysfunction, are reduced in P28 TSHI pups ($p<0.001$). EPO ligand to receptor ratios differ between brains exposed to TSHI and LPS. Gait analyses reveal that all groups (TSHI, LPS and TSHI+LPS) are ataxic with deficits in stride, paw placement, gait consistency and coordination (all $p<0.001$). Prenatal TSHI and TSHI+LPS lead to different patterns of injury with respect to myelination, axon integrity and gait deficits. Further study will contribute to stratification of injury mechanisms in preterm infants, and guide the use of promising therapeutic interventions.

Disclosures: L.L. Jantzie: None. **S. Robinson:** None.

Poster

518. Developmental Disorders: Animal Models I

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 518.28/X23

Topic: C.06. Developmental Disorders

Support: NJCBIR grant CBIR12MIG011

DOD–CDMRP-TSCRP grant TS110033

Title: Targeting the PI3K/Akt/mTOR pathway in an *in vitro* model of cortical dysplasia

Authors: *G. D'ARCANGELO, I. NIKOLAEVA, B. CROWELL, T. KAZDOBA
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Abstract: Cortical dysplasia is a group of developmental brain disorders characterized by anatomical malformations of the cerebral cortex and cellular overgrowth. These malformations are highly associated with intellectual disability and childhood epilepsy. The activity of the PI3K/Akt/mTOR pathway is elevated in dysplastic brain regions as a result of genetic or somatic mutations in several genes in this signaling cascade. To mimic this condition in the developing mouse forebrain, we conditionally disrupted the *Pten* gene that normally suppresses PI3K/Akt/mTOR signaling specifically in excitatory neurons of the forebrain. Homozygous knock out mice (NEX-Pten) exhibit megalencephaly and activation of the PI3K/Akt/mTOR pathway in forebrain neurons, and die shortly after birth. Forebrain neuronal cultures derived from mutant embryos exhibit dramatic cellular hypertrophy, including increased soma size and dendrite complexity. Cultured neurons also exhibit the expected activation of PI3K/Akt/mTOR signaling. In this study we first characterized NEX-Pten forebrain cultures by measuring morphometric parameters of cellular growth and the level of PI3K/Akt/mTOR signaling in mutant and control neurons at different stages of development and maturation. Second, we tested known compounds that inhibit these specific pathway components to identify agents that reduce the growth abnormalities of NEX-Pten neurons. We used FDA-approved compounds, such as the Akt inhibitor MK2206 and the mTORC1 inhibitor RAD001 (a rapamycin analog), alone or in combination, to establish the most effective form of intervention that suppresses the phenotype and restores normal signaling without affecting neuronal viability. These findings will help to develop novel pharmacological treatments for children affected by cortical dysplasia. This work is supported in part by multiprogrammatic grant CBIR12MIG011 from the New Jersey Commission on Brain Injury Research, and by Exploration-Hypothesis Development Award #TS110033 from the Department of Defense - CDMRP - TSCRP (G.D.).

Disclosures: G. D'Arcangelo: None. **I. Nikolaeva:** None. **B. Crowell:** None. **T. Kazdoba:** None.

Poster

518. Developmental Disorders: Animal Models I

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 518.29/X24

Topic: C.06. Developmental Disorders

Support: Lottery Health New Zealand

Title: Delayed post-treatment with bone marrow-derived mesenchymal stem cells is neurorestorative of striatal medium-spiny projection neurons and improves motor function after neonatal rat hypoxia-ischemia

Authors: *D. E. OORSCHOT, A. J. ALWAKEEL, L. GODDARD, C. E. HOBBS, E. K. GOWING, E. R. BARNETT, S. E. KOHE, R. J. SIZEMORE, S. H. CAMERON
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Abstract: Perinatal hypoxia-ischemia is a major cause of striatal injury and may lead to cerebral palsy. We investigated whether delayed administration of bone marrow-derived mesenchymal stem cells (MSCs), at one week after neonatal rat hypoxia-ischemia, was neurorestorative of striatal medium-spiny projection neurons and improved motor function. The effect of a subcutaneous injection of a high-dose, or a low-dose, of MSCs was investigated in stereological studies. Postnatal day (PN) 7 pups were subjected to hypoxia-ischemia. At PN14, pups received treatment with either MSCs or diluent. A subset of high-dose pups, and their diluent control pups, were also injected intraperitoneally with bromodeoxyuridine, every 24h, on PN15, PN16 and PN17. This permitted tracking of the migration and survival of neuroblasts originating from the subventricular zone into the adjacent injured striatum. Pups were euthanized on PN21 and the absolute number of striatal medium-spiny projection neurons was measured using stereological methods after immunostaining for DARPP-32 (dopamine- and cAMP-regulated phosphoprotein-32), double immunostaining for bromodeoxyuridine and DARPP-32, and after cresyl violet staining alone. The absolute number of striatal immunostained calretinin interneurons was also measured. There was a statistically significant increase in the absolute number of DARPP-positive, bromodeoxyuridine/DARPP-32-positive, and cresyl violet-stained striatal medium-spiny projection neurons, and fewer striatal calretinin interneurons, in the high-dose MSCs group compared to their diluent counterparts. Compared with previous stereological data on the absolute number of cresyl violet-stained striatal medium-spiny projection neurons in the normal uninjured brain, a high-dose of MSCs restored the absolute number of these neurons to normal uninjured levels. For the low-dose experiment, in which cresyl violet-stained striatal medium-spiny neurons alone were measured, there was a lower statistically significant increase in their absolute number in the MSCs group compared to their diluent controls. Investigation of behaviour in another cohort of animals showed that delayed administration of a high-dose of bone marrow-derived MSCs, at one week after neonatal rat hypoxia-ischemia, improved long-term motor function on the staircase test and the cylinder test. Thus, delayed therapy with a high- or low-dose of adult MSCs, at one week after injury, is effective in restoring the loss of striatal

medium-spiny projection neurons after neonatal rat hypoxia-ischemia and a high-dose of MSCs improved motor function. Funded by Lottery Health New Zealand.

Disclosures: **D.E. Oorschot:** None. **A.J. Alwakeel:** None. **L. Goddard:** None. **C.E. Hobbs:** None. **E.K. Gowing:** None. **E.R. Barnett:** None. **S.E. Kohe:** None. **R.J. Sizemore:** None. **S.H. Cameron:** None.

Poster

518. Developmental Disorders: Animal Models I

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 518.30/Y1

Topic: C.06. Developmental Disorders

Title: Combined effects of prenatal stress and maternal genotype on interneuron development

Authors: *P. HECHT[1], E. JASAREVIC[2], F. MATSUI[1], L. WELBY[1], J. MINK[1], M. WILL[1], D. BEVERSDORF[1]
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Abstract: Prenatal stress has been shown to have a profound and lasting impact on neurodevelopment and leads to an increased risk for several neuropsychiatric conditions, including autism spectrum disorder (ASD). It is crucial to understand how genetics may interact with this environmental trigger to identify those most susceptible to the increased risk and to create a better understanding of the underlying mechanisms and potential treatments of such disorders. A 44 base-pair deletion polymorphism located in the promoter region (5-HTTLPR) of the serotonin transporter gene (SLC6A4) results in decreased gene expression and has been associated with several anxiety related disorders. In an animal model, pregnant dams heterozygous for the serotonin transporter gene (Slc6a4 +/-) exposed to stress produced offspring that exhibited several autistic-like behaviors. It has been suggested that the GABAergic system plays a key role in the underlying pathophysiological process of ASD. Previous research has shown that prenatal stress and manipulation of the serotonergic system affects the proper development of the GABAergic system individually. However, the combined effects are unknown. In the present study, wild-type female mice and females heterozygous for the serotonin transporter gene (Slc6a4 +/-) were bred with wild-type males. Upon detection of a mating plug, mice were either placed in a prenatal stress or control condition. In the stress condition, animals were exposed to restraint stress beginning on embryonic day 12 and continued every day until embryo tissue collection or parturition. GABAergic interneuron development was

then analyzed using immunofluorescence. Results suggest that manipulations of the maternal serotoninergic system further exacerbate the deleterious effects of stress on the developing brain. Embryonic tissue in this group exhibited delayed interneuron migration and abnormal cortical layering into the cortex. These findings begin to reveal how stress exposure and genetics may interact to impact the development of neural circuits believed to be critical for social interaction.

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Poster

519. Developmental Disorders: Animal Models II

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 519.01/Y2

Topic: C.06. Developmental Disorders

Support: NIH Grant HD067379

Title: Learning and memory deficits in a novel mouse model of non-syndromic intellectual disability and autism spectrum disorder

Authors: A. W. OAKS[1], S. DI COSTANZO[1], D. E. TAMBUNAN[2], H. L. POND[1], D. GONZALEZ[2], C. A. WALSH[2], *M. MANZINI[1]
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Abstract: Autosomal recessive loss of function mutations in the *CC2D1A* gene cause a spectrum of fully penetrant cognitive phenotypes including mild to severe intellectual disability (ID), autism spectrum disorder (ASD), as well as seizures, suggesting that these conditions have shared developmental mechanisms. Cognitive and behavioral deficits are often attributed to hypomorphic or *de novo* heterozygous mutations, however complete loss of *CC2D1A* function identifies this gene as a critical participant in neurodevelopmental processes without any additional syndromic presentation. CC2D1A is an important regulator of endosomal trafficking and signaling and these functions appear to be essential for normal cognitive development. Previous attempts to identify the developmental role of this gene by genetic removal in mice have been partially prevented due to perinatal lethality in *Cc2d1a*-deficient animals. In order to overcome this challenge and to further elucidate the role of *CC2D1A* in humans, we generated a conditional knockout (cKO) line to remove *Cc2d1a* in the brain at different times. In these

animals the floxed *Cc2d1a* gene is disrupted following expression of a cre recombinase driven by the *nestin* or *CamKIIa* promoter. While the Cc2d1a^{nestin} cKO animals still die at birth, conditional postnatal removal of Cc2d1a in the forebrain via CamKII-cre generates animals which are viable and fertile and show grossly normal growth and development. Brain anatomy and basic behavioral function are comparable to wild type in adult Cc2d1a^{CamKII} cKO mice, but these animals display deficits in spatial learning and memory and a tendency toward hyperactivity and excessive grooming, which are consistent with other ASD/ID models. As detailed morphological and electrophysiological characterization is underway, we propose that the Cc2d1a^{CamKII} cKO mice could provide much needed insight into the developmental mechanisms underlying non-syndromic cognitive deficits.

Disclosures: A.W. Oaks: None. **S. Di Costanzo:** None. **D.E. Tambunan:** None. **H.L. Pond:** None. **D. Gonzalez:** None. **C.A. Walsh:** None. **M. Manzini:** None.

Poster

519. Developmental Disorders: Animal Models II

Location: Halls A-C

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Program#/Poster#: 519.02/Y3

Topic: C.06. Developmental Disorders

Support: FCT PTDC/SAU-GMG/112577/2009

Title: Absence of H3K4 demethylase rbr-2 in *C. elegans* results in defective GABAergic network morphology and function

Authors: A. J. RODRIGUES[1], C. BESSA[1], F. MARQUES[1], A. AMORIM[1], F. LOPES[1], *P. E. MACIEL[1,2]
[1]Life and Hlth. Sci. Res. Inst. (ICVS), Braga, Portugal; [2]ICVS/3B's - PT Government Associate Lab., Braga, Portugal

Abstract: Intellectual disability (ID) is one of the most frequent and disabling neurological impairments with an estimated prevalence of 1.5-2% in Western countries. Technological advances such as the use of array comparative genomic hybridization (aCGH) and massive parallel sequencing have allowed the identification of novel genetic causes of ID. This large-scale analysis originates an incredible number of novel genetic associations that most often require functional validation. We are using the simple round worm Caenorhabditis elegans (*C. elegans*) as a platform to functionally validate ID genetic associations and to better understand

the importance of target genes and proteins in the nervous system and neuronal function. So far, we have studied 35 mutant strains that correspond to 29 orthologues of human genes previously linked to ID. Absence of one candidate, rbr-2, the orthologue of human KDM5C/JARID1C, leads to anatomical reproductive defects, developmental delay (larval arrest), increased embryonic lethality and decreased life span. Interestingly, rbr-2 mutant worms also present several behavioural deficits which suggest an impairment of neuronal functions. Of notice, worms exhibit increased motor uncoordination and abnormal sensory chemotactic responses. By crossing the rbr-2 knock-out strain with reporter strains expressing GFP in specific neuronal sub-types, we found an increased occurrence of GABAergic network defects - namely abnormal neuronal process positioning and migration. In addition, these animals were more sensitive to Pentylenetetrazol (PTZ - a GABA antagonist) than wild-type worms. This correlates well with expression analysis of genes related to GABA metabolism or transport, some of which were found be altered in mutant worms. Overall, our data suggests that rbr-2, which encodes for a histone demethylase, plays a relevant role in nervous system development and specifically in GABA-dependent neuronal functions.

Disclosures: A.J. Rodrigues: None. **C. Bessa:** None. **P.E. Maciel:** None. **F. Marques:** None. **A. Amorim:** None. **F. Lopes:** None.

Poster

519. Developmental Disorders: Animal Models II

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 519.03/Y4

Topic: C.06. Developmental Disorders

Title: Shank2 mutation in a rat model decreases social interaction and increases hyperactivity and motivated behavior

Authors: *M. E. MODI[1], M. SCHMEISSER[2], D. REIM[2], T. BOECKERS[2], T. KISS[1], D. L. BUHL[1]
[1]Pfizer Inc., Cambridge, MA; [2]Univ. of Ulm, Ulm, Germany

Abstract: Many genes identified as anomalous in genetic screens of the autism population encode proteins that regulate synaptic plasticity, including the *SHANK* family of genes. The link between mutation of ubiquitously expressed synaptic genes and the social impairments of autism, though, is unclear. To investigate the relationship between decreased synaptic function and social impairment, the behavioral and molecular phenotype of transgenic rats expressing a

truncation mutation of the *Shank2* gene was characterized. *Shank2 m*utation resulted in alterations in social behavior throughout development. *Shank2* mutant rats engaged in less juvenile play than wild type rats (WTs), which was maintained into adulthood as evidenced by decreased social investigation and social recognition. Unlike *Shank2* deletion in the mouse, a decrease in social approach was not observed in *Shank2* mutant rats. *Shank2* mutant rats also exhibited several forms of hyperactive and repetitive behaviors, including increased locomotion and abnormal circling and checking behaviors. Independent of the hyperactivity, *Shank2* mutant rats showed increased motivation in a progressive ratio operant response task relative to WT rats, despite impairment in the initial acquisition of the task. Both increased circling and operant responses were significantly diminished by either dopamine D1 or D2 receptor antagonists. The observed hyperactivity/hypermotivation phenotypes may be due to the observed upregulation of mGluR1 and SHANK3 in the striatum. The alterations of mGluR1 expression are observed only in male mutant rats. Despite the alterations in locomotor behavior and synaptic receptor expression, *Shank2* mutant rats do not have any overt alterations in cortical EEG activity or in the sleep/wake cycle. However, as SHANK2 mutant rats display many of the behavioral features of autism, further investigation into behaviorally induced neuronal activity within neurocircuits associated with emotion will be explored for the development of electrophysiological endophenotypes of autism-related impairments.

Disclosures: **M.E. Modi:** A. Employment/Salary (full or part-time):; Pfizer Inc.. **M. Schmeisser:** None. **D. Reim:** None. **T. Boeckers:** None. **T. Kiss:** A. Employment/Salary (full or part-time):; Pfizer Inc. **D.L. Buhl:** A. Employment/Salary (full or part-time):; Pfizer Inc..

Poster

519. Developmental Disorders: Animal Models II

Location: Halls A-C

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Topic: C.06. Developmental Disorders

Support: NIH 2P20RR017702-061A1 (RMG, JC)

NIH HL-086662 (DG)

University of Louisville Research Initiation Grant (JC)

Title: Maternal exposure to sleep apnea-featured intermittent hypoxia leads to oxidative stress and perinatal white matter deficits in mouse brains

Authors: X. LI[1,3], R. WU[1], C. TUONG[1], D. GOZAL[4], *J. CAI[2]
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Abstract: Pregnancy has emerged as an important risk factor for sleep-disordered breathing (SDB). Little is known about the impact of untreated gestational SDB on the fetus/newborn. We hypothesize that maternal SDB during gestation will result in hypoxic/oxidative stress and white matter injury (WMI) in perinatal brains. To test this hypothesis, we examined hypoxic status/oxidative response and WM development in perinatal brains using maternal mouse models of sleep apnea-featured intermittent hypoxia (IH). Pulse oxyhemoglobin saturation (SpO2) changed in a recurrent manner and produced similar nadir hemoglobin oxygen saturations (60%~70%) as observed in moderate to severe sleep apnea patients. Earlier delivery occurred with small litter size in IH-exposed pregnant mice. The gestational IH-insulted neonates showed retarded somatic growth after birth. Hypoxic cells in fetal brain were dramatically labeled with tissue hypoxia marker-pimonidazole hydrochloride. Expressions of iNOS and eNOS, but not nNOS, were enhanced in maternal IH-exposed fetal brains. Intriguingly, p67phox and gp91phox, the cytosolic and membrane-bound components of NADPH oxidase, were significantly elevated in fetal/neonatal brains subjected to maternal IH exposures. Although barely detectable SOD2 was slightly increased in IH-insulted fetal brains, a large amount of superoxide was produced, coinciding with increased endogenous apoptosis in multiple brain regions. Furthermore, maternal IH exposures inhibited myelin synthesis and axonal maturation in offspring after birth, especially in the areas of corpus callosum and cerebellum, which may result in long-term neurobehavioral sequelae. The findings suggest that hypoxic/oxidative stress may play an important role in the pathogenesis of adverse fetal outcomes, including perinatal WMI.

Disclosures: X. Li: None. **R. Wu:** None. **C. Tuong:** None. **D. Gozal:** None. **J. Cai:** None.

Poster

519. Developmental Disorders: Animal Models II

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Topic: C.06. Developmental Disorders

Support: NIH Grant R56 NS082092

NIG Training Grant T32GM007752

Title: The role of lysophosphatidic acid signaling in initiating premature infantile post-hemorrhagic hydrocephalus

Authors: *N. C. STODDARD[1,2]
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Abstract: Post-hemorrhagic hydrocephalus (PHH) is a disease caused by intraventricular hemorrhage that presents with increased intracranial pressure leading to ventriculomegaly, severe cognitive disability, and possible death. This is a leading childhood neurological disorder, affecting 1 in 1500 newborns, with only palliative treatment options. Recent studies have linked the bioactive lipid, LPA, through its cognate receptors (LPARs) to the initiation of PHH during embryonic life. This lipid is a small molecule existing in cells and circulating at high concentrations within the blood. Here, we present a new LPA-induced mouse model of PHH that implicates LPA signaling at a much later period of human development. This is especially relevant, as premature infants have fragile vasculature in the subventricular zone and surrounding the brain, making them a high risk group for PHH. Neonatal mice, approximating infants born at 20-32 weeks, were injected intracranially with LPA, blood, serum, or vehicle. LPAR signaling initiated by intraventricular hemorrhage caused drastically altered ventricular morphology, disrupted ependymal lining, and decreased cell number surrounding the ventricles. Importantly, these effects are distinct from the fetal PHH model based on age of LPA exposure, maturity and localization of neural cell types, and spatiotemporal LPAR expression. Ongoing studies include identification of receptor selectivity along with efforts to determine the therapeutic usefulness of targeting these GPCRs as a preventative measure against PHH development.

Disclosures: N.C. Stoddard: None.

Poster

519. Developmental Disorders: Animal Models II

Location: Halls A-C

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Program#/Poster#: 519.06/Y7

Topic: C.06. Developmental Disorders

Support: NIHR01DA020796

Title: Cognitive flexibility and frontal cortical BDNF-TrkB signaling following prenatal cocaine exposure

Authors: *D. M. MC CARTHY[1], M. N. HUIZENGA[2], G. BELL[3], E. N. CANNON[1], K. P. LEE[1], J. ZHU[1], D. A. FADOOL[3], G. SADRI-VAKILI[2], P. G. BHIDE[1]
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Abstract: Prenatal exposure to cocaine produces lasting changes in brain structure and function. Studies using animal models of prenatal cocaine exposure have focused mainly on behavioral outcomes relevant to reward and addiction such as behavioral sensitization and conditioned place preference. However, prenatal cocaine exposure is likely to impact other cognitive functions such as working memory and cognitive flexibility, which are components of attentional and executive function networks. To address this gap in our knowledge, we used an olfactory reversal learning paradigm to assay cognitive flexibility in prenatally cocaine exposed adult mice. Water-deprived, adult (postnatal day 90) mice from prenatal cocaine- or saline-exposed groups were operant trained to associate water reward with a reinforcing odor (S+) and no reward with a non-reinforcing odor (S-). Following training, when the S+ and S- odors were reversed, the prenatally cocaine exposed mice demonstrated significant inability to elicit the correct responses, indicating deficiencies in cognitive flexibility. Plasticity in dopaminergic and GABAergic neuronal circuits in the prefrontal cortex is critical for cognitive flexibility. The neurotrophin BDNF plays critical roles in neuronal plasticity. Cocaine is known to alter BDNF signaling in multiple brain regions, including the frontal cortex. Therefore, we hypothesized that BDNF signaling via the TrkB receptor plays an essential role in mediating the effects of prenatal cocaine exposure on cognitive flexibility. We found that BDNF protein and mRNA as well as well as TrkB phosphorylation were significantly increased in the frontal cortex of prenatally cocaine exposed adult mice. Together our data show that prenatal cocaine exposure leads to lasting deficits in cognitive flexibility, which appear to be associated with alterations in BDNF signaling. Prenatal cocaine exposure also produces lasting changes in dopamine and GABA neuronal networks. Therefore, currently we are exploring whether the changes in cognitive flexibility are mediated solely by the elevated BDNF-TrkB signaling or whether other mechanisms such as frontal cortical dopamine and GABA neurotransmitter signaling are also involved.

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Poster

519. Developmental Disorders: Animal Models II

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Program#/Poster#: 519.07/Y8

Topic: C.06. Developmental Disorders

Support: MH087978

MH091372

Title: Perinatal high fat diet leads to DNMT1 deficits in the offspring prefrontal cortex: mRNA overexpression, reduced activity and cytoplasmic sequestration

Authors: *S. MCKEE, N. M. GRISSOM, N. BOWMAN, T. M. REYES
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Abstract: Maternal obesity and excess gestational weight gain increase the risk of mental disorders, including schizophrenia, autism, and attention deficit/hyperactivity disorder (ADHD). These disorders of cognition and attention implicate prefrontal cortex (PFC), a region important for action selection, executive function, and inhibitory control. Previously, we have shown that offspring of a maternal high-fat diet (HFD) have reduced whole-genome DNA methylation in multiple brain regions, including the PFC, coupled with promoter-specific hypomethylation and dysregulated gene expression. This suggests that DNA methylation deficits may contribute to the neurodevelopmental programming deficits induced by gestational exposure to a high-fat diet. Therefore, the present experiments were designed to systematically examine the expression and function of DNA methylation machinery in offspring born to dams fed a HFD during pregnancy and lactation. DNA methyltransferase 1 (DNMT1) was overexpressed in all examined brain regions, with the largest fold change observed in the PFC. In contrast, expression of DNMT3a and 3b was unchanged by maternal diet. Experimentally induced DNMT1 overexpression is typically associated with DNA hypermethylation, suggesting that the observed DNA hypomethylation in this model was due to impairments in DNMT1 function. In the PFC of HFD offspring, DNMT1 protein was elevated, while DNMT3a protein was unchanged. Via immunohistochemistry, we identified high levels of cytoplasmic DNMT1 in HFD offspring, while DNMT1 was largely contained in the nucleus in the PFC of control offspring. Methyltransferase activity of nuclear extracts of PFC was unchanged between HFD and control offspring, but the methyltransferase activity of cytoplasmic extracts was significantly lower in HFD offspring, suggesting that mechanisms impairing DNMT1 nuclear localization also impair its catalytic activity. Immunoprecipitation experiments revealed that DNMT1 from HFD offspring PFC was significantly less associated with the kinases AKT and casein kinase, both of which regulate DNMT1 nuclear import and catalytic function. Overall, these data indicate a profound impairment in the function of DNMT1 protein in adult PFC neurons due to gestational HFD. DNMT1 overexpression has been repeatedly observed in PFC in schizophrenia, and neuronal DNMT1 cytoplasmic sequestration has been noted in several forms of dementia. These

findings demonstrate that early life events can permanently alter the function of DNMT1 into adulthood, possibly predisposing to mental disorder.

Disclosures: **S. McKee:** None. **N.M. Grissom:** None. **N. Bowman:** None. **T.M. Reyes:** None.

Poster

519. Developmental Disorders: Animal Models II

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 519.08/Y9

Topic: C.06. Developmental Disorders

Support: DFG IS63/3-1

DFG IS63/4-1

NGFN-EMINET

Title: Developmental HCN/h-channel deficiency in the murine forebrain models structural and functional brain disorders

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Abstract: The hyperpolarization-activated cyclic nucleotide-gated nonselective cation (HCN) channels mediate I(h) and are important determinants of the biophysical properties of neurons. Changes in expression patterns, subcellular localization, or biophysical characteristics of HCN channels have been associated with neurological dysfunctions ranging from motor learning deficits to diseases such as epilepsy. Recently, de novo mutations in the HCN1 gene in human patients have been linked to epileptic encephalopathy with concomitant neurological abnormalities, including autistic features, ADHD, absence of language, behavioral disturbances, ataxia, and delay of motor development (1). We generated HCN/h channel-deficient mice in which we functionally suppressed I(h) in different developmental stages. The mice developed phenotypes that were dependent on the age of onset of I(h) suppression and that partially resembled comorbidities observed in human patients with different de novo mutations in the HCN1 gene (1). HCN/h channels are homo- or heterotetramers of HCN 1-4 subunits. By transgenic expression of a dominant-negative (non-conducting) HCN subunit (HCN-DN), we generated mice with a conditional and subunit-unspecific functional knock-out of I(h). HCN-DN

expression under the control of either the EMX1 or CaMKII alpha promoters enabled prenatal or peri-/postnatal ablation of I(h) in forebrain projection neurons. EMX1 promoter-mediated early prenatal ablation of the HCN/h current resulted in severe morphological abnormalities in the developing brain. Brain volume and cortex thickness were strongly reduced in HCN-DN-expressing mice. In contrast, CaMKII alpha promoter-driven early postnatal suppression of HCN channel-activity did not affect brain morphology, but resulted in behavioral abnormalities. HCN-DN mice displayed delayed somatosensory development with respect to sensorimotor reflexes, cognitive deficits in working memory and spatial learning and memory, as well as hyperactivity. Post-weaning onset of HCN-DN expression resulted in slight learning and memory deficits, but no hyperactivity. Our results demonstrate distinct roles of HCN/h-channel activity during pre- and postnatal development of the central nervous system of the mouse. The different age-dependent developmental phenotypes observed in our I(h)-deficient mice may provide a model to investigate the range and variability of neurological dysfunctions associated with HCN1 mutations in human patients. (1) C. Nava et al. (2014), De novo mutations in HCN1 cause early infantile epileptic encephalopathy, Nature Genetics; doi:10.1038/ng.2952

Disclosures: **A. Schlusche:** None. **A. Merseburg:** None. **D. Isbrandt:** None. **A. Neu:** None. **S. Sandke:** None.

Poster

519. Developmental Disorders: Animal Models II

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Topic: C.06. Developmental Disorders

Support: Swedish Research Council (VR)

Title: Developmental exposure to dexamethasone induces alterations in circadian rhythms which precede depression-like behaviour

Authors: *S. SPULBER[1], M. CONTI[2], C. DUPONT[2], N. ONISHCHENKO[2], S. CECCATELLI[2]
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Abstract: Glucocorticoids play a critical role during development, but foetal exposure to high levels increases the risk of cardiovascular, metabolic, neuroendocrine and psychiatric disorders

later in life. The aim of this study was to characterise the early and long-term behavioural alterations induced by prenatal exposure to a synthetic glucocorticoid analogue (dexamethasone, DEX). Pregnant female C57Bl/6 mice were exposed to 0.05 mg/kg/day DEX from gestational day (GD) 14 until delivery. The offspring underwent a battery of behavioural tests to assess spontaneous locomotion, social interactions, and depression-like behaviour, between 1 and 12 months of age. We found that DEX-exposed males were hyperactive and displayed impaired social recognition between 1 and 5 months of age. At 12 months, but not earlier, the DEX-exposed mice displayed depression-like behaviour in the forced-swimming test and impaired hippocampal neurogenesis, which were not reversed by chronic antidepressant treatment with fluoxetine. In humans, mutations in the clock genes have beebn associated with depression. Therefore, we investigated the circadian and ultradian rhythms at 1, 3, 5, and 12 months of age to identify possible alterations that could predict the depression-like phenotype. To this end we monitored the locomotor activity in the homecage using the TraffiCage™ system. The data were analysed offline for assessing the rhythmicity and complexity of fluctuations in spontaneous activity. We found that control mice have fractal-like scale-invariant fluctuations in spontaneous activity. In contrast, starting from 3 months of age, DEX-exposed mice display alterations in circadian and ultradian rhythms suggestive for defective internal clock function. In agreement with the behavioural data, the cyclic expression of clock genes was abolished in the hippocampi of DEX-exposed mice. In conclusion, we found that developmental exposure to DEX induces subtle alterations in the circadian and ultradian rhythms that can predict depression-like behaviour later in life.

Disclosures: S. Spulber: None. **M. Conti:** None. **N. Onishchenko:** None. **S. Ceccatelli:** None. **C. DuPont:** None.

Poster

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Support: Simons Foundation Autism Research Initiative (SFARI 9900060) to P.H.P

UC Davis (Research Investments in Science and Engineering (RISE) to AK McAllister

NARSAD Young Investigators Award to MD Bauman

Title: Brain pathology in a nonhuman primate model of maternal immune activation

Authors: *R. K. WEIR[1], R. FORGHANY[2], A. K. MCALLISTER[2], P. H. PATTERSON[3], C. M. SCHUMANN[4], M. D. BAUMAN[4]
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Abstract: Maternal infection during pregnancy is associated with an increased risk of having a child later develop autism or schizophrenia. Rodent models have played a critical role in establishing causal relationships and identifying mechanisms of altered brain development and behavior in pups prenatally exposed to maternal immune activation (MIA). To bridge the gap between rodent models and human patient populations, we have developed a novel, nonhuman primate model of MIA using a modified form of the viral mimic, polyIC. Pregnant rhesus monkeys (Macaca mulatta) received polyICLC injections at the end of the first trimester to produce a transient innate inflammatory response. A separate control group of pregnant rhesus monkeys received saline injections at these time points. Behavioral data from a large cohort of MIA-treated and control macaque offspring demonstrates that MIA in the rhesus monkey yields offspring with abnormal repetitive behaviors, communication, and social interactions. Despite these intriguing behavioral findings, we do not know the mechanisms by which MIA alters brain development in the nonhuman primate. Here we present our initial assessment of brain pathology in the nonhuman primate MIA model using archived brain tissue from a smaller cohort of MIA-treated and control offspring that were used to establish dosing parameters. Tissue from 8 animals (4 MIA-treated, 4 control) was processed using the Golgi-Cox method that stains approximately 2-5% of neurons in the cortex in an apparent random selection of cells. In brief, tissue blocks were placed in a solution of 1% potassium dichromate, 1% mercuric chloride and 0.8% potassium chromate for 10 weeks. Blocks were then embedded in parlodion, cut into 150μm coronal sections on a sliding microtome and mounted. For each case, 10 pyramidal cells in layer III of the dorso-lateral pre-frontal cortex (BA46) cortex were traced in their entirety using the Neurolucida software package, including all apical, oblique and basal dendrites and their spines. In addition, a more in-depth analysis of the apical dendrite was completed for 30 neurons/case by quantifying diameter, spine density and number of branches in a 30μm section located 100±10μm from the soma. While no significant group differences were found from the 10 neurons traced in their entirety, significant differences were detected in the more in depth analysis of apical dendrite morphology. Compared to controls, the apical dendrites of MIA-treated offspring were smaller in diameter and had a greater number of oblique dendrites. The data provide the first evidence that prenatal exposure to MIA alters dendritic morphology in a nonhuman primate MIA model.

Disclosures: R.K. Weir: None. **R. Forghany:** None. **A.K. McAllister:** None. **P.H. Patterson:** None. **C.M. Schumann:** None. **M.D. Bauman:** None.

Poster

519. Developmental Disorders: Animal Models II

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Topic: C.06. Developmental Disorders

Support: Canadian Institute for Health Research

Ontario Brain Institute

Title: Characterization of mice bearing humanized androgen receptor genes (h/mar) varying in q tract polymorphism length

Authors: *Z. BUCHWALD[1,2], J. ELLEGOOD[3], C. BURTON[2], D. ROBINS[4], A. RAZNAHAN[5], P. ARNOLD[2], J. LERCH[3]
[1]Mouse Imaging Ctr., Toronto, ON, Canada; [2]Hosp. for Sick Children, Toronto, ON, Canada; [3]Mouse Imaging Center, Hosp. for Sick Children, Toronto, ON, Canada; [4]Univ. of Michigan Med. Sch., Ann Harbor, MI; [5]Natl. Inst. of Mental Hlth., Bethesda, MD

Abstract: Background - The androgen receptor (AndR) is a transcription factor that is crucial for sexual development. AndR is linked to several disorders, including ADHD, Autism, and AIS. Mice bearing humanized AndR genes (h/mAr) with varying lengths of a polymorphic N-terminal glutamine (Q) tract have been created. The length of the Q tract is inversely proportional to the AndR activity. **Objectives –** To determine the behavioral and neuroanatomical phenotype of the AndR mouseline, which can lead to a better understanding of the initiation, progression, and treatment of androgen related disorders, as well as sex-related differences. **Methods –** *Mice* – Three separate mouselines have previously been created with varying Q tract lengths: 12Q, 21Q, and 48Q. Two separate cohorts of mice were used. The first cohort was naïve and sacrificed at P60 for MRI scanning; this cohort consisted of male hemizygotes, and female hetero- and homozygotes for each mouseline. The second cohort consisted of only male hemizygotes and were bred to ~P60 and subjected to 4 different behavior tests, sacrificed and set to undergo further MRI scanning to examine brain/behavior correlations *MRI Acquisition* - Scan parameters: T2- weighted, 3D fast spin-echo sequence (TR - 2000 ms, TEs - 14 ms, 6 echoes, 2 averages, FOV - 14 x 14 x 25 mm3, Matrix size = 250 x 250 x 450). This sequence yielded an image with 0.056 mm isotropic voxels (3D pixel). The same sequence was used on both the naïve and behavioral tested cohorts. *Behavioral Testing* - Tests were used to assess grooming (timed observation), anxiety (open field), sociability (3 chamber test), and compulsive behaviors (marble burying). **Results –** *Volume* - Measurements revealed that the hemizygote 12Q and

homozygote 48Q mice display the most volume differences compared to the WT, with the differences in the homozygote 48Q the most severe with 43 of the 62 regions found to be larger in size. The opposite was found in the hemizygote 12Q mice with 15 of the 62 regions smaller. *Behavior* – A dosage-effect with the 12Q and 48Q mice expressing an non- and anxious (respectively) phenotype was expected. This was affirmed in all measures of the open field test, repudiated by the marble burying and grooming measures, and the sociability test showed a trend towards it. **Conclusions** – The difference in Q tract length of the androgen receptor affects the brain differently in males versus females, with increase androgen activity causing decreases in the male brain and decrease androgen activity causing increases in the female brain. Behavioral investigation revealed a possible role for androgen activity both in anxiety-related and social behaviors, but not repetitive behaviors.

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Poster

519. Developmental Disorders: Animal Models II

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Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

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Topic: C.06. Developmental Disorders

Support: NIH R01 MH083807

NIH R01 DA027487

Title: Pharmacological and behavioral assessment of a selectively bred hyperactive mouse line to model ADHD

Authors: *P. MAJDAK, J. R. OSSYRA, J. M. OSSYRA, A. J. COBERT, T. K. BHATTACHARYA, J. S. RHODES
UIUC, Urbana, IL

Abstract: Attention deficit-hyperactivity disorder (ADHD) is a relatively common behavioral disorder characterized by developmentally inappropriate levels of hyperactivity, inattention and impulsivity. It is highly heritable, with broad sense heritability estimates approaching 75%. However, the specific genes and neurobiological risk factors remain a mystery, partly due to the fact that few animal models have been generated for the purpose of exploring the neurobiological

changes underlying a hyperactive phenotype. The goal of this study was to evaluate a line of mice selectively bred for increased locomotor activity in the home cage for face and construct validity as an animal model for ADHD. Following Zombeck et al. (2011) two lines of mice were maintained for over 15 generations. One line, referred to as High-Active, was subjected to within family selection each generation for increased total distance traveled in the home cage on days 5 and 6 of a six day test. Video tracking was used to precisely measure horizontal distance traveled continuously in the home cage over 6 days. The other line, referred to as unselected Control, were randomly bred (avoiding sibling mating) each generation. The purpose of the first experiment was to test face validity of the model by determining whether the High-Active mice also display impulsivity using the operant Go/No-go task as compared to Controls. The purpose of the second experiment was to test the construct validity of the model by determining whether administration of 0.25 mg/kg d-amphetamine during adolescence and adulthood ameliorates hyperactivity and impulsive behavior in the High-Active mice relative to Controls. Current evidence suggest that High-Active mice demonstrate significantly more impulsive behavior relative to Controls in the Go/No-go task, and that amphetamine administration produces a trend in alleviating hyperactivity of the High-Active mice. These preliminary results encourage the continued evaluation of our High-Active line as a potential model for ADHD.

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Poster

519. Developmental Disorders: Animal Models II

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Topic: C.06. Developmental Disorders

Title: Metabolic effects of creatine transporter deficiency in mice

Authors: A. N. KOKENGE[1], K. N. MILES[1], G. J. PYNE-GEITHMAN[2], Z. KHUCHUA[3], J. F. CLARK[4], *M. R. SKELTON[1]
[1]Neurol., [2]Radiology, [3]Mol. Cardio. Bio., Cincinnati Childrens Res. Fndn., CINCINNATI, OH; [4]Univ. of Cincinnati, Cincinnaiti, OH

Abstract: Creatine (Cr) is a guanidino compound required for rapid replenishment of ATP in cells with a high-energy demand. In humans, mutations in the Cr transporter (CrT;SLC6A8) prevent Cr entry into tissue and result in significant intellectual impairment, epilepsy, and

aphasia. CrT knockout ($CrT^{-/y}$) mice, a high-fidelty model of human CrT deficiency in that they lack brain Cr and show severe cognitive impairments, were used to evaluate the effect of a lack of Cr on both whole body and cellular metabolism. In regards to whole body metabolism, $CrT^{-/y}$ mice weigh significantly less than their WT counterparts though both groups of animals consume similar amounts of food. Paradoxically, $CrT^{-/y}$ mice have a two-fold increase in body fat percentage as measured by ECHO-MRI. $CrT^{-/y}$ mice have an increase in energy expenditure compared with WT mice both in a homecage environment and during treadmill running. Consistent with the increases in whole-body metabolic function, $CrT^{-/y}$ mice show changes in cellular metabolism as well. Increased mitochondrial respiration was observed in skeletal muscle fibers from $CrT^{-/y}$ mice. In cardiac fibers, reductions in mitochondrial respiration were observed. In regards to neurometabolism, mitochondrial respiration was increased in hippocampal lysates from $CrT^{-/y}$ mice. Despite the increase in mitochondrial respiration, ATP levels were significantly reduced in the brain. In order to begin to understand the molecular regulation of these metabolic changes within the hippocampus, next generation RNA sequencing (RNA-seq) was performed. RNA-seq analysis revealed that many genes involved with glycogenolysis, including glycogen phosphorylase, were up-regulated in the hippocampus of $CrT^{-/y}$ mice. Gene expression changes were also observed in mitochondrial proteins, antioxidant synthesis proteins, and monocarboxylate transporters. The results of these studies suggest that the loss of Cr has a significant effect on both whole-body and cellular metabolism. Future studies will be conducted to determine if the changes observed are related to cognitive deficits seen in $CrT^{-/y}$ mice and CTD patients.

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Poster

519. Developmental Disorders: Animal Models II

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Topic: C.06. Developmental Disorders

Support: Swedish research council

Title: Glucocorticoid programming of neural development involves a Tet3-dependent regulation of Wnt signaling and DNA methylation

Authors: *R. K. BOSE[1], S. SPULBER[1], P. KILIAN[1], N. HELDRING[1], P. LÖNNERBERG[2], A. JOHNSSON[2], O. HERMANSON[1], S. CECCATELLI[1]
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Abstract: Glucocorticoids (GC) play a critical role during development. However, fetal exposure to excess GC increase the risk of cardiovascular, metabolic, neuroendocrine and psychiatric disorders later in life. We have previously shown that exposure to dexamethasone (Dex, a synthetic GC analogue) decreases proliferation and neuronal differentiation in neural stem cells (NSCs) with concomitant senescence. These effects are heritable and associated with changes in global DNA methylation. Here we performed a genome-wide analysis of differentially methylated DNA regions (DMRs) in embryonic cortex-derived NSCs by immunoprecipitation of methylated DNA enriched by methyl binding domain protein 2, followed by next generation sequencing. We found that Dex induced genome-wide DNA hypomethylation in cortical NSCs *in vitro*, and global hypomethylation in cerebral cortex of 3 day-old mouse pups exposed to Dex *in utero*. We validated selected genes of particular interest by qPCR and methylation-specific PCR in proliferating NSCs.We found that Dex exposure up-regulated Dkk1, a negative regulator of Wnt-signaling, and that the DNA hypomethylation was associated withTet1-3 up-regulation and Dnmt3a down-regulation. These alterations persisted in daughter cells never directly exposed to Dex, and were confirmed *in vivo*.The upregulation of Tet3 was required for the up-regulation on Dkk1 and for the down-regulation of Dnmt3a induced by Dex in NSCs. In summary, we propose that GC elicit strong effects on genome-wide DNA methylation in fetal NSCs including a Tet3-dependent regulation of Wnt signaling.

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Poster

519. Developmental Disorders: Animal Models II

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Topic: C.06. Developmental Disorders

Support: Lurie Center for Autism

Title: Prenatal viral immune challenge to mimic autism-like behavioral and electrophysiological phenotypes in mice

Authors: S. M. LANDINO[1], Y. LI[1], *B. C. FINGER[1], V. Y. BOLSHAKOV[1], C. J. MCDOUGLE[2], W. A. CARLEZON, Jr[1]
[1]Dept. of Psychiatry, Harvard Med. School, McLean Hosp., Belmont, MA; [2]Lurie Ctr. for Autism, Massachusetts Gen. Hosp., Lexington, MA

Abstract: Clinical studies have demonstrated associations between maternal infection and inflammation during pregnancy and increased risk of autism. Furthermore, preclinical investigations of maternal immune activation and the development of autism-like phenotypes support the hypothesis of immunological involvement in the etiology of at least some forms of autism. Alterations in neural connectivity and abnormal functioning of numerous brain regions, such as the medial prefrontal cortex (mPFC), have been reported in autism spectrum disorders (ASDs). Further examination of the biological consequences of *in utero* infection may elucidate the specific role of the immune system in the onset of ASDs. To investigate whether *in utero* infection alters function of the prelimibic (PL) division of the mPFC in C57BL/6J mice, we administered the viral mimic polyinosinic:polycytidylic acid (Poly I:C) (20 mg/kg) or vehicle (phosphate buffered saline) to female C57BL/6J mice on day 12.5 of pregnancy. The offspring were tested for core behavioral features of autism, including deficits in communication (measured by analysis of ultrasonic vocalizations in early childhood and in adulthood), diminished social behavior (measured in the social interaction test), and increases in repetitive or stereotyped behaviors (measured in open field and marble burying tests). Following these behavioral tests, we conducted electrophysiology studies in brain slices of representative mice from both treatment conditions to examine the consequences of maternal immune activation on membrane excitability and glutamatergic synaptic transmission in the PL mPFC. Although membrane excitability of PL neurons remained unchanged, Poly I:C treated offspring had reductions in the amplitude of NMDA receptor-mediated synaptic responses at inputs to PL neurons without affecting AMPA receptor-mediated synaptic responses. Examining the behavioral consequences of *in utero* inflammatory responses together with cellular and molecular consequences may provide evidence for an etiologic subtype of neurodevelopmental disorders triggered by insults (e.g. exposure to viruses or bacteria, stress, toxins) that cause immune activation. Our results provide evidence that early immune challenge can trigger behavioral changes that share features with the core signs of autism and corresponding changes in brain glutamate function. Establishing cause-effect relationships among these findings may facilitate the development of interventions that target the immune system to more effectively treat, or even prevent, certain types of ASDs.

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Poster

519. Developmental Disorders: Animal Models II

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Title: Complex neurological phenotype in mutant mice lacking Tsc2 in excitatory neurons of the developing forebrain

Authors: *V. DAL POZZO, B. CROWELL, G. LEE, G. D'ARCANGELO
Rutgers, the State Univ. of New Jersey, Piscataway, NJ

Abstract: Tuberous Sclerosis Complex (TSC) is a genetic disease resulting from mutations in either the *TSC1* or the *TSC2* gene. The disease is characterized by a high frequency of epilepsy, intellectual disability and autism, and by the presence of brain malformations (tubers), frequent tumors and lesions in other organs. The TSC gene products form a complex that inhibits the growth-promoting complex mTORC1. This cellular mechanism may account for tumor susceptibility and growth abnormalities, however, it is not clear whether it underlies cognitive dysfunction in TSC patients. To better understand the role of TSC2 in forebrain development we generated a conditional mutant mouse line (NEX-Tsc2), which lacks *Tsc2* expression specifically in postmitotic excitatory neurons of the developing forebrain. Homozygous mutant mice exhibited neuroanatomical abnormalities in the cerebral cortex and hippocampus, became runt and mostly die during the second-third postnatal week. Mutants also displayed expected abnormalities in Akt and mTOR signaling, but no detectable alterations in glutamate receptor expression. Surprisingly, the mutants exhibited striking cellular abnormalities in non-neuronal populations of the brain, which were not directly affected by the gene knock out strategy. These findings provide new insights into the role of Tsc2 in the development of excitatory neurons, and in mechanisms controlling neuron-glia communication during brain development. This work is supported by Exploration-Hypothesis Development Award #TS110033 from the Department of Defense - CDMRP - TSCRP (G.D.).

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Poster

519. Developmental Disorders: Animal Models II

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Title: Pharmacological reversal of autism spectrum disorder social deficits in the SERT Ala56 mouse via p38α MAPK blockade

Authors: *M. J. ROBSON[1], D. M. WATTERSON[3], J. VEENSTRA-VANDERWEELE[2], A. M. POCH[1], R. D. BLAKELY[2]
[1]Pharmacol., [2]Pharmacol. and Psychiatry, Vanderbilt Univ. Sch. of Med., Nashville, TN; [3]Pharmacol., Northwestern Univ. Sch. of Med., Chicago, IL

Abstract: The reported incidence of autism spectrum disorders (ASD) in the United States pediatric population is currently 1 in 68 children. Exacerbating this problem is a lack of pharmacotherapies aimed at ameliorating aberrant behaviors relevant to ASD. One of the oldest biomarkers of ASD is peripheral hyperserotonemia, an effect linked to serotonin (5-HT) transporter (SERT) activity on platelets. Central 5-HT signaling is tightly regulated by SERT and changes in SERT expression and/or activity are linked to multiple neuropsychiatric disorders, including ASD. Genetic gain-of-function (GOF) variants in SERT have been identified in ASD, specifically in the context of rigid-compulsive behaviors and sensory aversion (Sutcliffe et al. 2005). Mice expressing the most prevalent of these GOF variants, SERT Ala56, display behavioral and biochemical phenotypes characteristic of ASD, including hyperserotonemia, social and communication deficits, and repetitive behaviors (Veenstra-Vanderweele et al. 2012). Additionally, SERT Ala56 animals display a p38 MAPK-dependent hyperphosphorylation of SERT within the CNS (Veenstra-Vanderweele et al. 2012). Pathways involving p38α MAPK signaling are important for responses to stressors and immune system activation and may represent a convergence point between environmental and genetic risk factors. Therefore, we asked whether pharmacological inhibition of p38α MAPK signaling attenuates ASD-like behaviors in the SERT Ala56 model. *In vitro* studies using CHO cells revealed that a novel,

selective p38α MAPK inhibitor, MW108 blocks anisomycin-induced increases in p38 MAPK phosphorylation. Furthermore, MW108 treatment was found to mitigate p38α MAPK-induced increases in SERT uptake in human SERT stably transfected SK-N-MC cells, providing evidence that human SERT is regulated in a similar manner by p38α MAPK as mouse SERT *in vitro*. Consistent with this idea, MW090, a p38α MAPK specific inhibitor with 10-fold lower potency than MW108, required a 10-fold higher concentration to attenuate p38α MAPK-induced increases in SERT activity in SK-N-MC cells. Importantly, *in vivo* behavioral studies revealed that chronic i.p. injections of MW108, as well as the less selective p38α/β MAPK inhibitor SB203580, reverse social behavior deficits in male SERT Ala56 mice in the Tube Test for Social Dominance. To our knowledge, our studies are the first to demonstrate pharmacologic rescue of ASD-like phenotypes by modulating p38α MAPK signaling, suggesting an ongoing versus developmental contribution of the pathway to social behavior deficits and the potential utility of p38α MAPK-based treatments for adult ASD subjects.

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Poster

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CONACYT Grant 129303 and 138663

Title: Electroencephalographic activity and social interaction in adult rats a model of autism spectrum disorder

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Abstract: Autism is a neurodevelopmental condition diagnosed by impaired social interaction, abnormal communication and stereotyped behaviors. In rodents *in utero* exposure to valproic acid (VPA) also causes neurodevelopmental abnormalities and is an important model of autism. In the present study, we aimed to investigate the effect of prenatal administration of VPA (500 mg/kg) at E12.5 on the social interaction behavior linked with electroencephalographic activity (EEG) in hippocampus (HIPP) and prefrontal cortex (PFC). At PD90, control (n 8) and *in utero* exposure to VPA rats (n 8), were stereotaxically implanted in both HIPP and PFC. Social interaction test was performed in pairs simultaneously with EEG recording, experiment was filmed to analyze the social behaviors. EEG activity was acquired on-line (300 Hz sampling rate) using an analog/digital conversion system (ADQCH4) designed in our laboratory. Frequency domain analysis of each chanel recording was performed off-line on the signals. Number, cumulative interaction time and latency of interaction behaviors were analyzed. Power numerical values were normalized against the maximum power of each band: 1-4 Hz, 5-12 Hz and 13-30 Hz and were averaged in 10 minutes epochs, We used the standard fast Fourier transform (FFT) to analyze long periods and the short FFT for short periods. The VPA rats showed an increase in the emission of social behaviors and an increased in latency to explore compared to the control group. Intra-group difference in latency to inactivity was showed in VPA group. The EEG power spectra of left HIPP decreased in the VPA animals in all bands. VPA rats showed an increase in the left PFC power in the 1-4 Hz band. Our study allows link specific brain activity with behavioral manifestations of autism and its changes during development, as well as, being consistent with the results obtained in the study of brain morphology and microcircuitry.

Disclosures: A. Valdés-Cruz: None. **M.E. Bringas:** None. **G. Flores:** None. **J.V. Negrete-Díaz:** None. **V.M. Magdaleno-Madrigal:** None. **D. Martínez-Vargas:** None. **O. Simón-García:** None. **G.L. Licea-Haquet:** None. **M. Atzori:** None.

Poster

519. Developmental Disorders: Animal Models II

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 519.19/Y20

Topic: C.06. Developmental Disorders

Title: Cellular and axonal constituents of neocortical molecular layer heterotopia

Authors: *R. L. RAMOS[1], N. SIU[1], W. J. BRUNKEN[2], K. T. YEE[3], L. A. GABEL[4], S. E. VAN DINE[1], B. J. HOPLIGHT[5]
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Abstract: Human neocortical molecular layer heterotopia consist of aggregations of hundreds of neurons and glia in the molecular layer (layer I) and are indicative of neuronal migration defect. Despite having been associated with dyslexia, epilepsy, cobblestone lissencephaly, polymicrogyria, and Fukuyama muscular dystrophy, a complete understanding of the cellular and axonal constituents of molecular layer heterotopia is lacking. Using C57BL/6J mice as a model, we identify diverse excitatory and inhibitory/GABAergic neurons as well as glia in heterotopia based on molecular profiles. Using immunocytochemistry, we identify diverse afferents in heterotopia from subcortical neuromodulatory centers. Finally, we document intracortical projections to/from heterotopia including callosal projections. These data are relevant toward understanding how heterotopia affect brain function in diverse neurodevelopmental disorders.

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Poster

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Topic: C.06. Developmental Disorders

Title: Mode of delivery at birth and behavioural outcomes: Rewiring of the brain-gut-microbiome axis?

Authors: *Y. E. BORRE[1], L. HECKE MORAIS[1], A. V. GOLUBEVA[1], E. PATTERSON[3], F. CRISPIE[3], R. D. MOLONEY[1], K. A. SCOTT[1], R. STILLING[1], N. P. HYLAND[1], G. CLARKE[2], C. STANTON[3], P. D. COTTER[3], T. G. DINAN[2,1], J. F. CRYAN[1]
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Abstract: Mode of delivery has been shown to impact the development of the microbiota and immune system in newborns with negative health associations being reported. Specifically, children delivered by Caesarean section (C-section) are more likely to suffer from immune and metabolic disorders including allergies, obesity and diabetes, which have been linked to the disrupted microbiota maturation. We and others, have shown that early-life perturbations of the developing gut microbiota can affect neurodevelopment and potentially lead to adverse mental health outcomes later in life. However, the relationship between the mode of delivery and behavioural function across lifespan remains to be fully elucidated. To investigate this question, we applied an inter-disciplinary approach using a mouse model of C-section, and consisting of several behavioural assays combined with microbiota, metabolic, immune and transcriptome analyses. Our studies establish that male C-section offspring exhibit stereotypical, anxiety- and-depression- like symptoms, social deficits, disrupted hypothalamic-pituitary-adrenal axis responsivity and perturbed intestinal permeability. Using high throughput DNA sequencing, we monitored the gut microbial composition and diversity, and assessed metabolic and immune consequences of the mode of delivery in the offspring. In addition, we examined the effects of C-section on gene expression within brain limbic structures that play a key role in emotion, stress response and cognition. Furthermore, we evaluated crucial changes in the systemic and mucosal immunity, which may play a role in rewiring the brain-gut-microbiota axis following a C-section. Taken together, these findings support a microbiome-gut-brain connection in a mouse model of C-section and identify potential novel mechanisms by which mode of delivery may contribute to rewiring the developing brain, and susceptibility to neurodevelopmental disorders.

Disclosures: Y.E. Borre: None. **L. Hecke Morais:** None. **A.V. Golubeva:** None. **E. Patterson:** None. **F. Crispie:** None. **R.D. Moloney:** None. **K.A. Scott:** None. **R. Stilling:** None. **N.P. Hyland:** None. **G. Clarke:** None. **C. Stanton:** None. **P.D. Cotter:** None. **T.G. Dinan:** None. **J.F. Cryan:** None.

Poster

519. Developmental Disorders: Animal Models II

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Program#/Poster#: 519.21/Y22

Topic: C.06. Developmental Disorders

Title: Npas4 contributes to stress-induced changes in the prefrontal cortex of mice

Authors: *L. COUTELLIER[1], R. SHEPARD[2]
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Abstract: The prefrontal cortex (PFC) is a brain structure strongly implicated in psychiatric conditions such as schizophrenia and depression. Many studies report that stress during childhood and adolescence induces changes in the PFC that could be responsible for the onset of the symptoms of these disorders. However, the molecular mechanisms underlying postnatal PFC development and its vulnerability to stressful events remain unknown. Here we suggest that the transcription factor Npas4 could play a significant role in PFC vulnerability to stress in early life. We first observed significant age-dependent changes in the level of Npas4 mRNA in the PFC of mice, with a significant increase from 3 weeks of age to adolescence and then to adulthood. Then, to determine whether low level of Npas4 in the brain during adolescence affects sensitivity to stress and adult PFC-dependent cognitive functions we used Npas4 heterozygotes (HET) and wild-type (WT) mice. WT and HET mice were exposed to chronic mild stress during adolescence (from 4 to 6 weeks old) or kept in standard rearing conditions (control). PFC-dependent memory was assessed in adulthood (10 weeks old) using the spontaneous alternation test and object/context mismatch test. We observed that WT mice were not affected by the chronic mild stress situation and have normal cognitive function in adulthood. However, adult Npas4 HET mice exposed to chronic mild stress during adolescence display severe PFC-dependent cognitive deficits, while they have only minor impairments when raised in standard conditions. Altogether, these findings support the importance of Npas4 for the development of the PFC during adolescence, and that disturbance of Npas4 expression, associated with stress can affect cognitive functions that rely on the PFC.

Disclosures: L. Coutellier: None. **R. Shepard:** None.

Poster

519. Developmental Disorders: Animal Models II

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Topic: C.06. Developmental Disorders

Support: NIH R01DC009607

Title: Hypoxic-ischemic injury alters auditory cortex function in neonatal rats

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Abstract: Subplate neurons (SPNs) are required for the formation and functional refinement of thalamocortical connections (Kanold & Luhmann 2010; Tolner, Sheikh et al. 2012). SPNs are selectively vulnerable to neonatal hypoxic-ischemic (HI) insults (McQuillen et al. 2003) and such insults impair critical period plasticity, at least in the visual cortex (Failor 2010). HI insults in human can give rise to auditory impairments, as well as a multitude of other developmental language disorders. Thus we investigated the consequences of developmental HI on auditory cortex function in rodents. HI was performed on rat pups on postnatal day (P) 1-2 and SPN loss was assessed at P5-P8 by immunohistochemistry to complexin 3, a SPN specific marker. We performed extracellular single-unit recordings in the auditory cortex of anesthetized control and HI animals between P20-33. The pure tone evoked response properties of auditory cortex neurons were altered consequent to HI suggesting either functional changes to cortical neurons or altered connectivity between neurons. To distinguish between these possibilities and to get a more in-depth understanding of the *in vivo* results on a cellular level, whole-cell patch clamp recordings were performed from layer 4 neurons between P18-23. We find that intrinsic properties of layer 4 cells are not significantly altered. Thus, the changes observed in-vivo may be due to differences in synaptic connectivity. Together our observations imply that SPNs are necessary for normal functional development of the auditory cortex.

Disclosures: **A. Sheikh:** None. **J. Liu:** None. **A. Isaiah:** None. **P. Kanold:** None.

Poster

519. Developmental Disorders: Animal Models II

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Topic: C.06. Developmental Disorders

Support: European Research Council

Fundação para a Ciência e a Tecnologia

Fundação Bial

Title: Effects of Foxp2 disruption in selected brain regions and in adulthood

Authors: *C. A. FRENCH[1], A. GOMEZ-MARIN[1], M. CORREIA[1], C. FELICIANO[1], V. B. PAIXÃO[1], X. JIN[2], S. E. FISHER[3], R. M. COSTA[1]
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Abstract: Disruptions of the FOXP2 gene cause a rare speech and language disorder. A core feature of this disorder is difficulty in producing the sequences of orofacial motor movements necessary for fluent speech, although other expressive and receptive language problems are also present. Imaging studies have shown structural abnormalities in the caudate nucleus and ventral cerebellum, as well as altered patterns of activation during language-based tasks. FOXP2 encodes a transcription factor which is expressed in the cortico-striatal and cortico-cerebellar circuits required for sensorimotor integration and motor-skill learning. This neural expression pattern, and the FOXP2 protein sequence, are highly conserved in other vertebrate species such as mice and songbirds. Mice carrying a heterozygous point mutation in Foxp2 (the KE-family mutation) have motor-skill learning deficits and lack striatal long-term depression. We also found aberrant striatal activity *in vivo* during the learning of a motor task. Juvenile zebra finches show increased FoxP2 expression in AreaX of the striatum during the song learning-period, and FoxP2 knockdown in AreaX results in inaccurate and incomplete imitation of the tutor bird's song. FoxP2 knockdown in AreaX of mature birds abolishes the mediation of song by social context and suggests that FoxP2 function is important in adulthood as well as during development. We previously generated a conditional Foxp2 line and have used it to disrupt Foxp2 in selected brain regions (cortex, striatum or cerebellar Purkinje cells), and at a defined time point (adulthood). This genetic approach is being combined with an operant motor-sequence learning task where mice must complete a sequence of 8 lever presses to obtain a food reward. After 12 days a time constraint is added and the sequence must be performed at increasingly high speeds. Both cerebellar and striatal Foxp2 mutants show a reduced rate of lever pressing during training compared to controls. However, analyses of behavioural microstructure revealed that whilst pressing of all speeds is altered in cerebellar mutants, in striatal mutants it is primarily rapid pressing that is affected. These data indicate that Foxp2 function in distinct subcircuits differentially affects motor-skill learning. We disrupted Foxp2 globally in adult mice using a tamoxifen-inducible Cre. Preliminary data indicate that homozygous adult knockouts are viable, unlike other Foxp2 homozygous mutants which die at 3-4 weeks of age. Work is ongoing to investigate motor-sequence learning and other aspects of behaviour in these animals.

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Poster

519. Developmental Disorders: Animal Models II

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Topic: C.06. Developmental Disorders

Support: Canadian Institutes of Health Research (CIHR)

Fonds de recherche du Québec – Santé (FRQ-S)

Foundation of Stars

Title: Group B streptococcus infection during gestation leads to gender specific neurodevelopmental and behavioural impairments

Authors: *M.-J. ALLARD[1], J. BERGERON[1], L.-C. FORTIER[1], C. POYART[2], G. SÉBIRE[1,3]
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Abstract: Background: A new preclinical animal (rat) model of gestational inflammation showed that exposure to inactivated Group B streptococcus (GBS) during gestation leads to perinatal brain injuries resulting in gender specific neurobehavioural impairments. To increase the clinical relevance, we refined this model by replacing inactivated by live GBS. The hypothesis is that GBS-induced gestational inflammation has a deleterious neurodevelopmental impact on offspring through a maternofoetal inflammatory response. **Methods:** Pregnant rats were intra-peritoneally injected at gestational day 19 with serotype 1a GBS (10^8 CFU) or saline. The maternal and foetal blood and placentas will be studied by ELISA and immunohistochemistry to characterize inflammatory responses. Brains were collected at postnatal day 40 (P40) for histological studies. Behavioural tests were performed from P7 to P40 to assess maternal attachment and neonatal communication (nest-seeking task, ultrasonic vocalizations), exploratory abilities (open field), social interactions and anxiety (elevated-plus-maze). **Results:** A decrease in body weight gain of dams exposed to GBS was noticed. An intra-uterine growth retardation was present in male, but not in female, pups at P1, and persisted until P4. An increased mortality was observed in GBS-exposed males as reflected by an unbalanced sex-ratio in GBS-exposed litters. GBS-exposed placentas displayed an increase of polymorphonuclear cells (PMN) compared to control; this PMN infiltration was more prominent in male than female. An increase of lateral ventricle size and a decrease of the corpus callosum thickness were detected in GBS-exposed males, but not females, at P40. An increase of myelin basic protein (MBP) detection was observed in the external capsule of GBS-exposed rats at P40. GBS-exposed males, but not females, at P7 showed a decrease of ultrasonic vocalisations. GBS-exposed males at P20 and GBS-exposed females at P25 showed hyperactivity, disorganised exploratory behaviour and decreased level of anxiety, compared to control. **Conclusion:** GBS-

induced inflammation resulted in gender dichotomic neurodevelopmental and behavioural impairments. Communication and social interactions will be further characterized by ongoing studies. Such neurobehavioural characterization correlated to inflammatory mechanistic studies will help to target future neuroprotective strategies.

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Poster

519. Developmental Disorders: Animal Models II

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Topic: C.06. Developmental Disorders

Support: NIH Grant NS063956

Title: Systemic endotoxin exposure exacerbates longterm neurobehavioral deficits after neonatal cerebral ischemia

Authors: M. SHABANINIA[1], M. PORAMBO[1], J. MARX[1], M. BUELOW[1], J. CRAWFORD[2], M. PLETNIKOV[2], M. V. JOHNSTON[1], *A. FATEMI[3]
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Abstract: Background: We recently reported an ischemic mouse model of Neonatal White Matter Injury (NWMI) induced by unilateral neonatal carotid artery ligation. Several studies have suggested that neuroinflamation has a critical role in the development of NWMI. The aim of this study was to investigate the combinatorial effect of neonatal ischemia with systemic inflammation on longterm neurobehavioral outcomes. Methods: At postnatal days P4/P5, 54 CD1 mouse pups were stratified in 4 groups, they received either an intraperitoneal injection of lipopolysaccharide (LPS, 1 mg/kg) (LPS group, N=18), or underwent neonatal unilateral carotid artery ligation and intraperitoneal injection of PBS (ISCH group, N=18), or unilateral carotid artery ligation and intraperitoneal injection of LPS 1 mg/kg (LPS+ISCH group, N=18), or were injected with PBS only (control group). The effects of these interventions were evaluated by Rotarod, Y-maze, prepulse inhibition (PPI) and open field testing at 8 weeks. Results: On rotarod testing, LPS group preformed significantly worse than controls, while ISCH group did not, however, the LPS+ISCH group performed dramatically worse compared to controls. ISCH and ISCH+LPS groups spent significantly less time in the novel arm of Y maze compared to

controls, while the LPS group did not. Total activity in open field testing was significantly lower in all injury groups and comparable within these three groups. On PPI testing, LPS group did not show a significant difference while ISCH group showed a significant decrease in startle amplitude. This decrease was most pronounced in the LPS+ISCH group. Conclusion: Mice undergoing neonatal ischemia combined with systemic inflammation express a pronounced phenotype with higher deterioration of motor learning skills, spatial memory, and sensorimotor gating. This animal model can facilitate the study of disease pathogenesis and conduction of trials to identify candidate therapeutic for NWMI.

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Poster

519. Developmental Disorders: Animal Models II

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Topic: C.06. Developmental Disorders

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Title: An N-terminal domain essential for PKG- and p38 MAPK-mediated regulation of the serotonin transporter revealed by studies of inbred mouse strain variation in transporter structure

Authors: ***M. A. QUINLAN**[1], R. YE[1,2], Z. JIN[1], R. D. BLAKELY[1,2,3]
[1]Pharmacol., [2]Silvio O. Conte Ctr. for Neurosci. Res., [3]Psychiatry, Vanderbilt, Nashville, TN

Abstract: Disruption of serotonin (5-HT) signaling is implicated in multiple neuropsychiatric and neurodevelopmental disorders, including anxiety and major depressive disorder, obsessive-compulsive disorder, and autism spectrum disorder (ASD). Regulation of 5-HT levels at the synaptic cleft is tightly controlled by high-affinity 5-HT uptake through the presynaptic, antidepressant-sensitive 5-HT transporter (*SLC6A4*, SERT). Studies of human SERT (hSERT) have shown that activation of PKG- and p38-MAPK signaling pathways lead to increases in SERT activity, changes made constitutive by multiple SERT coding variants associated from

individuals with ASD, including SERT Ala56. Our previous studies revealed various pharmacological, neurochemical, and neurobehavioral differences between mouse strains carrying the ER (DBA/2J = E39, R152) and GK (C57BL/6J = G39, K152) SERT. Additionally, site-directed mutagenesis studies of mouse SERT (mSERT) indicated that E39 in the transporter N-terminus dictates functional variation between the two mouse inbred strains. In the present study, we report that these mSERT haplotypes dictate PKG- and p38 MAPK-dependent regulation of SERT activity. Specifically, PKG- and p38 MAPK-dependent regulation is abolished in the ER mSERT, despite the fact that hSERT also encodes E39 and R152 at their corresponding positions. In an attempt to explain the latter discrepancy, we employed a species-scanning-mutagenesis strategy, converting the 15 residues of the N terminus of mSERT to their corresponding identifies in hSERT while maintaining the ER haplotype. *In vitro* 5-HT uptake analysis of the ER mSERT mutants reveal that multiple residues in the hSERT N-terminus overcome the inhibitory influence of E39 to permit regulation via PKG- and p38-MAPK-mediated pathways. Interestingly, this structural domain is localized around SERT Ala56, in which PKG- and p38-MAPK-dependent regulation of SERT is constitutive, consistent with the region as important in PKG/p38MAPK regulation of the transporter. Ongoing studies seek to build on these findings to elucidate sequence-specific interactions of this region with regulatory proteins.

Disclosures: **M.A. Quinlan:** None. **R. Ye:** None. **Z. Jin:** None. **R.D. Blakely:** None.

Poster

519. Developmental Disorders: Animal Models II

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Topic: C.06. Developmental Disorders

Support: Wittenberg Psychology Department

Title: Effects of oxytocin on serotonin 1B agonist-induced social interaction deficits in mice

Authors: S. K. LAWSON, *N. S. WOEHRLE
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Abstract: Autism spectrum disorder (ASD) is a group of developmental disabilities characterized by communication deficits, repetitive, compulsive behaviors with narrowed interests, and social difficulties. Despite the prevalence of ASD, few highly-valid animal models

and no pharmacological treatments for the social deficits in ASD currently exist. The serotonin system has been highly implicated in the pathophysiology of ASD, and, in particular, the serotonin 1B receptor may be involved. Drugs that activate the serotonin 1B receptor, such as FDA-approved treatments for migraine, have been shown to exacerbate ASD symptoms. Moreover, these same drugs induce ASD-like perseverative behavior in mice. Thus, serotonin 1B agonist-induced behavior may represent a novel pharmacological animal model of aspects of ASD. However, the effects of serotonin 1B receptor activation on social interaction behavior in mice have not been assessed. Here, we examine the effects of the serotonin 1B agonist RU24969 on social behavior in the three-chambered social test and on rearing behavior in the open field. ASD is associated with non-selective attention deficits, and rearing behavior is used as an index of non-selective attention in mice. In addition, we examine the effects of a putative treatment for ASD, oxytocin (OT), on serotonin 1B agonist-induced behavior. OT is a neuromodulator that has been linked to numerous pro-social behaviors including emotional bonding, affiliation, and social attachment. Moreover, OT has been proposed as a novel pharmacological treatment for the social deficits in ASD. Our results show that serotonin 1B agonist treatment induces deficits in sociability and social novelty, decreases rearing behavior, and increases locomotion and perseveration in mice. Moreover, we show that OT reverses serotonin 1B agonist-induced deficits in social novelty, but not sociability.

Disclosures: S.K. Lawson: None. **N.S. Woehrle:** None.

Poster

519. Developmental Disorders: Animal Models II

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Topic: C.06. Developmental Disorders

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T32 MH076690 (MM)

Title: Postnatal role for histone deacetylase 1 and 2 in behavioral and neuronal homeostasis

Authors: *M. MAHGOUB, M. ADACHI, L. M. MONTEGGIA
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Abstract: Histone deacetylases (HDACs) compress the chromatin structure, restricting access of transcription factors to the DNA and consequentially repressing gene expression. Previous studies using pharmacological agents have demonstrated that inhibition of Class I HDACs results in enhancements in learning and memory with recent attention focused on HDAC1 and HDAC2. Deletion of both HDAC1 and HDAC2 selectively in the brain during embryogenesis results in major structural abnormalities in cortical, hippocampal and cerebellar development with death at postnatal day 7, suggesting HDAC1 and HDAC2 together play a critical and redundant role during neuronal proliferation. More recent work has identified distinct roles for HDAC1 and HDAC2 in complex behaviors. Brain specific conditional HDAC2 knockout mice have enhanced learning and memory, as well as enhanced synaptic plasticity, with no effect on these measures observed in the conditional HDAC1 knockout mice. These data have generated interest in the development of HDAC2 selective inhibitors for enhancing learning and memory and as a possible therapeutic approach for neurodegenerative disorders such as Alzheimer's disease. However, given the high degree of sequence identity (85%) between HDAC1 and HDAC2, it is unknown whether the deletion of both of these genes in postnatal brain would be detrimental similar to the embryonic deletion or whether the late stage deletion would show beneficial effects on learning and memory. We generated conditional brain-specific HDAC1 and 2 double knockout (DKO) mice and found they die at approximately 9 weeks of age, although survival is not impacted in the single knockouts. The DKO mice have behavioral abnormalities including hypoactivity and heightened anxiety shortly after the deletion of HDAC1 and 2. Hematoxylin and Eosin (H&E) staining reveals abnormal neuronal morphology as represented by disruptions in cortical lamination and cell layering in the hippocampus. Additionally, the DKO mice have a significantly smaller brain mass that appears to be due to apoptosis in the brain. Further characterization of the DKO mice is on going in the laboratory and data will be discussed. Our results so far show redundant functions of HDAC1 and HDAC2 in postmortem brain and highlight the need for caution in the development of pharmacological inhibitors of HDAC1 and HDAC2 as therapeutic tools in treating symptoms of neurodegenerative diseases.

Disclosures: **M. Mahgoub:** None. **M. Adachi:** None. **L.M. Monteggia:** None.

Poster

519. Developmental Disorders: Animal Models II

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Topic: C.06. Developmental Disorders

Support: NJ Gov Council on Autism

Title: Engrailed 2 mutant mice exhibit abnormal hippocampal inhibitory circuits at cellular and synaptic levels

Authors: *M. GENESTINE SCHMITT[1], G. VAIL[2], S. HU[1], Z. PANG[3], M. PLUMMER[2], J. H. MILLONIG[4], E. DICICCO-BLOOM[1]
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Abstract: The ENGRAILED 2 (EN2) gene, a homeobox transcription factor that is critical to normal hindbrain formation, is genetically associated with Autism Spectrum Disorder (ASD). En2 knockout (KO) mice exhibit behavioral and structural phenotypes relevant to neurodevelopmental disorders. Behavioral abnormalities in the KO include deficits in social interactions, fear conditioning, prepulse inhibition and spatial learning and memory (Brielmaier et al., 2012). In addition, we found reduced norepinephrine (NE) fiber innervation and neurotransmitter in the KO hippocampus. Given the importance of NE and the hippocampus in these behaviors, we hypothesized that the KO hippocampus would exhibit abnormal neuronal circuitry and function including GABA interneurons, synaptic activity and neurogenesis. One target population of NE in the hippocampus is GABA inhibitory neurons. Preliminary data indicate that GAD65+ interneuron number was unaffected in hippocampal subregions. However, GABA interneuron differentiation was apparently altered since there were 25% less parvalbumin+ cells, which might lead to functional changes. Indeed, acute recording from hippocampal slices in the KO revealed increased baseline synaptic transmission (+65%) associated with reduced paired pulse facilitation (-20%). Moreover, we previously defined dysregulation of hippocampal neurogenesis in the KO. We now find that newly generated Dcx+ neurons migrate 25% further from the KO subgranular zone than wild type, and some localize ectopically in the molecular layer. This enhanced outward migration is associated with decreased expression of the chemokine CXCR4 receptor that might underlie the phenotype. A similar migration abnormality occurs following kainate-induced seizures, a protocol to which the KO are especially sensitive (Tripathi et al., 2009). These studies identify reduced GABA interneurons, altered synaptic transmission and ectopic new born granule neurons in the KO hippocampus. These changes in the excitatory/inhibitory balance may be responsible for previously reported increased susceptibility of the KO to seizures, which are well-described comorbid symptoms in ASD (up to 35%) and other disorders. Since there are NE deficits in the KO forebrain, we are determining whether increasing NE pharmacologically can reverse reduced hippocampal inhibition. Interestingly, we recently found that NE re-uptake inhibitor desipramine reversed the En2 KO deficits in social and hippocampal dependent tasks (Brielmaier et al., 2014). Thus our studies of the En2 mutant identify neurobiological abnormalities that may be relevant to human neurodevelopmental disorders such as ASD.

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Poster

520. Animal Models of Epilepsy II

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Title: Characterization of the seizure phenotypes for the D1866Y Scn1a mouse model of genetic epilepsy with febrile seizures plus (GEFS+)

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Abstract: Mutations in the *SCN1A* gene, which encodes the voltage-gated sodium channel (VGSC) $Na_V1.1$, have been linked to a variety of epilepsy conditions including genetic epilepsy with febrile seizures plus (GEFS+) and Dravet Syndrome. Within a GEFS+ family, affected members can display a variety of epilepsy phenotypes that differ in severity and age of onset. The D1866Y (DY) *SCN1A* GEFS+ mutation was first identified in an Italian family with four affected members displaying different epilepsy phenotypes. The DY mutation is located in the C-terminus of the channel and is predicted to alter channel function and possibly affect interaction with auxiliary β subunits. To gain a better understanding of the effect of this mutation on seizure susceptibility, mice containing the human GEFS+ DY mutation were generated and evaluated for their susceptibility to seizures induced by the proconvulsant flurothyl, the 6 Hz electroconvulsive paradigm, and hyperthermia. Heterozygous mutants exhibited increased seizure susceptibility to all three seizure-induction paradigms when compared to their wild type (WT) littermates. We also determined the effect of the DY mutation on mouse weight and survival by routine observations and weighing starting at postnatal day 5 (P5). All three genotypes gained weight at a similar rate until P23, after which homozygous mutants began to lose weight and exhibited

premature lethality. Heterozygous mutants and WT littermates exhibited normal lifespans and comparable body weights. Our findings demonstrate that the DY mutation alters the function of the $Na_v1.1$ channel, leading to increased seizure susceptibility in the heterozygous mutants and reduced lifespan of the homozygous mutants.

Disclosures: **N. Browder:** None. **A. Escayg:** None. **S. Dutton:** None.

Poster

520. Animal Models of Epilepsy II

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Topic: C.07. Epilepsy

Title: Behavioral characterization of a zebrafish model of febrile seizures

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Abstract: Febrile seizures (FS) are the most common type of seizures in humans, affecting up to 5% of children before the age of 5. Current evidence suggests a link between prolonged FS and an increased risk of developing temporal lobe epilepsy, although the magnitude of the risk is not accurately known. Animal models are a vital in the search for mechanisms underlying FS and their epileptogenic effects. The zebrafish has become an important model species for epilepsy (1) as they show similar sensitivity to a convulsants and anticonvulsants as mammalian models. A recent study by Hunt et al. (2) found that transient exposure of larval zebrafish to hyperthermia induces acute electrographic seizures. We extended the zebrafish FS model by identifying the optimum conditions for behavioral febrile seizures and characterizing the ictal behavioral phenotype in detail. **Method** Larval zebrafish were placed in embryo medium in well plates and exposed to increasing temperatures in an incubator bath. We examined the effects of larval age and rate of temperature increase. Trials were recorded and assessed for the percentage of larval zebrafish reaching each seizure stage, latency to seizure stages, temperature at seizure onset, duration of seizure bouts, and average number of seizure bouts. For detailed analysis of seizure phenotype, a high-speed camera was used to capture tail motion. Zebrafish were immobilized in agarose with the tail free and four types of behavior were compared: spontaneous swim behavior and escape response induced by an abrupt touch in controls, seizures induced by PTZ, and

seizures induced by hyperthermia. Custom software in MATLAB was used for analysis of tail movement with parameterization of deflection angle, speed, acceleration, and angular velocity. **Results** Macroscopic observations of zebrafish FS resembled the three-stage seizure behavior established in the literature. Zebrafish at 5 days post fertilization exhibited FS when exposed to a temperature increase of 2 °/min. The proportion of fish reaching Stage 2 and the duration of Stage 2 bouts showed a clear developmental trajectory. The latency to reach seizure Stage 2 was shortest at larval day 5 and temperature at each seizure stage onset was consistent across the larval ages with the average temperature reaching 36.2°C and 40.3°C for Stage 1 and 2. Analysis of tail movement showed that spontaneous swimming comprised symmetrical displacements with alternating right-left oscillations. In contrast PTZ-induced seizures produce a large, slow, and asymmetrical tail movement. **References** 1. Kalueff A et al. Prog Neuor-Pyschoph. 2014 PMID: 24593944. 2. Hunt R et al. Exp Neurol. 2012; 237:199-206.

Disclosures: J.F. Ullmann: None. **G. Leanage:** None. **F. Sepehrband:** None. **D. Reutens:** None.

Poster

520. Animal Models of Epilepsy II

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Topic: C.07. Epilepsy

Support: NINDS grant 1U54NS079202

Title: Mouse model of tetramethylenedisulfotetramine (TETS)-induced status epilepticus

Authors: *D. ZOLKOWSKA[1], H. WULFF[2], B. D. HAMMOCK[3], P. J. LEIN[4], M. A. ROGAWSKI[1]
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Abstract: Tetramethylenedisulfotetramine (TETS) is a highly lethal convulsant toxin that acts by noncompetitive block of GABA-A receptors. Although TETS is banned throughout the world, accidental and intentional exposures are not uncommon. In humans, poisoning with TETS causes generalized clonic-tonic convulsions and persistent status epilepticus (SE) that is often refractory to treatment. Acute administration of TETS to mice and rats produces generalized convulsions

that are rapidly lethal. It has therefore been difficult to model TETS-induced SE in rodents. Here we report that pretreatment of mice with a single dose of riluzole (10 mg/kg IP) 10 min prior to administration of a lethal dose of TETS (0.2 mg/kg IP) reproducibly results in continuous seizure activity, characterized by a progression of behavioral seizure signs and EEG seizure discharges. Mice were implanted with right frontal and parietal cortical screw EEG recording electrodes. An EMG electrode was implanted in the neck. Recordings were conducted with respect to a screw reference electrode located in the left parietal cortex area. After a recovery period of 1 week, animals were treated with riluzole/TETS and monitored by video-EEG recording. Abnormal behavioral and EEG activity began approximately 3 min after TETS administration. During an initial period of about 20 min, the animals exhibited myoclonic jerks and isolated spikes, sharp waves, and spike and slow wave complexes, as well as isolated clonic seizures. Subsequently, merging seizures and continuous ictal discharges were observed for nearly 1 h. The EEG typically then transitioned into periodic epileptiform activity on a flat background and some animals died about 100 min after receiving TETS. Riluzole is an antiseizure agent that affects the function of many ion channels. It is known to inhibit voltage-gated sodium channels and it also activates small-conductance calcium-activated potassium channels. As is the case with other sodium channel blocking antiseizure agents, riluzole is weak or ineffective at preventing seizures induced by GABA-A receptor antagonists. However, riluzole can protect against the rapidly lethal effects of TETS in mice, allowing persistent SE. The utility of this model in the identification of therapeutic agents for the treatment of TETS-induced SE will be examined.

Disclosures: D. Zolkowska: None. **M.A. Rogawski:** None. **H. Wulff:** None. **B.D. Hammock:** None. **P.J. Lein:** None.

Poster

520. Animal Models of Epilepsy II

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Topic: C.07. Epilepsy

Support: HRA_POR/2012/56

Title: Characterization of a novel model of neonatal hypoxia-induced seizures in mice

Authors: *N. RODRIGUEZ, E. JIMENEZ-MATEOS, D. HENSHALL
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Abstract: Seizures are most prevalent in the neonatal period, with an incidence of 1-3.5/1,000 live births. Up to 60% of neonatal seizures are associated with hypoxic/ischaemic encephalopathy (HIE) (exposure to hypoxia or hypoxia/ischemia) and are related with later life epilepsy, neurological and/or cognitive deficits. The hippocampus is particularly vulnerable to damage in HIE, and injury may produce permanently enhanced excitability in the brain. Critically, no drug has been developed specifically for neonatal seizures, and a new therapeutic approach is required. Improved understanding of the pathophysiology and treatment of neonatal seizures would be achieved via the availability of a simple, reliable mouse model of neonatal hypoxia-induced seizures. Here, we present a characterization of a novel model of neonatal seizures induced by hypoxia in mice. Hypoxia was induced in post-natal (P7) mice by placing pups inside an air-tight chamber and exposing them to 100% N2 with the flow rate adjusted to create graded global hypoxia (4-6%) for 15 minutes. Seizure activity was detected using cortical EEG recordings from implanted subdermal electrodes and behavioral seizures induced by hypoxia were scored by a modified Morrison scale, specifically developed for immature mice . Levels of the immediate early gene *c-fos* were analyzed as a marker of seizure activity, and levels of vascular endothelial growth factor (VEGF) as a marker of hypoxia, using quantitative real-time PCR, and damage to the brain was assessed by Silver staining. Hypoxia was reliably induced and maintained in P7 mouse pups with minimal mortality or morbidity. 95% of mice displayed seizures during hypoxia which were characterized as brief bursts of epileptiform activity. Brain tissue from mice displayed increased markers of hypoxia, neuronal activity and neuronal injury but neuronal death was not evident. Also, we evaluated whether hypoxia during neonatal period results in an increase of long-term susceptibility to chemoconvulsant, kainic acid. We conclude that perinatal hypoxic seizures at P7 significantly increase susceptibility to later life kainate-induced seizures and demonstrate enhanced neuronal injury. Brief global hypoxia can be used to reliably elicit neonatal seizures in P7 mice. This model may be useful to investigate the pathophysiology of neonatal seizures and novel treatments and can be used to study transgenic mice.

Disclosures: **N. Rodriguez:** None. **E. Jimenez-mateos:** None. **D. Henshall:** None.

Poster

520. Animal Models of Epilepsy II

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Title: Epileptiform activity and psychiatric comorbidity in a mouse model of atypical absence epilepsy

Authors: *S. JUNG, D. JEON
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Abstract: Purpose: Atypical absence epilepsy (AAE) showing slow spike-and-wave discharges (SWD) is characterized by severely abnormal cognition and neurodevelopmental or neurological outcomes in humans. However, despite the severe behavioral outcomes in AAE, the relationship between AAE and social-behavioral dysfunctions and seizure development have not defined well, either experimentally or in patients with AAE. Experimentally, AAE can be produced by administering AY-9944 (AY), a cholesterol biosynthesis inhibitor. In this study, we characterized social behavior and epileptiform activity during the postnatal development in the AY mouse model of AAE. Method: AAE in the mouse was induced by repeated postnatal administration of AY every 6 days from postnatal day (P) 2 to P20. AY-treated mice performed tasks involving sociability/social novelty preference, social interaction with a juvenile conspecific, observational fear, and resident-intruder aggression. We also measured the electrophysiological properties of hippocampal CA1 neurons which are treated with AY-9944 using patch-clamp technique. Results: They showed behavioral dysfunction in social interactions with a juvenile conspecific and sociability/social novelty preference tasks. They also exhibited reduced social fear learning in observational fear conditioning. Interestingly, they showed increased levels of offensive behaviors in a resident-intruder task. However, AY-treated mice displayed normal levels of anxiety in light/dark transition and the elevated plus maze tasks, and showed slightly increased locomotor activity in an open-field task. AY-treatment on hippocampal slices changed membrane electrical property of hippocampal CA1 neurons toward increasing excitability resulting in abnormal action potential firing patterns during a long depolarization. Conclusion: These results demonstrate social dysfunction and epileptic activity during developmental stage in the AY-induced AAE model. Our study can also provide valuable information about Lennox-Gastaut syndrome, in which AAE is a component. Thus, our findings may help to understand behavioral pathogenesis or characteristics of patients with AAE.

Disclosures: S. Jung: None. **D. Jeon:** None.

Poster

520. Animal Models of Epilepsy II

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RO1 GM089893 to PJW

RO1 GM076327 to PJW

Title: A mouse model of Xq22.1 deletion syndrome displays epilepsy and cortical circuit dysfunction

Authors: *E. M. GOLDBERG[1,2], J. ZHOU[3], C. YUE[1], P. J. WANG[3], D. A. COULTER[1]
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Abstract: X-linked inheritance is an increasingly recognized pattern of intellectual disability (ID) in humans, constituting ~10% of all cases; over 90 genes on the X chromosome are associated with intellectual disability. X-linked ID is often accompanied by epilepsy. Experimental models of epilepsy and ID provide an opportunity to investigate disease pathomechanisms. In this study, we aim to investigate the mechanisms of epilepsy in a novel mouse model of epilepsy due to a 1.1 Mb microdeletion of chromosome Xq22.1. This region in mouse is syntenic to the deleted region in human Xq22.1 deletion syndrome, which is characterized by developmental delay/ID, epilepsy, dysmorphic features, and an X-linked pattern of inheritance. Interestingly, the deleted region includes multiple members of the G-protein coupled receptor (GPCR) associated protein (GPRASP, or GASP) family, which regulate the trafficking of various GPCRs. Males harboring the deleted region of Xq22.1 exhibited respiratory failure, cleft palate, and neonatal lethality. Heterozygous females exhibited cleft palate and growth retardation; many (~75% of) females died in the first 3-4 weeks of life. Females surviving to post-natal day 30 exhibited epilepsy, including handling and stress-induced seizures as well as spontaneous seizures. 6-channel continuous intracranial electroencephalography confirmed that adult heterozygous females had, on average, 0.5 ± 0.2 seizures per 24 hours. Electrographic seizures appeared to initiate from the hippocampus. Histological analysis revealed normal anatomy and layering of the neocortex and hippocampus. Voltage sensitive dye imaging in brain slices prepared from heterozygous female mice revealed hyperexcitability in dentate gyrus in response to perforant path stimulation. In conclusion, we show that deletion of a 1.1 Mb region of Xq22.1 reproduces the salient features seen in patients harboring this deletion. Epilepsy in these mice may be due to dysfunctional trafficking of G-

protein coupled receptors leading to hyperexcitability in cortical networks. While Xq22.1 deletion syndrome is likely a very rare cause of epilepsy and ID, analysis of the mouse model may yield important insights into the role of GASP/GPRASPs in cortical excitability and mechanisms of epilepsy.

Disclosures: E.M. Goldberg: None. **D.A. Coulter:** None. **C. Yue:** None. **P.J. Wang:** None. **J. Zhou:** None.

Poster

520. Animal Models of Epilepsy II

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Topic: C.07. Epilepsy

Support: European Science Foundation EUROCORES Programme EuroEpinomics

University of Eastern Finland, Doctoral Program in Molecular Medicine

Title: Alternations in hippocampal and cortical transcriptome during post traumatic epileptogenesis

Authors: *A. LIPPONEN[1], N. HUUSKO[1], K. DęBSKI[4], A. KASPI[5], H. KAIPANANICKAL[5], I. KHURANA[5], M. ZIEMANN[5], J. PAANANEN[2], M. HILTUNEN[3], A. EL-OSTA[5], K. LUKASIUK[4], A. PITKÄNEN[1,6]
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Abstract: Traumatic brain injury (TBI) is a significant cause of acquired epilepsies. After the initial damage by direct mechanical force to the head, secondary brain damage leads to molecular changes affecting reorganization of neuronal networks in epileptogenesis. We hypothesize that expression of genes that control formation of neuronal circuits alters network features after TBI in both, hippocampus and cortex, leading to increased seizure susceptibility and epilepsy.TBI was induced with lateral fluid-percussion injury to adult rats (n=5). Five sham-operated rats served as controls. At 3 months post-TBI two 2-mm-thick coronal slices were sectioned to sample the hippocampus and cortex for RNA extraction. Sequencing of

transcriptome was carried out with Illumina Genome Analyzer IIx. In the hippocampus, 4280 genes and cortex 176 genes were expressing differently when compared to controls (adj.p<0.05). The expression of 140 same genes was altered significantly in both brain areas. Most common functional terms in among of these differential expressing genes were glycoprotein, signal, plasma membrane and ion/metal/cation binding. Our result reveals long-lasting alternations after TBI in gene expression in the hippocampus and cortex.

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Poster

520. Animal Models of Epilepsy II

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Topic: C.07. Epilepsy

Support: European Science Foundation EUROCORES Programme EuroEpinomics

University of Eastern Finland, Doctoral Program in Molecular Medicine

Title: Long-lasting alterations in DNA methylome during posttraumatic epileptogenesis

Authors: ***N. E. HUUSKO**[1], A. LIPPONEN[1], K. DęBSKI[4], A. KASPI[5], H. KAIPANANICKAL[5], I. KHURANA[5], M. ZIEMANN[5], J. PAANANEN[2], M. HILTUNEN[3], A. EL-OSTA[5], K. LUKASIUK[4], A. PITKÄNEN[1]
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Abstract: Traumatic brain injury (TBI) is estimated to cause 10-20% of all acquired epilepsies. After the initial damage caused by direct mechanical force to the head, secondary damage develops over time consisting of molecular changes that underlie the subsequent reorganization of neuronal networks. The little evidence available suggests that epigenetic regulation controls some part of the alterations found in the expression of hundreds of genes after TBI. We hypothesize that DNA methylation alters gene expression which regulates post-injury reorganization of neuronal circuits, eventually leading to the development of hyperexcitability

and epilepsy. TBI was induced with lateral fluid-percussion injury to adult rats (n=5). Five sham-operated rats served as controls. At 3 months after TBI sampling of the hippocampus and cortex was done for DNA extraction. Sequencing of methylome was carried out with Illumina Genome Analyzer IIx. After TBI, methylation was changed in the gene body area in 21 genes in the hippocampus and in 45 genes in the cortex (adj.p-value<0.05). Three promising candidate genes with altered gene expression and methylation level were identified for further validation. Our results demonstrate long-lasting change after TBI in DNA methylation which can explain altered gene expression levels.

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Poster

520. Animal Models of Epilepsy II

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CURE

Title: State-dependent respiratory effects of seizures contribute to death in a mouse model of SUDEP

Authors: K. I. CLAYCOMB, M. A. HAJEK, D. A. RAPPOPORT, *G. F. BUCHANAN
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Abstract: Sudden unexpected death in epilepsy (SUDEP) is the leading cause of death in patients with chronic refractory epilepsy. SUDEP tends to occur at night, but the mechanisms of how this happens are unknown. It is known that seizure-related respiratory and cardiac arrest are the most-likely etiologies for SUDEP, and control of both breathing and cardiac function is subject to sleep state-dependent and circadian regulation. In addition, seizure themselves are modulated in a state- and circadian-dependent manner. Here we set out to determine whether there are state- and/or circadian-dependent effects of seizures on cardiac and respiratory function that contribute to seizure-related death. EEG, EMG and EKG electrodes were implanted in adult

male mice with or without a bipolar stimulating/recording electrode in the right centrolateral nucleus of the amygdala. Seizures were induced with maximal electroshock (MES; 50 mA, 200 ms, 60 Hz) or amygdala kindling (100-180 μA, 1 ms biphasic square wave, 1 s, 60 Hz) during wakefulness, non-rapid eye movement (NREM) sleep and rapid eye movement (REM) sleep, and EEG, EMG, EKG, and breathing were assessed. Vigilance state was determined on-line in real time based on EEG and EMG characteristics using standard parameters. A separate set of animals were made to spontaneously seize with pilocarpine (275-400 mg/kg, i.p.) induced status epilepticus and epileptogenesis. For these animals, seizures were analyzed post-hoc for vigilance state and circadian time of occurrence and effects on breathing and cardiac activity were assessed. In the MES model, seizures that occurred during sleep were more likely to be fatal, especially those induced during REM. Death was due to primary respiratory arrest. Seizures induced during NREM in the MES and kindling models were associated with increased respiratory rate variability and increased occurrence of apneas. Seizures were not readily induced during REM in the kindling model. Seizures occurred rarely during REM in the spontaneously seizing model, but those that did were associated with increased cardiac and respiratory variability. These data indicate that seizures that occur during sleep can have detrimental effects on breathing and cardiac activity, which may contribute to increased seizure related death.

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Poster

520. Animal Models of Epilepsy II

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Topic: C.07. Epilepsy

Support: NIH/SUDEP Center Without Walls

Title: Postictal hypoventilation is a common cause of death in multiple mouse models for SUDEP

Authors: *Y. KIM[1,2], L. P. SOWERS[1], G. F. BUCHANAN[4], G. B. RICHERSON[1,3]
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Abstract: Sudden unexpected death in epilepsy (SUDEP) is the most common cause of death in chronic refractory epilepsy patients and is a great concern for the epilepsy community. The primary cause of SUDEP is unclear, because most cases occur at night without witnesses, and recordings of physiological data have rarely been obtained at the time of death. The recent MORTality in Epilepsy Monitoring Unit Study (MORTEMUS) reported the largest series of SUDEP cases that occurred in epilepsy monitoring units (EMUs), and included video, EEG, and EKG. For all of these cases breathing rate was estimated using post-hoc observation of the videos, but was not measured directly. Tidal volume, ventilation, oxygen saturation or other measurement of breathing were not made. Of 16 SUDEP cases, 100% occurred after a generalized seizure. All of these deaths associated with cardiac dysfunction, but it could not be determined whether the cardiac changes were the primary cause of death or were secondary to respiratory dysfunction and hypoxia. If data on breathing had been included, then it could have been easier to determine the primary mechanism of the SUDEP cases. Sudden death occurs in some mice after seizures. Investigation of sudden death after seizures in mouse models may help to define the primary mechanisms of SUDEP in humans. We designed and built a mouse EMU that included 2 EEG electrodes, nuchal EMG, EKG, animal movement, video, body temperature, chamber temperature and humidity, and whole-body plethysmography pressure (breathing) traces. We used multiple mouse models that are prone to sudden death in response to seizures, including both non-epileptic and epileptic mice: 1) audiogenic seizures induced in DBA/1 mice, 2) maximal electroshock (MES) seizures induced in $Lmx1b^{f/f/p}$ conditional knock-out mice, 3) MES seizures induced in C57BL6 mice, and 4) MES seizures induced in $Scn1a^{R1407X/+}$ mutant mice (Dravet Syndrome model). In sudden death cases, seizures caused respiratory arrest in all 4-mouse models. EKG activity was present for 3 to 5 minutes after terminal apnea. We interpret these data as indicating that the primary cause of sudden death was central apnea that began during the seizures, and the resulting hypoxia and hypercapnia later caused bradycardia and asystole. It is important to know whether different mouse models share the same mechanisms of SUDEP to investigate methods of prevention. To provide better understanding of SUDEP mechanisms, we now plan to expand these studies to epileptic mice in which SUDEP occurs spontaneously, since SUDEP in humans is due to spontaneous, not induced seizures.

Disclosures: **Y. Kim:** None. **L.P. Sowers:** None. **G.F. Buchanan:** None. **G.B. Richerson:** None.

Poster

520. Animal Models of Epilepsy II

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Bloorview Children's Hospital Foundation

Title: Infantile spasms: Rescue of an animal model

Authors: *K. JOSHI[1], L. SHEN[2], M. A. CORTEZ[2], J. H. EUBANKS[3], O. C. SNEAD 3RD[2]
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Abstract: Infantile spasms (IS) is a catastrophic childhood seizure disorder characterized by a distinct developmental window of onset [4-12 mos.], a unique clinical semiology [flexor and/or extensor spasms], electroencephalographic [EEG] signature [hypsarrythmia] and unique pharmacology. The latter consists of the sole effectiveness of ACTH or vigabatrin in IS. The mechanism/circuitry contributing to the fundamental pathogenesis of IS is unknown. Children with Down Syndrome (DS) are especially vulnerable to IS. Therefore we have chosen to use a genetic mouse model of DS, the Ts65Dn mutant mouse (Ts) as a substrate to develop an animal model of IS. We have demonstrated that administration of the GABABR/GIRK2 agonist, γ-hydroxybutrytic acid (GHB) to the Ts65Dn mouse results in an EEG behavioral/pharmacology phenotype that replicates IS in humans. One of the genes triplicated in the Ts mouse line is the Kcnj6/Girk2 gene. We hypothesize that the overexpression of GIRK2 (G-protein inward rectifying potassium channel) is necessary and sufficient for the GABABR/ GIRK2 agonist induced IS phenotype in the Ts mouse model. We reduced the GIRK2 channel activity in the Ts mice pharmacologically using a general GIRK 1-4 antagonist tertiapin Q (TPQ). The TPQ treated Ts mice were resistant to the IS-inducing effect of GHB. To further validate the role of GIRK2, we knocked down the GIRK2 gene in the Ts mouse. Specifically, we used GIRK2 null mice to engineer the Ts mouse to be disomic for the GIRK2 gene (Ts65Dn GIRK2+/+/-) and measured the difference in the GHB-induced IS-like behavioral and EEG seizure activity between theTs65Dn mice, Ts65Dn disomic mice and the Wild Type controls. We found that the genetically knocked down GIRK2 in the Ts mice rendered them resistant to the GHB-induced IS phenotype . In summary we have shown that the GHB-treated Ts model of IS can be rescued by knocking down GIRK2 function, either pharmacologically or genetically. The data suggest that overexpression of GIRK2 leading to excess hyperpolarization plays a major role in the pathogenesis of IS. We are now creating a transgenic GIRK2 overexpressing mouse line to further strengthen our hypothesis. We hypothesize that the GIRK2 overexpressed mice will be exquisitely sensitive to the IS-inducing effects of GHB. This study is important, as the GIRK2 expression profile information in various brain regions could help us identify the circuitry

involved in IS. This study also has important implications for future therapies for IS and Down's syndrome.

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Poster

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Title: Methyl-CpG-binding domain protein 3 (MBD3) in normal brain and in the rat model of temporal lobe epilepsy

Authors: *J. BEDNARCZYK, K. LUKASIUK
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Abstract: MBD3 belongs to a family of nuclear proteins which are characterized by the presence of a methyl-CpG-binding domain. This multisubunit complex combines reading of DNA methylation marks with modifying histones participating in regulation of gene expression. The aim of this project was to define the composition of MBD3-containing protein complex in the brain, cellular localization of MBD3 mRNA and protein as well as a characterization of the expression pattern of MBD3 protein in normal rat brain and in the amygdala stimulation model of epilepsy. Epilepsy was induced in Sprague-Dawley rats by amygdala stimulation induced status epilepticus evoked by 100-ms train of 1-ms biphasic square-wave pulses (400 μA peak to peak) delivered at 60 Hz, every 0.5 s for 30 min. Animals were sacrificed 14 days after stimulation. Composition of MBD3-containing protein complex was studied with mass spectrometry using normal brain tissue. Obtained data was analyzed in the IPA and validated using Western-blot. To characterize the expression of mRNA MBD3 in the brain *in situ* hybridization for MBD3 combined with immunohistochemistry for markers of neurons (NeuN), oligodendrocytes (Olig2) or astrocytes (GFAP) was carried out. To determine cellular localization and level of expression of MDB3 protein immunofluorescent staining of control

(n=5) and stimulated (n=5) animals was performed. Cell number and staining intensity in the amygdala and piriform cortex were evaluated using ImageJ software. Mass spectrometry revealed that in the brain MBD3 is a part of conventional NuRD complex and immunoprecipitation combined with Western-blot confirmed that MBD3 co-precipitates with HDAC1 and MTA2 proteins. MBD3 mRNA is expressed in neurons and oligodendrocytes in the normal brain. In control and epileptic brains MBD3 immunoreactivity was observed in nuclei of neurons, astrocytes and oligodendrocytes but not in microglia. Analysis of expression pattern of MBD3 protein in the third layer of the piriform cortex, in the lateral and central nuclei of amygdala revealed that 98,5±1,41%, 99,3±0,52% and 99,7±0,67% of NeuN-positive neurons respectively contain MBD3 in their nuclei. The intensity of MBD3 immunofluorescence was significantly increased in epileptic group in the third layer of the piriform cortex (contra 230±48%; ipsi 208±24%, $P<0.01$, t-test), lateral nucleus of amygdala (contra 186±38%; ipsi 196±28%, $P<0.01$) and central nucleus of amygdala (contra 176±41%, $P<0.05$; ipsi 166±19%, $P<0.001$) compared to controls. MBD3 is expressed in the brain in cell specific manner, participates in conventional NuRD-containing MTA2 and HDAC1 complex and its expression is affected in epileptic brain.

Disclosures: **J. Bednarczyk:** None. **K. Lukasiuk:** None.

Poster

520. Animal Models of Epilepsy II

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Topic: C.07. Epilepsy

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CURE

DSF

Dravet.org

Vanderbilt University CTSA

Title: The mutant Γ2(Q390x) subunit protein was produced in the GABRG2(Q390X) knockin epilepsy mouse model

Authors: *J.-Q. KANG[1], W. SHEN[1], C. ZHOU[1], X. HUANG[2], D. XU[3], R. L. MACDONALD[1]
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Abstract: GABRG2(Q390X) is a mutation associated with the most severe kind of epilepsy, Dravet syndrome in two independent pedigrees. We have previously demonstrated that the mutant γ2(Q390X) subunit produces dominant negative suppression on wildtype partnering subunits and the mutant subunit protein is stable and aggregated *in vitro*. Using protein structure modeling, the 4th hydrophobic, transmembrane α-helix (YARIFFPTAFCLFNLVYWVSYLYL) of the wildtype γ2 subunit was deleted and a new α-helix with many charged amino acids (KDKDKKKKNPAPTIDIRPRSATI) was found to assume its location in the mutant γ2(Q390X) subunit. The new α-helix was found to be unstable in the membrane, and the remaining hydrophobic surfaces in the α-helices of the mutant subunit were shown to become "sticky". To further understand the pathophysiology of GABAA receptor mutations and epilepsy, we created Gabrg2(Q390X) knockin mice. Similar to homozygous Gabrg2-/- knockout mice, homozygous Gabrg2(Q390X)/Q390X mice were not viable. Heterozygous Gabrg2 +/Q390X mice had spontaneous generalized tonic clonic seizures starting from about P20 and had increased mortality throughout life. Brain slice recording identified that GABAergic mIPSCs in the cortex layer VI neurons were reduced. Biochemistry and surface biotinylation has demonstrated that both surface and total wildtype γ2 and α1 subunits were reduced in the Gabrg2+/Q390X mice. Quantitative RT-PCR(qPCR) demonstrated that total mRNA of Gabrg2 in the mutant mice were not changed compared with the wildtype mice. Additionally, Gabrg2 mRNA abundance of the mutant allele was the same as the wildtype allele in the heterozygous Gabrg2 +/Q390X mice. This is likely due to the fact that since the mutation is located in the last exon of Gabrg2 gene, the mutant γ2 (Q390X) subunit mRNA is not degraded by nonsense mediated mRNA decay (NMD). Instead, the mutant γ2(Q390X) subunit protein was produced, accumulated intracellularly in heterozygous Gabrg2 +/Q390X mice.

Disclosures: J. Kang: None. **W. Shen:** None. **C. Zhou:** None. **X. Huang:** None. **D. Xu:** None. **R.L. Macdonald:** None.

Poster

520. Animal Models of Epilepsy II

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Topic: C.07. Epilepsy

Support: Conacyt Grant 106402 (to MLLM)

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Title: Seizure susceptibility in developing rats prenatally exposed to valproic acid

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Abstract: Epilepsy is a neurological condition characterized by recurrent epileptic seizures, whereas autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized by social deficits, impaired communication, and stereotyped and repetitive behaviors. Clinical and epidemiologic evidence has shown comorbidity between ASD and epilepsy. Prenatal exposure to valproic acid (VPA) induces neural tube defects and impairment in social behaviors related to ASD in humans and rodents, which make it a useful animal model to study autism. The goal of this project was to evaluate seizure susceptibility in developing rats prenatally exposed to VPA (pregnant females were injected with 600 mg/Kg, i.p., during the twelfth embryonic day, E12), by using two epilepsy models, pentylenetetrazole (PTZ, 80 mg/Kg, i.p.) and lithium-pilocarpine (Li-Pilo, 3 mEq/Kg, i.p. and 100 mg/Kg, s.c., respectively). Control animals were expose to saline solution during E12. Seizures were induced in fourteen-days-old rat pups (both sex). Results showed two subgroups with different PTZ-induced seizure susceptibility in rats exposed to VPA ($p<0.0001$): a high susceptibility (HS) subgroup [28/42, seizure severity 4.9 ± 0.04, corresponding to generalized clonic-tonic seizures (GCTS)] and a low susceptibility (LS) subgroup [14/42, seizure severity 2.1 ± 0.01, displaying myoclonic seizures). There were not differences between HS subgroup and the control group (39/42, seizure severity 4.8 ± 0.1), but LS subgroup had less susceptibility to PTZ-induced convulsions when compared to the control group ($p<0.0001$). Animals exposed to VPA from the HS subgroup exhibited an increased latency ($p<0.0006$) to the GCTS (120.6 ± 5.2 s) when compared to control animals (106 ± 6.2 s). Additionally, HS animals had more that one GCTS (13/28, $p<0.05$) and status epilepticus (19/28, $p=0.006$) than control animals (10/42 and 14/42, respectively). When all animals exposed to VPA were considered (HS and LS subgroups), there was a decrease in the average PTZ-induced seizure severity (4.0 ± 0.2, corresponding to generalized clonic seizure; $p<0.01$) and in the number of rats displaying GCTS (27/42; $p=0.0018$) when compared to the control group [4.8 ± 0.1 (i.e. mostly GCTS) and 39/42, respectively]. No differences in seizure severity, latency or duration of status epilepticus induced by Li-Pilo were detected between VPA and control animals. In conclusion, prenatal expose to VPA in rats modifies the susceptibility to seizures induced by PTZ during the second week of age, but not the status epilepticus induced by Li-Pilo. This effect could involve an alteration in the GABAergic system.

Disclosures: **A.A. Puig Lagunes:** None. **J. Medel-Matus:** None. **R. Toledo:** None. **J. Manzo:** None. **M. López-Meraz:** None.

Poster

520. Animal Models of Epilepsy II

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Support: Austrian Science Fund (FWF) Grant P26680

Title: Development of epilepsy after spatially restricted viral vector-mediated silencing of parvalbumin-expressing GABAergic interneurons in the mouse

Authors: ***M. DREXEL**[1], S. WEGER[2], R. HEILBRONN[2], P. WULFF[3], R. O. TASAN[1], G. SPERK[1]
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Abstract: Parvalbumin (PV)-expressing GABAergic interneurons in the CA1 sector of the hippocampus and in the subiculum comprise basket cells and axo-axonic cells targeting the pyramidal cell soma and the axon initial segment, respectively. There they potently control the firing frequency and timing of pyramidal cells. In patients with temporal lobe epilepsy (TLE) and in animal models of TLE degeneration of PV-expressing interneurons in the hippocampal formation has been described. The goal of this study was to test the hypothesis that impaired function of PV-GABA interneurons in the hippocampal/parahippocampal region of mice may be causative for the development of epilepsy. Our approach was to specifically silence synaptic output of PV-expressing interneurons in different subregions of the hippocampal/parahippocampal region. This was achieved by locally restricted stereotaxic injection of an AAV-vector containing the GFP-tagged tetanus toxin light chain (TeLC) reading frame (inverted in a flip-excision (FLEX) cassette) into the brain of mice expressing Cre-recombinase under the PV promoter. TeLC-mediated cleaving of vesicle associated membrane protein 2 (VAMP2) stops vesicle fusion thereby inhibiting GABA release from PV-interneurons. Respective viral vectors containing GFP alone were used for control-injections. Mice were implanted with transmitters for telemetric EEG-recording and continuous video- and EEG-recordings were carried out for 30 (up to 50) days after virus-injection. Double-fluorescence

immunohistochemistry confirmed strong expression of TeLC in PV-expressing interneurons at the injection site. During the first 30 days after virus-injection, about 40% of mice developed at least 2 spontaneous recurrent seizures (latency 5 - 28 days). In these mice, the mean number of seizures per week was 2.2 ± 0.33. Each seizure was preceded by several minutes of low-frequency population spikes. Seizures occurred mostly in clusters followed by seizure-free intervals of 4-6 days. In animals recorded for 50 days (n = 4), seizure numbers per week did not increase over time although duration and intensity of individual seizures increased to some extent. The current results underline the crucial role of PV-expressing GABAergic interneurons for the control of principal cell firing in the hippocampal/parahippocampal region. We show that even a spatially restricted loss of function of these interneurons may lead to the development of spontaneous recurrent epileptic seizures. Malfunction or loss of PV-interneurons may also contribute to the development of human temporal lobe epilepsy.

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Poster

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Support: Simons Foundation

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Title: Scn1a mutation in parvalbumin-positive interneurons causes autistic-like social deficits in a mouse model of Dravet syndrome

Authors: ***S. HAN**, M. H. RUBINSTEIN, J.-Y. BAE, W. A. CATTERALL
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Abstract: Autism spectrum disorders (ASDs) are developmental neuropsychiatric diseases with characteristic symptoms of impaired social interaction, stereotyped behaviors, and delayed language development, often accompanied by cognitive deficit. One hypothesis is that the core behavioral features of autism are caused by an imbalance of excitatory over inhibitory neurotransmission in the brain. Dravet Syndrome (DS) is caused by heterozygous loss-of-

function mutation of the Scn1a gene, which encodes brain specific voltage-gated sodium channel type 1. Mutation in Scn1a in forebrain inhibitory interneurons is sufficient to cause autistic-like behaviors and cognitive deficits in a mouse model of DS. More than 20 different classes of interneurons are involved in forebrain inhibitory circuits, yet it is not known which subtype(s) of interneurons are responsible for the autistic-like traits. To address this question, we generated conditional mutant mice for the Scn1a gene using the Cre-LoxP strategy, and we performed a battery of behavioral tests, including the three-chamber test for social interaction, the open field test for social interaction and repetitive behaviors, and the contextual fear conditioning test for spatial learning and memory. We observed that mice with heterozygous Scn1a mutation in paralbumin (PV)-positive interneurons display autistic-like social deficits, but not hyperactivity, whereas mice with heterozygous Scn1a mutation in somatostatin (SST)-positive interneurons display hyperactivity, but not social deficits. Mice with Scn1a mutation both in PV- and SST-expressing interneurons have both autistic-like social deficits and hyperactivity. Interestingly, neither PV- nor SST-selective heterozygous Scn1a mutant mice had deficits in fear-associated spatial learning and memory. Our results indicate that different classes of interneurons may be responsible for the different behavioral deficits in DS and other ASDs.

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Poster

520. Animal Models of Epilepsy II

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Title: A model of epilepsy based on optogenetic kindling

Authors: *E. CELA[1,2,3], A. J. CHUNG[1,3], P. J. SJÖSTRÖM[1,3]
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Abstract: Epilepsy is a neurological disease characterized by pathological firing of neurons. However, the local circuit changes that are associated with epileptogenesis are not clear. To study these changes, we developed a novel optogenetic animal model of epilepsy. Using this model, we tested the hypothesis that repeated high-frequency stimulation (kindling) might pathologically rewire local circuits by excessive recruitment of Hebbian learning. We first targeted Channelrhodopsin-2 (ChR2) to primary motor cortex (M1) of male C57BL/6J mice by stereotactic delivery of AAV-CaMKIIa-hChR2-E123T/T159C-p2A-EYFP. Following a 21-day recovery period, we began stimulating M1 with 445-nm laser pulses while recording EEG in awake behaving animals. Seizures were not elicited in early stimulation sessions, but seizures were consistently evoked (6 out of 6 animals) after ~15 sessions. Seizures were defined as periods longer than 3 seconds where EEG FFT power exceeded 0.3 standard deviations. In one representative animal, we observed no seizures in the first five sessions but 10.8 ± 1.4 seizures in the last five out of 26 sessions ($p < 0.01$). In the same animal, we also observed spontaneous seizures, a hallmark of epilepsy, after session 26 (1.67 ± 0.14 seizures/33 minutes, n=3 days). We quantified seizure duration by EEG, and their severity by a modified Racine scale in the same animal, and found that the duration ($r = 0.75$, $p < 0.001$) and severity ($r = 0.77$, $p< 0.001$) of seizures increased with stimulation sessions. This progressive development of seizures over sessions was qualitatively similar across animals. To our knowledge, our results are the first demonstration that repeated optogenetic stimulation can elicit epilepsy in awake behaving animals. This may occur through repeated recruitment of Hebbian plasticity by driving excessive neuronal activity. Our optogenetic approach allows the identification of directly activated cells, so that the role of specific cell populations in epileptogenesis can be investigated.

Disclosures: E. Cela: None. **A.J. Chung:** None. **P.J. Sjöström:** None.

Poster

520. Animal Models of Epilepsy II

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Topic: C.07. Epilepsy

Title: Progressive remodeling of hippocampal mossy fibres and dentate gyrus granule neuron dendritic arbors in the brain-derived neurotrophic factor overexpressing mice

Authors: *C. ISGOR, P. COOMBS, K. GUTHRIE
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Abstract: Mice that overexpress brain-derived neurotrophic factor (BDNF) under the alpha-calcium/calmodulin-dependent protein kinase IIa promoter (termed TgBDNF mice) develop a mild cognitive deficit that is evident by 2-3 months of age that progresses to emergence of spontaneous seizures at ~6 months of age. Slow developing nature of behavioral disruptions observed in TgBDNF mice led to the hypothesis that chronic and sustained elevations in local BDNF are critical for progressive remodeling of hippocampal circuits implicated in epileptogenesis. We have previously shown that the mossy fibres (MF), axonal projections from the dentate gyrus granule neurons that innervate the CA3 field, are expanded in volume in TgBDNF mice compared to wildtype (WT) controls at 2-3 months of age. Using whole brain Golgi-Cox immersion technique and a computer-interfaced image analysis system (Neurolucida, Microbrighfield, VT), we also showed that granule neurons of 2-3 month-old TgBDNF mice had remarkably higher number of spines compared to WTs, even though dendritic complexity appeared to be comparable between genotypes. Detailed analyses showed that increase in number of spines was observable in mushroom-type spines, suggesting that excess BDNF may be associated with increased synaptic input from entorhinal cortex onto granule neuron dendritic arbors in mature circuitry even before seizure development. In this study, we challenged ~6 month-old mice with a subthreshold dose of pilocarpine (200 mg/kg) and observed that TgBDNF mice were susceptible to seizure induction at this low dose compared to WT conspecifics. We expanded our examination of MF terminal fields and granule neuron dendiritic arbors to include ~6 month-old time point to further delineate the progressive nature of structural remodeling. These studies map out comparisons between the early (2-3 month-old) and late (~6 month-old) circuit disruptions in the hippocampus of the TgBDNF mice; and morphological correlations with the intensity of seizures in response to a subthreshold dose of pilocarpine in the latter time point. This work is supported by a collaborative FAU Seed Grant awarded to Drs. Isgor & Guthrie.

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Poster

520. Animal Models of Epilepsy II

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Topic: C.07. Epilepsy

Title: Mice lacking the xCT subunit of system x_c^- display delayed epileptogenesis in the self-sustained status epilepticus model

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Abstract: System x_c^- is an amino acid antiporter that mediates the exchange of extracellular L-cystine and intracellular L-glutamate across the cellular plasma membranes. The xCT catalytic light chain is responsible for the transport function of the system x_c^- complex. Previously published data indicate that system x_c^- plays an important role in seizure susceptibility and may contribute to glutamate excitotoxicity in the hippocampus. In the present study we used mice lacking the xCT subunit (xCT KO) of system x_c^- to determine its role in epileptogenesis, which had not been studied so far. We used the self-sustained status epilepticus (SSSE) model, which is a well-established model of epileptogenesis induced by electrical stimulation. Briefly, the xCT KO mice and their wild-type (WT) littermates were surgically implanted with EEG electrodes: a bipolar stimulation electrode (basolateral amygdala) and a cortical monopolar electrode. After recovery from the surgery, mice underwent electrical stimulation through the amygdala-implanted electrode (monophasic train stimulation: duration - 1 s, single pulse duration - 1 ms, frequency - 50 Hz) to establish their afterdischarge threshold (ADT). An ascending series of current stimulations with 5 μA steps was applied once every 2 min until an afterdischarge of at least 5 s duration was elicited. Three days later SSSE was induced by a 90 min stimulation paradigm with 100 ms trains of 1 ms alternating current pulses (50 Hz), 2 trains per s and 250 μA peak current intensity. SSSE characterized by a continuous convulsive and electrographic activity was stopped after 150 min with diazepam (ip, 10mg/kg). Subsequently, the mice were continuously video-EEG monitored for 28 days. Eight mice of each genotype were included in the seizure monitoring studies. We did not observe any difference in seizure susceptibility and comparable ADT values were obtained in both xCT WT and KO mice. Also, there were no differences in either electrographic activity during the SSSE or convulsive seizure severity during this phase. Remarkably, however, the xCT KO displayed significantly longer latency to the onset of the first spontaneous seizure and displayed overall reduced number of spontaneous recurrent seizures during the 4-week monitoring period. Our data indicate that system x_c^- plays an important role in epileptogenesis and could be an attractive target for therapies aiming at the prevention of epilepsy. Acknowledgements: We are grateful to Prof. H. Sato from the Department of Food and Applied Life Sciences, Faculty of Agriculture, Yamagata University, Japan for providing access to the xCT KO mice.

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Poster

520. Animal Models of Epilepsy II

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Title: The effects of 1400W and diapocynin on the hippocampus in rat models of acute organophosphate toxicity: Electographic and immunohistochemical analyses

Authors: *S. SHARMA[1], S. PUTTACHARY[1], A. VELLAREDDY[2], B. KALYANARAMAN[3], A. G. KANTHASAMY[1], T. THIPPESWAMY[1]
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Abstract: Diisopropylfluorophosphate (DFP), an organophosphate (OP), is considered as a surrogate to nerve gas soman. It is a potent seizurogenic neurotoxin, which causes irreversible brain damage due to hyper-excitability of neurons, reactive gliosis and neurodegeneration. If these are not adequately controlled at an early stage, they will lead to the development of epilepsy. The objective of this project is to prevent long term neurological effects of DFP intoxication by administering symptomatic drugs (atropine, 2-PAM, diazepam) with two novel neuroprotectants, 1400W (inducible nitric oxide synthase inhibitor) and diapocynin (NADPH oxidase inhibitor) to reverse neurological changes that follows the acute toxicity. We used two models of DFP intoxication in Sprague Dawley adult male rats (in accordance with IACUC). One with pyridostigmine (PST) pre-treatment and the other without to mimic military personnel (MP) and civilian population (CP) real-life OP intoxication scenarios. 2-PAM (25mg/kg, i.m.) and atropine (10mg/kg, i.p.) were given soon after DFP administration and seizures were scored

using modified Racine scale. Diazepam (10mg/kg, i.p.) was administered 2h later to mimic real-life scenario to control seizures and reduce mortality. 1400W (20mg/kg, i.p.) and diapocynin (300mg/kg, oral) were given twice a day for three days to reverse the damage produced by acute DFP exposure. Some rats were previously implanted with radiotransmitter telemetry device (DSI®), 10 days prior to DFP, and were video-EEG monitored continuously for 4 weeks. Rats were perfused with 4% paraformaldehyde under terminal anesthesia (pentobarbital, 100mg/kg, i.p.) and processed for immunohistochemistry (IHC). IHC and EEG were analyzed to reveal structural and functional changes in hippocampus at different time points. Increased EEG spike rate, reactive gliosis and neurodegeneration were observed 1 week after DFP exposure in both models. CP model rats showed similar behavioral and EEG changes as MP model, but at a 50% lower dose of DFP (n=3-5/group). FJB+NeuN and IHC staining revealed further neurodegenerative changes, astrogliosis and microgliosis in the hippocampus in spite of treating with symptomatic drugs. Both 1400W and diapocynin prevented these changes and significantly reduced the epileptiform spike rate during the first week of DFP exposure ($p<0.0001$) and reduced the number of spontaneous recurrent seizures (SRS) during the rest of the month ($p< 0.05$). 1400W and diapocynin, when given with symptomatic drugs, reduced neurodegeneration and gliosis in the hippocampus and reduced the number of SRS episodes demonstrated their therapeutic importance in OP toxicity.

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Poster

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Fondazione Pisa

Title: Dysfunction of hyperexcitable networks: Altered sensory processing accompanied by long-term biochemical and anatomical changes in neocortical epilepsy

Authors: *L. RESTANI[1], E. VANNINI[1,2], M. PIETRASANTA[1], O. ROSSETTO[3], S. MIDDEI[4], M. CALEO[1]
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Abstract: Epilepsy is characterized by a disruption in the balance of excitation and inhibition that leads to propensity for spontaneous seizures. Pathological remodeling of cortical circuitry may also lead to impaired information processing. However, how plastic rearrangements within the epileptic focus trigger cortical dysfunction and hyperexcitability is only partly understood. Here we have used a model of focal epilepsy induced by tetanus neurotoxin (TeNT) injection into the adult mouse visual cortex to examine alterations in sensory processing and underlying biochemical and neuroanatomical changes. In this model, spontaneous electrographic seizures are evident both during and at the completion of TeNT effects. We found persistent depletion of the TeNT substrate VAMP/synaptobrevin and upregulation of several GABAergic markers in epileptic mice as compared to vehicle-injected controls. At the anatomical level, there was a significant remodeling of the dendritic arbors of pyramidal neurons in superficial layers, with increased dendritic length and branching, and overall reduction in spine density but significant preservation of mushroom, mature spines. Functionally, spontaneous neuronal discharge was increased, and visual responses were dampened and less reliable in the epileptic cortex. Moreover, electrophysiological and behavioural visual acuity was consistently impaired in TeNT-treated mice. These data demonstrate robust, long-term remodelling of both inhibitory and excitatory circuitry associated with specific disturbances of neuronal function and visual perception in non-lesional neocortical epilepsy.

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Poster

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Title: Increased expression of growth associated protein 43 after seizures in rodent model of irradiation-induced cortical dysplasia

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Abstract: Epilepsy is the fourth most common neurological disorder in the United States and 30-40% of patients are not responsive to pharmacological treatment. In many cases, patients with medically intractable epilepsy have underlying pathological abnormalities such as cortical dysplasia (CD). Patients with CD have a decreased threshold for seizures, and may become epileptic after a second hit such as trauma, infection or ischemia. Growth-associated protein 43 (GAP-43), a protein expressed in growth cones during axonal outgrowth and synaptogenesis, is up-regulated following stroke and traumatic brain injury. GAP-43 is also up-regulated in response to seizures, and may contribute to the process of epileptogenesis in CD brains. Methods Pregnant rats were irradiated with 145cGy of cesium-137 on E17. Pups were grown to adulthood and served as experimental models of CD. Rats were then injected with pentylenetetrazole (PTZ) at a sub-convulsive dose (40 mg/kg, i.p.) which does not typically cause seizures in normal rats, but because CD rats have a decreased threshold, they go into *status epilepticus.* Rats were monitored with chronic video/EEG. Seizures were analyzed and interictal spikes were counted until each endpoint. Rats were sacrificed at 2 days,15 days and 30 days following seizure induction to observe the acute and chronic expression of GAP-43. Immunohistochemistry was performed on brain slices and they were graded for staining intensity. Results The expression of GAP-43 is higher in CD rats than NL rats without PTZ in both the hippocampus and the cortex. Normal rats revealed an acute up-regulation of GAP-43 at day 2, which then decreased at days 15 and 30 suggesting a recovery. However, GAP-43 continued to be up-regulated from day 2 to day 15 and day 30 after PTZ injection in the CD rats. In addition at each time point, GAP-43 is always higher in the CD rats. In parallel of higher GAP-43 protein in CD rats, the interictal spikings are more frequent in CD rats than normal rats at each time point. Within CD rats, the interictal spikings continued to increase at each time point parallel to the trend of GAP-43 increase except for day 30. Conclusions CD rats have an intrinsic ability to up-regulate GAP-43 expression. The second hit (PTZ injection) only causes GAP-43 expression in an acute phase in normal rats; however it triggers continuous up-regulation of GAP-43 in CD rats. Interictal spiking (markers for potential epileptogenicity) correlates with higher GAP-43 not only between CD rats and normal rats but also among CD rats at each time point. The data suggests that GAP-43 contributes to the epileptogenic mechanisms and could be used as a bio-marker for epileptogenesis in CD.

Disclosures: A. Nemes: None. **Z. Ying:** None. **K. Ayasoufi:** None. **W. Guo:** None. **I. Najm:** None.

Poster

520. Animal Models of Epilepsy II

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 520.23/Z21

Topic: B.02. Ligand-Gated Ion Channels

Support: HRA_POR/2012/56, HRA_POR/2010/123

IRCSET - RS/2012/323

Title: The regulation and function of the ATP-gated P2X7 receptor in epilepsy

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Abstract: Epilepsy is a common and disabling neurologic disorder which affects about 1% of the population. Seizures are the result of abnormal electrical activity in the brain and can be profoundly disabling, affecting work, social activity and increasing risk of harm. In at least 30-40 % of patients, treatment is inadequate, with patients continuing to experience seizures; therefore, there is an important need to develop new anti-epileptogenic drugs. Increased P2X7 receptor expression has been reported in the hippocampus and cortex after prolonged seizures in animal models and P2X7 receptor antagonists reduced seizure-induced damage to these brain regions. Here we show that P2X7 receptor mRNA and protein level is up-regulated in the hippocampus and cortex of epileptic mice. Using GFP-expressing P2X7 receptor reporter mice we localized the increased expression mainly to neuron and microglia in epileptic animals. In isolated synaptosomes, we observed increased presence and, using FURA-2 Ca+ imaging, functional activation of P2X7R in epileptic mice. These findings support a role for P2X7R in the pathophysiology of chronic epilepsy and suggest P2X7 receptor antagonists may have therapeutic effects against recurrent seizures or progression of disease pathology.

Disclosures: A. Jimenez Pacheco: None. **A. Sanz:** None. **M. Diaz-Hernandez:** None. **T. Engel:** None. **D.C. Henshall:** None.

Poster

521. Anticonvulsant and Antiepileptic Therapies

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 521.01/Z22

Topic: C.07. Epilepsy

Support: In-House Laboratory Independent Research

Title: Refractoriness of convulsive status epilepticus induced by soman, DFP and paraoxon to diazepam treatment

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Abstract: Organophosphates inhibit the enzyme acetylcholinesterase, cause accumulation of acetylcholine at the neuromuscular junctions and central synapses and induce seizures and status epilepticus in humans and animals. Diazepam is clinically used to control seizures induced by organophosphates. The present study evaluated the response of Sprague Dawley rats to diazepam treatment given at 40 min after the onset of seizures induced by soman, diisopropylfluorophosphate (DFP) and paraoxon. Rats were instrumented with cortical electrodes for monitoring electroencephalographic (EEG) activity 7 days prior to induction of seizures. Rats pretreated with HI-6 (125 mg/kg, ip) 30 min prior to soman challenge (180 ug/kg, sc) were treated with atropine methylnitrate (2.0 mg/kg, im) 1 min after soman administration. Rats pretreated with pyridostigmine bromide (0.026 mg/kg, ip) 30 min prior to DFP challenge (4.0 mg/kg, sc) were treated with atropine sulfate (AS; 0.2 mg/kg, im) and 2-PAM (25 mg/kg, im) 1 min after DFP injection. Rats exposed to paraoxon (1.05 mg/kg, sc) were treated with AS (2.0 mg/kg, im) and 2-PAM (25 mg/kg, im). All rats received diazepam (10 mg/kg, im) at 40 min after the onset of seizures. Rats that did not receive diazepam served as controls. While convulsive status epilepticus (CSE) induced by soman did not respond to diazepam treatment, DFP- and paraoxon-induced CSE was effectively treated with diazepam. Brain injury in rats treated with diazepam at 40 min after DFP- or paraoxon-induced CSE was reduced at 24 hr after seizure onset. In contrast, soman-exposed, diazepam-treated rats exhibited severe brain injury in the piriform cortex, amygdala and thalamus. Soman, DFP and paraoxon can induce CSE in rats, but only soman-induced CSE is refractory to diazepam treatment. DFP and paraoxon models were developed as surrogate animal models for chemical warfare nerve agent biomedical research for evaluation of putative therapeutics. The fact that only soman-induced CSE is refractory to diazepam treatment suggests that DFP and paraoxon are not suitable simulants for discovery of novel anticonvulsants to control on-going seizures. Initial screening of novel anticonvulsants using DFP and paraoxon may not be predictive of their effectiveness in terminating nerve agent-induced CSE.

Disclosures: **T.L. Dao:** None. **J.A. Leuschner:** None. **S.W. Kaski:** None. **L.J. Shumway:** None. **L.J. Shumway:** None. **C.R. Braue:** None. **R.K. Kan:** None.

Poster

521. Anticonvulsant and Antiepileptic Therapies

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 521.02/Z23

Topic: C.07. Epilepsy

Support: NIH Grant U01NS058162

Title: The case against diazepam as a treatment for nerve agent-induced seizures and neuropathology; comparison with UBP302

Authors: ***S. L. MILLER**[1], J. P. APLAND[3], V. ARONIADOU-ANDERJASKA[2], T. H. FIGUEIREDO[2], F. ROSSETTI[4], M. F. BRAGA[2]
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Abstract: Exposure to nerve agents induces prolonged status epilepticus (SE), which can lead to death or cause brain damage. Diazepam (DZP) is the presently FDA-approved drug for the cessation of nerve agent-induced SE. Using a rat model of nerve agent exposure, we compared the efficacy of DZP with that of UBP302 –an antagonist of the kainate receptors that contain the GluK1 subunit– against seizures, neuropathology, and behavioral deficits induced by soman. DZP, administered 1 h or 2 h post-exposure, terminated the SE, but seizures returned; thus, in the DZP-treated group, the total duration of SE within 24 h after soman exposure was similar to (DZP at 1 h) or longer than (DZP at 2 h) in the soman-exposed rats that did not receive anticonvulsant treatment. UBP302 stopped SE with a slower time course than DZP, but reduced dramatically the total duration of SE within 24 h. UBP302, but not DZP, reduced neuronal degeneration in a number of brain regions, as well as neuronal loss in the basolateral amygdala and the CA1 hippocampal area, and prevented the loss of interneurons in the basolateral amygdala. Anxiety-like behavior, assessed in the open field and by the acoustic startle response, 30 days after soman exposure, was increased in the group that did not receive anticonvulsant treatment and the DZP-treated group, but not in the UBP302-treated group. The results argue against the use of DZP for the treatment of nerve agent-induced seizures and brain damage, and suggest that targeting GluK1-containing receptors is a more effective approach.

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Poster

521. Anticonvulsant and Antiepileptic Therapies

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 521.03/Z24

Topic: C.07. Epilepsy

Support: NIH Grant 5U01NS058162-07

Title: LY293558 prevents soman-induced pathophysiological alterations in the basolateral amygdala and the development of anxiety

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Abstract: Without immediate treatment, nerve agent exposure can cause countless casualties, as it occurred in the recent sarin attack in Syria. Nerve agent-induced seizures become rapidly refractory to muscarinic antagonists, but also to benzodiazepines; therefore, novel treatments are necessary to stop seizures and prevent brain damage and the lasting health consequences of the exposure. We have previously shown that LY293558, a GluK1/AMPA receptor antagonist, is a very effective anticonvulsant and neuroprotectant against nerve agent exposure. In the present study, we examined if the protection against nerve agent-induced seizures and neuropathology conferred by LY293558 translates into protection against pathophysiological alterations in the basolateral amygdala (BLA) and the development of anxiety, which is the most prevalent behavioral deficit resulting from exposure. LY293558 (15 mg/kg) was administered to Sprague-Dawley rats along with atropine and the oxime HI-6, at 20 min after exposure to soman (1.2 x LD50). At 24 h, 7 days and 30 days after exposure, soman-exposed rats that did not receive LY293558 had small but prolonged evoked field potentials in the BLA, as well as increased paired-pulse ratio, reflecting neuronal damage and impaired synaptic inhibition. In contrast, rats that received LY293558 did not differ from control rats in these parameters. Similarly, long-term potentiation (LTP) of synaptic transmission was impaired at 7 days after exposure in the soman-exposed rats that did not receive anticonvulsant treatment, while this impairment was not present in the LY293558-treated rats. Anxiety-like behavior determined by the open field and acoustic

startle response tests was increased in the soman-exposed rats at 30 and 90 days after exposure, while soman-exposed rats treated with LY293558 did not differ from controls. These data substantiate the remarkable efficacy of LY293558 in counteracting nerve agent-induced seizures, neuropathology, pathophysiological alterations in the BLA, and anxiety-related behavioral deficits.

Disclosures: T.H. Figueiredo: None. **E.M. Prager:** None. **R.P. Long II:** None. **V. Aroniadou-Anderjaska:** None. **J.P. Apland:** None. **M.F.M. Braga:** None.

Poster

521. Anticonvulsant and Antiepileptic Therapies

Location: Halls A-C

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Program#/Poster#: 521.04/Z25

Topic: C.07. Epilepsy

Support: NIH UO1 Grant NS074926-01

Title: The anticonvulsant, anti-epileptic and protective effects of midazolam and ketamine combination treatment after soman-induced seizures in rats

Authors: F. ROSSETTI[1], M. K. SCHULTZ[2], M. DE ARAUJO FURTADO[1], A. R. FURMAN[2], M. F. STONE[2], C. R. SCHULTZ[2], J. E. SCHWARTZ[2], A. R. BOURNE[2], M. C. MOFFETT[2], T. TURNER[2], L. K. WRIGHT[2], C. G. WASTERLAIN[3], *L. A. LUMLEY[2]
[1]Walter Reed Army Inst. of Res., Silver Spring, MD; [2]Neurobehavioral Toxicology, USAMRICD, GUNPOWDER, MD; [3]West Los Angeles VA Med. Center, UCLA, West Los Angeles, CA

Abstract: Introduction: Chemical warfare nerve agents (CWNA), such as soman (GD), produce *status epilepticus* (SE), resulting in extensive neuropathology and long-term performance deficits if seizures are not controlled. Current treatments for CWNA exposure increase survival, but are not fully protective. Although seizure is thought to be initiated by cholinergic mechanisms, the neuropathology resulting from CWNA exposure is believed to be due to glutamate release and subsequent excitotoxic damage. We investigated the neuroprotective effects of the noncompetitive NMDA antagonist ketamine in combination with the benzodiazepine midazolam in rats exposed to GD. **Material and Methods**: Rats (n=11-12/group) received 1.2 LD50 GD (0.6 ml/kg, 220 µg/ml); atropine (2 mg/kg, im) and HI-6 (93.6 mg/kg, im) 1 min after GD exposure; midazolam (MDZ, 3 mg/kg, ip) combined with ketamine

(KET, 30 mg/kg, ip) 40 min post-seizure onset , or MDZ or KET separately with vehicle (water). Amount of time spent in EEG seizures during the first 72 h after SE onset, power spectra of Delta (0.1-4Hz), Theta (4.1-8Hz), Alpha (8.1-12Hz) and Beta (12.1-25Hz) bands during the first 24 h, latency of the first EEG spontaneous recurrent seizures (SRS) appearance and number of SRS until 36 days were analyzed. Behavioral tests of motor activity (beam, home cage, open field), sensorimotor (acoustic startle response) and acquisition of spatial memory (Morris water maze) were conducted in the weeks after exposure. Neuropathology assessments included various histological stains. **Results:** The EEG analysis showed that combination MDZ/KET treatment increased the latency to first SRS appearance (26.49±7.00 days; X±SEM) and reduced the number of SRS (0.50±0.40) in comparison with MDZ or KET alone (MDZ: 10.82±2.47 days and 10.90±5.29; KET: 9.45±2.48 days and 17.1±8.77). Time spent in seizures during the first 72 h after GD exposure tended to be reduced in MDZ/KET-treated rats. The MDZ/KET combination treatment reduced Delta, Theta and Alpha bands power spectra during the 24-hour period after GD exposure compared to MDZ or KET treatment alone, and reduced the Theta and Alpha power spectra, but not Delta and Beta, in relation to baseline. MDZ/KET combination reduced GD-induced spatial memory impairment and reduced GD-induced hyperactivity that typically develops in the weeks after GD exposure. **Conclusion:** The antiepileptic, anticonvulsant, and protective effects of combination treatment with MDZ/KET compared to either treatment alone provide further support that combination therapy with an NMDA antagonist and a benzodiazepine is an effective therapeutic strategy against GD-induced SE and epileptogenesis.

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Poster

521. Anticonvulsant and Antiepileptic Therapies

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 521.05/Z26

Topic: C.07. Epilepsy

Support: NIH Grant NS 40109-13

Title: Modulation of NKCC1 and KCC2 co-transporters for control of catastrophic drug-resistant seizures

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Abstract: Epilepsy after brain injuries caused by hypoxia-ischemia or trauma respond poorly to anticonvulsants, including those that target the inhibitory chloride-permeable $GABA_A$ receptor ($GABA_A$-R). A low intracellular chloride concentration ($[Cl^-]_i$) is an important determinant of inhibitory postsynaptic $GABA_A$-R signaling. Both hypoxia and acute brain trauma are associated with large increases in $[Cl^-]_i$ and a consequent positive shift in the reversal potential for $GABA_A$-R mediated responses (E_{GABA}). Depolarizing shifts in E_{GABA} in populations of injured neurons may contribute to failure of inhibition, seizures, epileptogenesis and anticonvulsant resistance. The Na^+-K^--$2Cl^-$ co-transporter NKCC1 and K^+-Cl^- co-transporter KCC2 are responsible for the return of $[Cl^-]_i$ and E_{GABA} to baseline after synaptic signaling. Immediately after injury, NKCC1 and KCC2 may also contribute to cytoplasmic chloride increase. Suppressing NKCC1 activity acutely and/or chronically enhancing KCC2 activity may be a useful therapeutic strategy to reduce $[Cl^-]_i$, restore GABAergic inhibition in pathological neurons, suppress seizures and prevent epileptogenesis. We compared acute and chronic anticonvulsant efficacy of NKCC1 blocker bumetanide and KCC2 agonist CLP257 in *in vitro* model of post-traumatic epileptogenesis. We found that: (i) bumetanide reduced the frequency and power of early recurrent seizures; (ii) CLP257 abolished early recurrent seizures and reduced the frequency of interictal discharges in a concentration-dependent manner; (iii) KCC2 antagonist VU0726722 strongly potentiated spontaneous interictal and ictal-like discharges; (iv) CLP257 reduced the amount of late chronic seizure activity in a concentration-dependent manner, assayed by lactate and LDH production. Our results validate KCC2 as a promising target of investigation for anticonvulsive and antiepileptogenic therapies, and highlight the need to investigate the mechanism of positive modulators of membrane transport.

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Poster

521. Anticonvulsant and Antiepileptic Therapies

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Topic: C.07. Epilepsy

Support: NINDS Grant R01 NS074772-04

NINDS Grant R01 NS034700-22

Title: Time course of the neuronal sodium concentration in post-traumatic epileptogenesis *in vitro*

Authors: *T. BALENA, K. J. STALEY
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Abstract: Post-traumatic increases in intracellular Cl- can result in GABA becoming depolarizing, which could lead to disinhibition and early post-traumatic epileptic seizures. Increases in intracellular anions are likely to be balanced by an increase in intracellular cations, and the consequent salt accumulation could underlie cytotoxic edema. We investigated post-traumatic changes in intracellular Na+ concentration ([Na+]i) using acute hippocampal slices and organotypic hippocampal slice cultures from wild-type C57BL/6J mice, imaged with the Na+ dye SBFI. Two-photon imaging was used to excite SBFI at both Na+-sensitive and -insensitive wavelengths, allowing for the ratiometric determination of the [Na+]i. Propidium iodide (PI), an indicator of cell death, was used as a costain in most experiments to exclude damaged neurons from the analysis. In acute slices hippocampal neurons had significantly higher [Na+]i than has been reported in undamaged neurons, particularly near the cut surface of the slice. In organotypic slice cultures, [Na+]i returned to low levels ~2 days after slice trauma. At longer incubation times, during which slices become epileptic, [Na+]i increased to levels seen immediately post-trauma; blockage of seizure activity via chronic application of 3 mM kynurenate did not prevent the [Na+]i increase. By 20 days after trauma, [Na+]i returned to low levels. These trends were correlated with an increase in the number of PI-positive neurons. PI staining was also higher closer to the cut surface, and PI-positive neurons had very high [Na+]i. Acute perfusion of 10 μM of the Na+/K+ ATPase inhibitors ouabain and strophanthidin, 100 μM of the KCC2/NKCC1 antagonist furosemide, or 100 μM of the Na+/Ca2+ exchange antagonist benzamil increased [Na+]i. 10 μM of the NKCC1 antagonist bumetanide increased [Na+]i during the first ~10 days after trauma, and thereafter decreased [Na+]i. Thus, in the days after trauma NKCC1 and the Na+/Ca2+ exchanger operate in the reverse of their canonical directions by exporting Na+ and, presumably, the cotransported ions. Application of 8 μM fluorescein conjugated to large dextran molecules indicated severe membrane disruption in only a small number of neurons, but a moderate amount of damage in a larger number of neurons, particularly as epileptogenesis progresses. The correlation between the levels of [Na+]i and PI staining after acute trauma suggests that increased [Na+]i may represent an early step in, or serve as an early biomarker of, neuronal death in post-traumatic epileptogenesis. Increases in intracellular [Na+]i could alter the concentrations of cotransported ions and may contribute to cerebral edema and cell death.

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Poster

521. Anticonvulsant and Antiepileptic Therapies

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Topic: C.07. Epilepsy

Support: NINDS 5R01NS077908

NINDS 5R21NS072258

Title: An *in vitro* screen for antiepileptogenic compounds utilizing organotypic hippocampal slice cultures

Authors: ***Y. SAPONJIAN**[1], Y. BERDICHEVSKY[2], W. SWIERCZ[1], K. STALEY[1]
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Abstract: Traumatic brain injury (TBI) is a major cause of medically intractable acquired epilepsy. There is an urgent need to identify new therapeutic interventions. We utilized the accelerated course of epileptogenesis in the *in vitro* organotypic hippocampal slice culture model of severe post-traumatic epilepsy to conduct a moderate-throughput screen of an array of drugs for anticonvulsant, antiepileptic and neuroprotective effects. Organotypic hippocampal slice cultures were prepared from postnatal day 6 or 7 Sprague-Dawley rats and were maintained in poly-D-lysine coated 6-well tissue culture plates on a rocking platform in a humidified chamber at 37oC and 5% CO2. The culture medium consisted of Neurobasal-A, B27, 0.5 mM GlutaMAX, and 30 µg/ml Gentamicin and was changed every 3-4 days. Chronic-application experiments were carried out for 28 days *in vitro*. Drugs were dissolved in DMSO (final concentration 0.1%) and added to the media starting on DIV 3. All experiments included DMSO control slice cultures derived from the same animal. The lactate concentration in spent culture medium was strongly correlated with electrographic seizure activity and therefore used in subsequent experiments as an assay for seizure activity. The lactate dehydrogenase (LDH) concentration in spent culture medium was correlated with propidium iodide assays for cell death and used in subsequent experiments as an assay for the rate of cell death and neuroprotective effects. We investigated over 150 high-interest drugs, obtained primarily from the National Institute of Neurological Disorders and Stroke (NINDS) custom collection of known bioactives and FDA-approved drugs. Drugs were typically tested at 3 concentrations: the most likely effective concentration as well as

1 log above and below this concentration. Positive screens were repeated to confirm the findings. Drugs exhibiting significant anticonvulsant activity were further analyzed in wash-out experiments to differentiate anticonvulsant from antiepileptogenic effects. Several drugs exhibited dose-dependent anticonvulsive or proconvulsive effects, as well as neuroprotective or neurotoxic effects. The timing of drug application was an important determinant of the action of several drugs, supporting the idea that epileptogenesis results from a sequence of processes, each of which may respond to unique interventions. Anticonvulsant and neuroprotective effects were strongly correlated, presumably due to reductions in ictal cell death. This technology comprises a promising strategy for the rapid investigation of drug efficacy in chronic epilepsy, and could be further scaled with available robotic technologies.

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Poster

521. Anticonvulsant and Antiepileptic Therapies

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Program#/Poster#: 521.08/Z29

Topic: C.07. Epilepsy

Title: Carisbamate modifies the proteomic profile of the hippocampus in a model of temporal lobe epilepsy

Authors: *E. MARQUES-CARNEIRO[1,2], D. S. PERSIKE[1], J.-C. CASSEL[2], A. NEHLIG[3], M. J. S. FERNANDES[1]
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Abstract: *Purpose:* Epileptogenesis is a term used to define a series of events that occur between factors that causes symptomatic epilepsy and the appearance of the spontaneous seizure. In adult rats the administration of pilocarpine (Pilo) followed lithium reproduces most clinical and neuropathological features of human temporal lobe epilepsy (TLE). Carisbamate (CRS) is a drug with present neuroprotective and a potent epileptogenesis-modifying effect (François et al., 2011). Fifty percent of rats subjected to pilocarpine-induced *status epilepticus* (SE) develop absence-like seizure instead of motor seizures when treated with CRS. The mechanism of action of CRS is not yet known. The aim of this study was to perform a proteomic analysis of the

hippocampus of rats receiving lithium-pilocarpine presenting motor seizures or absence-like seizures following CRS treatment. *Methods*: Fifteen adult male Sprague-Dawley rats were used. SE was induced by pilocarpine injection (30 mg/kg, sc) associated with lithium (127.17mg/kg, i.p.). CRS treatment was initiated at 1 h after SE onset and maintained for 7 days. Four groups were obtained: Control (CT n=3), CRS with absence seizures (CRS-AE n=4), CRS with motor seizures (CRS-TLE n=4) and epileptic untreated (TLE n=4). Proteomic analysis was conducted by 2D-SDS-PAGE. Spots were analysed with PDQuest software. Analysis was conducted using UniProt website, GENEmania, QuickGO and IntAct systems, the entire data was integrated using the Cytoscape software. *Results*: Eight proteins linked to glycolysis pathway were down regulated in CRS-AE, such as aldolase-A; aldolase-C; glyceraldehyde-3-phosphate dehydrogenase; RGD1565368; enolase-2; pyruvate dehydrogenase; ATP synthase and aconitate hydratase, although two proteins were up-regulated, as Pyruvate-Kinase and L-lactate dehydrogenase, when compared to control group. *Conclusion*: The partial seizures occurrence is directly related to the energy consumption involving the excitatory/inhibitory balance. Te global reduction of glycolysis and ATP production found in CRS-AE rats is a finding which could explain, at least in part, the epileptogenesis-modifying effect of the Carisbamate.
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Poster

521. Anticonvulsant and Antiepileptic Therapies

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Program#/Poster#: 521.09/Z30

Topic: C.07. Epilepsy

Support: SIP-IPN 20141344

Title: Pharmacological screening of the anticonvulsant activity of the (o-phenyl)-1,3-isoindolindione, a thalidomide analogue

Authors: *C. CAMPOS[1], S. ZAMUDIO[2], S. GUZMAN-VELAZQUEZ[2], J. TRUJILLO-FERRARA[3], E. RAMIREZ-SAN JUAN[2]
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Abstract: Thalidomide is a molecule consisting of a pthaloyl ring and a glutarimide ring, it was proven as antiepileptic drug, and recently has been confirmed this effect in preclinical and clinical research in epilepsy. In addition, it possesses pharmacological properties as antiemetic, sedative, hypnotic, and currently as immunomodulating and anti-inflammatory, however, it has caused congenital abnormalities which inhibited its use. Since thalidomide is an active molecule, nowadays scientists have synthetized new drugs based on thalidomide structure, such as (o-phenyl)-1,3-isoindolindione (OFI), synthesized with the aim of preserving the pharmacological effects on the central nervous system, particularly the effects of antiepileptic drugs, eradicating or reducing adverse effects. For the pharmacological screening, male CD1 mice of 25-30 g in weight were used, which were maintained with food and water ad libitum, in an environment with controlled conditions of temperature (22 ± 2 °C), humidity (55 ± 5%) and 12-hour light/dark cycle. We determined: 1) the effect of the OFI on locomotor activity and motor coordination; and 2) the anticonvulsant activity against seizures induced with pentylenetetrazole (PTZ; 80 mg/kg); strychnine (STR; 3 mg/kg) and pilocarpine (380 mg/kg) in different groups (n=12): control (vehicle OFI: corn oil; orally administrated.), groups that received doses of OFI (421.7 and 562.3 mg/kg, orally administrated) and one group was administrated with sodium valproate (300 or 550 mg/kg; given ip.). The results showed that 421.7 and 562.3 mg/kg doses of OFI significantly reduce the locomotor activity due to the distance traveled and the time of ambulation are decreased significantly with respect to the control; conversely resting time is increased significantly with respect to the control. OFI does not modify significantly the motor coordination of the mice at the doses tested in relation to the group treated with vehicle. The treatment with OFI do not antagonized meaningfully seizures induced by PTZ, STR and pilocarpine, since no dose significantly modifies the time to seizure onset, the number of seizures and/or the percentage of convulsing mice, the seizure total duration, or the death latency. In conclusion, the OFI induces a sedative effect from the dose of 421.69 mg/kg, it does not cause neurological deficit, and do not have anticonvulsant effect on the doses and models tested.

Disclosures: C. Campos: None. **S. Zamudio:** None. **S. Guzman-Velazquez:** None. **J. Trujillo-Ferrara:** None. **E. Ramirez-San Juan:** None.

Poster

521. Anticonvulsant and Antiepileptic Therapies

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 521.10/Z31

Topic: C.07. Epilepsy

Title: Stiripentol and comorbidities in Dravet syndrome: Experimental approaches in animal models

Authors: *V. RIBAN, W. DEFFAINS, I. HEULARD, M. VERLEYE
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Abstract: Dravet syndrome or severe myoclonic epilepsy of infancy (SMEI) is an encephalopathy characterized by refractory epileptic seizures associated with cognitive and motor impairments. Autistic traits and Attention Deficits and Hyperactivity Disorder (ADHD) are also common comorbidities in this syndrome. Seizures management is achieved with a combination of antiepileptic drugs, classically an association of valproate, clobazam and stiripentol, a positive allosteric modulator of GABA-A receptors (Fisher, 2011) which was granted orphan drug status for Dravet syndrome in the European Union in 2001. The aim of this study was to assess the effects of acute or chronic stiripentol administration (at anticonvulsant doses) on two animal models predictive of the main comorbidities found in Dravet syndrome: the spontaneously hypertensive rat (SHR) for ADHD (Sagvolden, 2005), and rats prenatally exposed to sodium valproate (VPA) for social behavior impairments (Rodier, 1995). Acute intraperitoneal administration of stiripentol (200 mg/kg) in juvenile SHR rats decreased locomotor activity and the number of rearings measured in an actimetry test ($p < 0.05$). In the open field test, the number of rearings and zone changes was also decreased after 200mg/kg stiripentol administration. These effects of stiripentol were not observed in the control strain (Wistar Kyoto), and were dissociated from any myorelaxant effects, as measured in the grip test. In rats prenatally exposed to VPA, chronic oral administration of stiripentol (400 mg/kg, from P12 to P32) reversed the diminution of social contacts observed in this model whereas it did not have any effect in the control animals ($p<0.05$).These preliminary results show that, at anticonvulsant doses, stiripentol may have beneficial effects in two comorbidities associated with Dravet syndrome: hyperactivity and impaired social contacts.

Disclosures: **V. Riban:** A. Employment/Salary (full or part-time):; Biocodex. **W. Deffains:** A. Employment/Salary (full or part-time):; Biocodex. **I. Heulard:** A. Employment/Salary (full or part-time):; Biocodex. **M. Verleye:** A. Employment/Salary (full or part-time):; Biocodex.

Poster

521. Anticonvulsant and Antiepileptic Therapies

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 521.11/Z32

Topic: C.07. Epilepsy

Support: PAPIIT Grant IN200614

Title: Effects of anti-seizure drugs on pro-inflammatory cytokines expression in the rat hippocampus

Authors: *C. D. GÓMEZ MARTÍNEZ, R. M. BUIJS, M. SITGES
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Abstract: In the present study the effects of the two classical antiepileptic drugs, carbamazepine and valproic acid, and the non-classical anti-seizure drug, vinpocetine were investigated on the expression of the pro-inflammatory cytokines IL-1beta and TNF-alpha in the hippocampus of rats by PCR or Western blot after the administration of one or seven doses. Next, the effects of the anti-seizure drugs were investigated on the rise in cytokine expression induced by LPS inoculation *in vivo*. To validate our methods the changes induced by the pro-convulsive agents: 4-aminopyridine, pentylenetetrazole and pilocarpine were also tested. Finally, the effect of the anti-seizure drugs on seizures and on the concomitant rise in pro-inflammatory cytokine expression induced by 4-aminopyridine was explored. Results show that vinpocetine and carbamazepine reduced the expression of IL-1beta and TNF-alpha from basal conditions, and reduced the increase in both pro-inflammatory cytokines induced by LPS. In contrast, valproic acid, failed to reduce both, the expression of the cytokines from basal conditions and to prevent the rise in IL-1beta and TNF-alpha expression induced by LPS. Tonic-clonic seizures induced either by 4-aminopyridine, pentylenetetrazole or pilocarpine increased the expression of IL-1beta and TNF-alpha markedly. 4-aminopyridine-induced changes were reduced by all the tested anti-seizure drugs, although valproic acid was less effective. We conclude that the anti-seizure drugs, vinpocetine and carbamazepine, whose mechanisms of action involve a decrease in ion channels permeability, also reduce cerebral inflammation.

Disclosures: **C.D. Gómez Martínez:** None. **R.M. Buijs:** None. **M. Sitges:** None.

Poster

521. Anticonvulsant and Antiepileptic Therapies

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 521.12/Z33

Topic: C.07. Epilepsy

Title: Effects of Lacosamide and Eslicarbazepine acetate metabolites on voltage-gated sodium channel expressed in a neuroblastoma cell line

Authors: *P. GHISDAL, N. NOEL, Y. QUESNEL, I. NIESPODZIANY, C. WOLFF
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Abstract: Lacosamide (LCM) is a third generation anti-epileptic drug with a molecular mode of action described to enhance slow inactivation of voltage-gated sodium channels (VGSC). Eslicarbazepine and its metabolites have also recently been shown to modulate VGSC gating properties. In this study we characterized and compared the effects of LCM and the active metabolites of eslicarbazepine acetate, S-licarbazepine (ESL) and R-licarbazepine (LIC), on the steady-state fast inactivation and slow inactivation properties of sodium current (I_{Na}) in mouse N1E-115 neuroblastoma cells. The whole-cell patch-clamp technique was used to evaluate and compare the effects of LCM and of the metabolites of Eslicarbazepine acetate (ESL and LIC) at different concentrations on VGSCs expressed in N1E-115 cell line. The sodium current (I_{Na}) recording was performed on an automated patch-clamp platform (PatchXpress 7000A, Molecular Devices). We have first evaluated the effect of compounds on the voltage dependency of the fast inactivation properties of VGSCs. Half inactivation potential of I_{Na} fast inactivation curve (V_{50}) was significantly shifted to hyperpolarized potentials after application of 100µM carbamazepine (-12mV±2mV, mean±SEM; n=5), or 300µM ESL (-8mV±1mV, n=7) or 300µM LIC (-8mV±1mV, n=6). The leftward shift of fast inactivation V_{50} measured in the 300µM LCM group (-4 mV±1mV, n=8) and the time-matched control group (-4mV±1mV, n=7) were not different. Voltage-dependent I_{Na} slow inactivation V_{50} value was significantly shifted by 300µM LCM (-42mV±4mV, n=6), ESL (-15mV±4mV, n=6) and LIC (-27mV±5mV, n=8) compared to the time-matched control group (-3 mV±4, n=6). Applied at a concentration of 100µM, LCM and ESL induced a I_{Na} slow inactivation V_{50} shift of -20 mV±1mV (n=4) and -6mV±1mV (n=7), respectively. Finally, we have tested the compounds efficacy on the tonic block of I_{Na} current when VGSCs were activated from a membrane potential corresponding to fast inactivation V_{50} of VGSCs individually measured for each recorded cell. A concentration of 300µM of either LCM, ESL, or LIC inhibited I_{Na} by 52±4% (n=6), 37±5% (n=6) and 24±5% (n=10), respectively. ESL 30µM had no effect on I_{Na} whilst 30µM LCM decreased it by 11±2% (n=6). In conclusion, this study revealed that both ESL and LIC, the minor metabolite of eslicarbazepine acetate, modulate fast and slow inactivation properties of VGSCs expressed in N1E-115 cells. Our results also confirmed that LCM enhances slow inactivation of VGSCs and does not modify their fast inactivation properties. Additionally, the slow inactivation was shown to be more significantly affected by LCM compared to ESL and LIC.

Disclosures: **P. Ghisdal:** A. Employment/Salary (full or part-time):; UCB BioPharma SPRL. **N. Noel:** A. Employment/Salary (full or part-time):; UCB BioPharma SPRL. **Y. Quesnel:** A. Employment/Salary (full or part-time):; UCB BioPharma SPRL. **I. Niespodziany:** A. Employment/Salary (full or part-time):; UCB BioPharma SPRL. **C. Wolff:** A. Employment/Salary (full or part-time):; UCB BioPharma SPRL.

Poster

521. Anticonvulsant and Antiepileptic Therapies

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 521.13/Z34

Topic: C.07. Epilepsy

Title: Anticonvulsant effect of three artificial sweeteners on Pentylenetetrazole-induced seizures in mice

Authors: *B. I. ALDANA[1], A. ENRIQUE[2], A. TALEVI[2], L. BRUNO-BLANCH[2], L. ROCHA[1]
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Abstract: Antiepileptic drugs (AEDs) with higher efficacy and lower toxicity are urgently needed as it is estimated that available medication does not adequately control seizures in up to 30% of individuals with epilepsy. In the search for new AEDs, a virtual screening campaign recently predicted several artificial sweeteners as potential anticonvulsants. The prediction was experimentally validated here by testing three artificial sweeteners (sodium cyclamate, saccharin, and acesulfame potassium) in the model of acute generalized tonic-clonic seizures induced by pentylenetetrazole (PTZ) in the mouse. With this purpose, the behavioral changes following PTZ administration at a convulsive dose of 60 mg/kg i.p. were visually evaluated during 30 min in animals pretreated i.p. either with the tree sweeteners at increasing doses or with the vehicle used to dissolve them (70% H2O/ 30% PEG 400). We found that cyclamate at the dose of 10 mg/kg increased the latency (293%) to the first myoclonic seizure induced by PTZ, compared with vehicle. At the dose of 30 mg/kg, cyclamate increased the latency to both myoclonic (285%) and generalized tonic-clonic seizure (434%); and avoided the generalized tonic-clonic seizures in 82% of the animals. Saccharin at a dose of 10 mg/kg increased the latency to PTZ-induced myoclonic (456%) and tonic-clonic (422%) seizures, and avoided the generalized seizures in 43% of the animals. Finally, acesulfame at a dose of 10 mg/kg, increased the latency to

myoclonic (403%) and tonic-clonic (274%) seizures induced by PTZ. In summary, the three artificial sweeteners studied here induce anticonvulsant effects being cyclamate the most effective of the compounds to reduce PTZ-induced seizures. Considering these results, it would be worthwhile to investigate the effect of these compounds in animal models of epilepsy.

Disclosures: B.I. Aldana: None. **A. Enrique:** None. **A. Talevi:** None. **L. Bruno-Blanch:** None. **L. Rocha:** None.

Poster

521. Anticonvulsant and Antiepileptic Therapies

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 521.14/Z35

Topic: C.07. Epilepsy

Support: FAPESP

CAPES

CNPq

INNT

CINAPSE

Title: Fish oil supplementation decreased inflammatory markers in animal model of epilepsy

Authors: *F. SCORZA[1], M. NEJM[1], A. HAIDAR[1], M. MARQUES[1], A. HIRATA[1], M. NAFFAH-MAZACORATTI[1], R. CYSNEIROS[2], E. CAVALHEIRO[1]
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Abstract: Sudden unexpected death in epilepsy (SUDEP) is the most common cause of death related in people with epilepsy. Its cause is still unknown. A number of studies have shown that pro-inflammatory and anti-inflammatory interleukins are able to orchestrate inflammatory responses in the heart. Regarding the importance of omega-3 on inflammation and immunomodulation we suggested that fish oil treatment could promote a reduction on pro-inflammatory interleukins expression in heart of rats with epilepsy. After 3h of SE the animals were randomly separated into the following groups: animals with epilepsy treated daily with vehicle (PV) or with 85mg/kg of fish oil (PO85) and control animals treated daily with vehicle

(CV) or with 85mg/kg of fish oil (CO85). After 90 days, it was investigated protein expression (western blot) of IL-6, IL-1β and TNF-α and protein level (ELISA) of IL-6 on the heart. The IL-6 expression was significantly higher in experimental group as compared to control ($F(1,12)=40.3$; $p<0.0001$) and fish oil treatment decreased it ($F(1,12)=6.8$; $p<0.05$) with no interaction between factors. The protein expression of TNF-α was significantly higher in experimental group as compared to control ($F(1,12)=5.6$; $p<0.05$), with no effect of treatment nor interaction between factors. The epileptic condition or treatment did not change the IL-1β expression. The IL-6 levels was significantly higher in experimental group comparatively to control ($F(1;16)=5.7$; $p<0.05$) and decreased under fish oil treatment ($F(1;16)=16.2$; $p<0.01$), with no interaction between factors. Proinflammatory cytokines are increased in heart of epileptic animals and fish oil decreased these cytokines, which could contribute to reducing the incidence of SUDEP.

Disclosures: F. Scorza: None. **M. Nejm:** None. **A. Haidar:** None. **M. Marques:** None. **A. Hirata:** None. **M. Naffah-Mazacoratti:** None. **R. Cysneiros:** None. **E. Cavalheiro:** None.

Poster

521. Anticonvulsant and Antiepileptic Therapies

Location: Halls A-C

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Program#/Poster#: 521.15/Z36

Topic: C.07. Epilepsy

Support: FAPESP

CAPES

CNPq

Title: Oxidative stress in animal model of epilepsy induced by pilocarpine: Effect fish oil supplementation

Authors: *M. B. NEJM[1], A. HAIDAR[1], A. HIRATA[1], R. CYSNEIROS[2], E. CAVALHEIRO[1], F. SCORZA[1]
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Abstract: Temporal lobe epilepsy (TLE) is the most common form of epilepsy. Brain injury observed in TLE may be the result of an overproduction of free radicals. NADPH oxidase

enzyme has emerged as a potential source of reactive oxygen species (ROS) generation in neurons. Previous studies of our group demonstrated that chronic treatment with fish oil in animal model of TLE exhibited neuroprotective and neuroplastic effects. This study evaluate if NAD(P)H oxidase expression is altered in TLE and if fish oil supplementation can exhibits protection effect against its ROS production. Animals were subjected to the animal model of TLE by pilocarpine administration (350 mg/kg, i.p) and the controls received saline. After 3h of SE the animals were randomly divided into the following groups: animals with epilepsy treated daily with vehicle (PV) or with 85mg/kg of fish oil (PO85) and control animals treated daily with vehicle (CV) or with 85mg/kg of fish oil (CO85). After 90 days, it was investigated the superoxide anion production using dihydroethidium (DHE) fluorescence dye in hippocampus, protein expression of NAD(P)H oxidase subunits (p47PHOX and gp91PHOX) by western blot and its RNAm expression. The superoxide anion production was significantly higher in CA1 and CA3 fields of the hippocampal formation of experimental group as compared to control ($F(1;16)=12.8$; $p<0.05$ and $F(1;16)=5.2$, $p<0.05$, respectively). The fish oil treatment significantly decreased the superoxide anion production as compared to vehicle in CA1 and CA3 fields of the hippocampal formation ($F(1;16)=5.7$; $p<0.05$ and $F(1;16)=5.8$, $p<0.05$, respectively). The protein expression of NAD(P)H oxidase subunit p47PHOX was significantly higher in experimental group as compared to control ($F(1;12)=8.3$; $p<0.05$), with no effect of treatment ($F(1;12)=1.5$; NS) nor interaction between factors. For the gp91PHOX expression, a significant effect of interaction was noted ($F(1;12)=6.6$; $p<0.05$). The gp91PHOX expression increased in the experimental group and decreased under the fish oil treatment. It was not found alteration on gene expression. The results shows that the expression of NADP(H) oxidase is increased in hippocampus of epileptic animals, being only mildly affected by the fish oil treatment and that the superoxide anion production increased in epileptic hippocampus and decreased by fish oil treatment.

Disclosures: **M.B. Nejm:** None. **A. Haidar:** None. **A. Hirata:** None. **R. Cysneiros:** None. **E. Cavalheiro:** None. **F. Scorza:** None.

Poster

521. Anticonvulsant and Antiepileptic Therapies

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 521.16/AA1

Topic: C.07. Epilepsy

Title: Effect of the most commonly-used antiepileptic drugs on the MTLE mouse model of human temporal lobe epilepsy: An EEG study

Authors: *C. ROUCARD[1], B. POUYATOS[1], C. BOUYSSIÈRES[1], C. DUMONT[1], A. DEPAULIS[2], V. DUVEAU[1]
[1]SYNAPCELL, La Tronche, France; [2]INSERM U836 Grenoble Inst. of neurosciences, Grenoble, France

Abstract: Mesial Temporal Lobe Epilepsy (MTLE) is the most common form of drug-refractory epilepsy. To better understand and treat this syndrome, the use of predictive drug-resistant animal models is mandatory. The MTLE mouse model induced by unilateral intrahippocampal injection of kainate reproduces most of the morphological and electroclinical features of human MTLE. Here, we present a comprehensive pharmacological characterization of the response of the MTLE mouse to the major antiepileptic drugs (AEDs) currently on the market. Using depth EEG recordings, we tested the dose-response effects of nine AEDs with different mechanisms of action on the occurrence of hippocampal paroxysmal discharges in the mouse model of MTLE. AEDs effects on ictal and interictal power spectra were as well studied using quantitative EEG methods. The MTLE mouse displayed a wide range of sensitivity to the tested AEDs. Depending on their effects on our model, AEDs can be classified in two categories. 1) Valproate, carbamazepine, lamotrigine and levetiracetam suppressed HPDs in a dose-dependent way, but only at high doses (10 to 20 times those used in patients) and associated with modifications of the general behaviour and/or EEG interictal activity for most of them. 2) Phenobarbital, pregabalin, tiagabine, vigabatrin and diazepam decreased in a dose-dependent manner the occurrence of HPDs at doses similar to the ones used in clinical practice and without obvious behavioural changes or interictal EEG perturbations. These data show that this mouse model of MTLE displays a specific pharmacology, with a resistance to several AEDs and sensitivity to others. This model provides a critical tool to identify new treatment for pharmacoresistant forms of focal epilepsy.

Disclosures: C. Roucard: None. **B. Pouyatos:** None. **C. Bouyssières:** None. **C. Dumont:** None. **A. Depaulis:** None. **V. Duveau:** None.

Poster

521. Anticonvulsant and Antiepileptic Therapies

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 521.17/AA2

Topic: C.07. Epilepsy

Title: Celecoxib reduced seizure activity and mrna expression of hmgb1 and tlr-4, in a model of recurrent seizures induced with kainic acid in developing rats

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Abstract: Clinical and experimental evidence establishes inflammation as a key factor in epileptogenesis; it promotes in the brain an increase in neuronal excitability, reduces the seizure threshold and it's involved in molecular and structural changes of epileptogenesis. The functional role of brain inflammation has been influenced by the use of anti-inflammatory and inmunosupressive treatments during neonatal period, when the brain is more susceptible due to the anatomical and physiological features that facilitate the development of seizures. The aim of the present study was to evaluate the effect of the anti-inflammatory drug, celecoxib, on the mRNA expression of HMGB1 and TLR-4, and seizure activity in a model of recurrent seizures produced by kainic acid (AK) which induces epileptogenesis in developing rats. 48 male Sprague Dawley rats 10 days old were used. (20-25g).They were divided into six groups: Sham group (GC), Kainic Acid group (GAK), Celecoxib group (CCX), Experimental group 1 (AK+CCX), Experimental group 2 (AK+PB), Experimental group 3 (AK+CCX+PB). The AK was administred for 5 days (1.4mg/kg), to induce tonic-clonic seizures. Seizure activity was assessed during 60 minutes evaluation after administration of AK in each treatment. The hippocampus and cerebral cortex was removed and the RNA was extracted (Trizol® Reagent Protocol). cDNA was synthesized for analysis of gene expression by PCR. The results showed that celecoxib (AK+CCX) reduces the latency and frequency of tonic and tonic-clonic seizures. It also appeared to reduce the expression of the genes of proinflammatory proteins compared with AK group, in cerebral cortex and hippocampus ($p<0.05$). The results show that celecoxib has a neuroprotective effect on seizure activity and on the expression of proinflammatory proteins, in agreement with the hypothesis of the role of inflammation in epileptogenesis.

Disclosures: **M. Morales:** None. **I.A. Feria:** None. **A. Vega:** None. **S.A. Orozco:** None.

Poster

521. Anticonvulsant and Antiepileptic Therapies

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Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 521.18/AA3

Topic: C.07. Epilepsy

Support: JSPS 24590114

Title: Metabolic control of intractable epilepsy by LDH enzyme

Authors: N. YOSHIDA, *T. INOUE
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Abstract: Epilepsy is a neurological disorder caused by hyperexcitation of neurons in the brain. About 30% of epilepsy patients suffer from intractable drug-resistant epilepsy. To date, it is known that ketogenic diets are effective for intractable epilepsy. However, there are no antiepileptic drugs that mimic ketogenic diets. In this study, we therefore explored a metabolic enzyme which controls intractable epilepsy. To address this issue, we first explored a metabolic enzyme to control membrane potentials in hippocampal and subthalamic neurons, using slice patch-clamp techniques. Our *in vitro* recording revealed that neurons are hyperpolarized by application of oxamate, an LDH inhibitor. We then examined effects of LDH inhibition on seizure mouse models. *In vivo* recording revealed that LDH inhibition suppresses pilocarpine-induced generalized seizures. LDH inhibition also suppressed intractable epilepsy in a model of hippocampal sclerosis. We then explored clinically-used antiepileptic drugs that can inhibit the LDH enzyme. Enzymatic assay revealed that stiripentol, an orphan drug for Dravet syndrome, is a LDH inhibitor. These results show that LDH inhibition controls electrical activity in neurons and suppresses intractable epilepsy *in vivo*, and also that the LDH enzyme is inhibited by antiepileptic drug stiripentol.

Disclosures: N. Yoshida: None. **T. Inoue:** None.

Poster

521. Anticonvulsant and Antiepileptic Therapies

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 521.19/AA4

Topic: C.07. Epilepsy

Title: Targeting GSK-3 in the 6-Hz corneal kindling model

Authors: *I. J. SMOLDERS[1], N. AOURZ[1], L. WALRAVE[1], A. MASSIE[1], A. VAN EECKHAUT[1], C. V. ESGUERRA[2], P. A. M. DE WITTE[2], Y. MICHOTTE[1], A. D. CRAWFORD[2,3], F. VAN LEUVEN[2]

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Abstract: GSK-3 is widely recognized as a key component of a surprisingly large number of cellular processes and is a tempting therapeutic target for several neurological and neurodegenerative disorders. Less than a handful of studies have linked increased GSK-3 activity to epileptic phenomena. Nevertheless, effects of GSK-3 pharmacological inhibition, of genetic deletion or overexpression in a model for epilepsy have never been investigated to date. We used the chronic 6-Hz corneal kindling model in which mice were stimulated (6 Hz, 3 s) twice daily at a predetermined fixed subconvulsive threshold current. Mice are considered kindled when they exhibit at least 10 consecutive generalized seizures (stage 3-5 Racine's scale). The selective and potent GSK-3 inhibitor BIO-acetoxime significantly reduced seizure severity in 6-Hz corneally kindled mice in a dose dependent manner. As such we here provide the first evidence for anticonvulsant actions of a selective GSK-3 inhibitor. We also compared differences in seizure susceptibility between GSK-3β neuronal knockout (GSK-3β $^{-/-}$) mice and their wild type littermates (GSK-3β $^{+/+}$), and between GSK-3β neuronal overexpression (GSK-3β OE) mice and their corresponding wild types (WT) in the same electrical kindling model. Although GSK-3β OE mice are more rapidly kindled, they display less severe seizures compared to their corresponding WT mice. Surprisingly, compared to GSK-3β $^{+/+}$ mice, GSK-3β $^{-/-}$ mice clearly show an increased susceptibility to the electrically-induced seizures. This observation raises the hypothesis that GSK-3β $^{-/-}$ mice develop a compensatory mechanism that might be responsible for their higher seizure susceptibility. Altogether our data sustain that GSK-3 is a complex and multi-faceted enzyme and that its potential role in epilepsy mechanisms is exciting and deserves further investigations.

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Poster

522. Traumatic Brain Injury: Mechanisms and Therapeutics I

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Topic: C.10. Trauma

Support: Veteran's Affairs Office of Research and Development Medical Research Service

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University of Washington Royal Research Fund

Title: Real-time *in vivo* imaging of blast-induced mild traumatic brain injury reveals focal microglial activation, associated blood-brain barrier permeability and elevated cytokine responses

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Abstract: Introduction: Many soldiers deployed to modern military conflicts have experienced repetitive mild traumatic brain injuries (mTBIs) caused by blast overpressure (BOP) events. There is great concern that such repetitive blast exposure may be similar to the repetitive subconcussive impacts that are common among boxers and football players that develop chronic traumatic encephalopathy (CTE). A growing body of evidence argues that persistent neuroinflammation plays a role in the pathophysiology of repetitive blast mTBI. In order to better understand the mechanism of pathogenesis in blast-induced mTBI we have developed a murine model of blast-mTBI and have used this model to evaluate microglia in the setting of single and repetitive blast exposure. Methods: *In vivo* experiments were performed with non-targeted Qdots 655 delivered intravenously to male CX3CR1-GFP+/- mice immediately prior to single BOP events. Repetitive blast exposure consisted of 3 BOP events with an interval of 24 hours between each blast. Following BOP exposure or sham treatment a thinned skull cranial window was introduced 2 mm posterior to the bregma and 2 mm lateral to the midline. Anesthetized mice were imaged with two-photon scanning confocal microscopy for periods of up to 6 hours. Morphologic and biochemical analyses were carried out using standard immunostaining and western blot methods. Results: At BOPs of 17 psi (7ms positive phase) 100% of the mice survived both single and repetitive blast exposure. Two photon imaging revealed increased regional blood-brain barrier permeability with increased Qdot florescence in the perivascular parenchyma, with markedly increased microglial uptake of Qdots in 8/8 BOP exposed mice. Microglia demonstrated a range of changes associated with activation including hyper- and de-ramified morphologies. Immunofluorescence microscopy of 50 um perfusion fixed sections demonstrated microglial morphologic changes (Iba-1 stain) after a single mild BOP exposure. Microglial processes (filaments) were shorter, occupy less volume and had a smaller surface area indicating partial activation. After a single blast exposure there is an increase in TNF-α at 24 hours. After two weeks only mice exposed to repetitive blast had significantly elevated TNF-α. Conclusion: These findings indicate repetitive blast results in persistent microglial activation and suggests that each additional blast exposure can enhance

expression of inflammatory cytokines from primed microglia leading to persistent elevation of inflammatory cytokines and microglial morphological changes long after the initial injury.

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Poster

522. Traumatic Brain Injury: Mechanisms and Therapeutics I

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

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Topic: C.10. Trauma

Support: Department of Veterans Affairs (Veterans Health Administration, Office of Research and Development, Biomedical Laboratory Research and Development and Rehabilitation Research and Development)

Bay Pines Foundation

Florida Department of Health James and Esther King Biomedical Research Program

Title: Potential neuroprotective benefits of coffee nano-particles in a mouse model of closed head traumatic brain injury: A morphometric analysis of dendritic parameters

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Abstract: Millions of traumatic brain injuries (TBI) occur every year, 75% of which are considered mild traumatic brain injuries (mTBI). mTBIs are often seen in athletes, military personnel, and people involved in accidents. The cognitive effects of TBI are still untreatable. Neuronal loss and functional deficits are common attributes. Previous studies have suggested

that coffee/caffeine can have neuroprotective, and even therapeutic, potential for brain diseases such as Alzheimer's disease. Here, we investigated whether the beneficial effects of coffee may extend to treatments of mild TBI. There were 4 groups of mice: (1) vehicle + sham TBI; (2) vehicle + TBI; (3) coffee-treated + sham TBI; (4) coffee-treated + TBI. The coffee-treated mice were administered coffee nanoparticles (i.p.) at 30 minutes, 3 days, and 7 days following a mild TBI (a single 50 g weight drop-induced impact to the skull overlying the right temporal cortex). Brains were harvested 30 days following TBI. The Golgi impregnation method was used to visualize cortical layer V pyramids of the parietal cortex and the granule cells of the hippocampus for quantitative analysis of dendritic branching. Results showed that in the neocortex the dendritic domains of the layer V pyramids were significantly larger in both coffee groups (e.g., coffee + TBI and coffee + sham), suggesting either neuronal plasticity caused by the coffee nano-particles and/or compensatory dendritic hypertrophy associated with TBI-related neuronal death. Either - or both - events can occur independently of each other. In the hippocampus, both of the TBI groups had significantly less dendritic arbor than either of the sham TBI groups. This suggests TBI-related granule cell dendritic atrophy, but this cell population fails to exhibit any signs of neuroprotective properties in the coffee groups. Future stereology studies are planned to elucidate neuronal loss and compensatory mechanisms.

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Poster

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VA Career Development Award-2

Title: Developmental changes in adult-born neurons following controlled cortical impact injury

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Abstract: Traumatic brain injury (TBI) often results in long-term cognitive impairment. In mammals, the generation of adult-born hippocampal neurons persists into late adulthood, and experimental TBI increases the number of these newly-generated dentate granule cells. Because hippocampal neurogenesis is important for certain forms of memory, TBI-induced neurogenesis could reflect a compensatory mechanism contributing to cognitive recovery. However, it is unknown whether TBI-induced adult-born neurons functionally integrate into the existing hippocampal network. Additionally, as hippocampal neurons born after experimental seizures demonstrate altered maturation and integration, it is possible that neurons born after TBI aberrantly wire into the hippocampus. Here, we used 2-month-old male and female POMC-EGFP (C57Bl/6J) mice, which express EGFP in young adult-born hippocampal granule cells, to examine the development and integration of adult-born neurons following a controlled cortical impact injury (CCI). Mice were anesthetized with isoflurane and a 4 mm craniotomy was made on the right hemisphere between bregma and lambda. A 0.9 mm cortical deformation (speed 4.4 msec/ 3mm diameter) was made using an electromagnetic impact device in accordance with IACUC-approved protocols. Sham mice were anesthetized but did not undergo craniotomy or injury. Consistent with previous reports, the number of newborn neurons increased two weeks post-injury. Moreover, CCI enhanced the migration and dendritic arborization of adult-born dentate granule cells compared to sham-treated mice, suggesting that CCI alters the development of these new neurons. Whole-cell recordings demonstrated that these adult-born neurons integrate into the hippocampus. Their functional impact on the hippocampal circuitry is under investigation.

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Poster

522. Traumatic Brain Injury: Mechanisms and Therapeutics I

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Support: Department of Veterans Affairs (Veterans Health Administration, Office of Research and Development, Biomedical Laboratory Research and Development and Rehabilitation Research and Development)

Bay Pines Foundation

Florida Department of Health James and Esther King Biomedical Research Program

Title: Modulation of neuroprotective signaling and morphological changes in neurons after mild traumatic brain injury and treatment in mice

Authors: *B. A. CITRON[1,2], R. F. MERVIS[4,3], S. K. FOLEY[4,3], L. RACHMANY[5], V. RUBOVITCH[5], C. G. PICK[5], J. N. CHANG[1,2]
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Abstract: At least 0.5% of the population sustains a traumatic brain injury each year and the incidence is 30 times greater among deployed military service personnel. The majority of TBIs are mild and these can result in deleterious cognitive effects for which there are currently no effective treatments. Our targeting of specific transcription factors has produced improved outcomes after mild TBI. We have now characterized changes associated with neuronal health at the intracellular level and by measurements of changes in the dendritic arbors of neurons. Several different transcription factor modulators that we have tested have proven to be neuroprotective in an *in vitro* TBI model. For example, inhibiting p53, or c-Jun or upregulating PPARγ or Nrf2 has resulted in improved health of model neurons after injury. With a weight drop induced-closed head injury model that includes rotation we have significantly reduced memory loss by treatment with tBHQ (tertiary butylhydroquinone) to increase the activation of an inflammatory responsive transcription factor, Nrf2, beginning 30 minutes after injury and identified factors, e.g., HSP70, that participate in the neuroprotection. We will report the effects of the injury and treatment on dendrites and spines in the hippocampus and cortex, characterized by Golgi staining. To help distinguish between compensatory hypertrophy or preferential susceptibility that could account for phenomena such as higher levels of branching complexity that we found after TBI, but not after TBI plus treatment, we are complementing these examinations with stereological counts of the neurons. Overall, our goal is to help identify therapeutic approaches that would benefit individuals suffering mild traumatic brain injury.

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Poster

522. Traumatic Brain Injury: Mechanisms and Therapeutics I

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Topic: C.10. Trauma

Support: CNRM funding

Title: Identification of microstructural subdomains by diffusion MRI microscopy in the perilesional cortex following controlled cortical impact in the mouse

Authors: *E. B. HUTCHINSON[1], S. SCHWERIN[2], E. SHINDELL[2], M. KOMLOSH[1], A. AVRAM[1], S. JULIANO[2], C. PIERPAOLI[1]
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Abstract: Objectives: Following focal brain trauma, multiple and evolving cellular and tissue changes affect the microstructure of the perilesional brain tissue. Some of these changes indicate damage while others are associated with a protective or regenerative response. The purpose of this work was to use high resolution diffusion MRI of ex-vivo mouse brains taken at variable times following controlled cortical impact (CCI) to identify distinct MRI markers of tissue changes, validate them with histology in the same brains and characterize their spatial pattern and evolution after injury. Methods: A total of 19 perfusion-fixed mouse brains were obtained following mild CCI at 24 hours, 1 week, 4 weeks or 12 weeks, controls were surgically naive. For each brain, 297 diffusion weighted MRI volumes were collected with b-values ranging from 100 to 10,000 s/mm2. The resolution of these images was 100 microns isotropic. Standard diffusion tensor imaging (DTI) maps of fractional anisotropy (FA) and mean diffusion (MD) were calculated as well as mean apparent propagator (MAP) index maps, including return to the origin probability (RTOP), which probes water restriction. Histological processing is ongoing. Results: Both DTI and MAP-MRI revealed spatially distinct and highly reproducible abnormalities in the perilesional cortex (PLCX) following injury. Abnormal MD values were most prominent at 24 hours post-CCI with a central region of high MD surrounded by a region of low MD that extended for several mm. At the edge of this low MD region there was a distinct thin band of high RTOP. At later time points MD was low and RTOP was high in a thin band near the cavity. FA abnormalities were non-overlapping with MD and RTOP abnormalities. At

24 hours, FA was low on the side of injury, but at 1 week a distinct patch of increased FA was evident. At 4 and 12 weeks all brains demonstrated a region of high FA that encapsulated the cavity and extended several mm in all directions. Tissue orientation within this region was radial to the cavity wall and comparison with glial fibrillary protein (GFAP) indicated only partial overlap of oriented astrocytes and high FA. Conclusions: We have used high-resolution diffusion MRI to identify subregions within the perilesional cortex that differ in diffusion based metrics. In particular, MD, FA and RTOP appear to offer complementary and non-overlapping information about the altered structural environment. Ongoing histological validation promises to better define the source of diffusion changes in the identified subdomains. In this study, diffusion MRI microscopy has demonstrated sensitivity to the spatial organization of cellular changes that follow brain injury.

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Poster

522. Traumatic Brain Injury: Mechanisms and Therapeutics I

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Topic: C.10. Trauma

Support: CURE Taking Flight Award

VA RRD Merit

Title: Multi-channel neurophysiology of the hippocampus in awake behaving swine after diffuse brain injury

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Abstract: We have recently developed acute recording methodology to interrogate hippocampal circuitry post diffuse brain injury in a swine model of rotational injury. However, these investigations are inherently limited by the anesthetized preparation. Furthermore, as diffuse TBI is predominantly a disease of the white matter, understanding how network level interactions

break down after injury is critical. Therefore we are developing a method of stereotactic multi-channel electrode implantation in order to examine these networks in swine and correlate activity to behavior post injury. To replicate the range of forces experienced at different injury levels by the brain during mild traumatic brain injury (mTBI), we utilize a range of coronal rotational accelerations (180260 rads/sec) that induce little or no loss of consciousness (< 15 min), yet exhibit axonal pathology. Acute multi-channel recordings in the hippocampus post injury revealed changes in synaptic function and excitability under anesthesia at terminal time points up to 14 days, as well as after repetitive injury. In order to capture both single unit activity and local field structure in the hippocampus, we developed a 32-channel electrode with an ideal configuration for the laminar structure of the hippocampus. Tetrodes are arranged in layer CA1 as well as in the dentate and hilus, with other contacts equally spaced in dendritic layers to examine changes in laminar structure and cortical EEG. We then modified a large animal stereotax to include skull pins for stereotaxic placement of this chronically implantable probe after injury. A surgical approach for sterile implantation was developed including a burr-hole craniotomy, and mapping of the hippocampus using tungsten electrodes prior to probe placement. Fixation of the connector and cable were achieved with Geristore acrylic, while leaving the electrode implanted in the brain parenchyma. Units >150uV were detected on the tetrodes and clearly separable. Unit and field recordings prior to and post electrode fixation were stable across this time period. Further histopathological examination will indicate potential tissue reaction to this implantation over the recording period. Successful development of this technique, combining awake behaving recordings with this injury model, may reveal changes in network function due to previously reported changes in hyperexcitability and axonal injury after diffuse brain injury. Furthermore, this paradigm may be useful in the future for multiple brain regions to correlate activity with behavioral changes post injury, potentially revealing network dysfunction underlying cognitive changes after mTBI.

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Poster

522. Traumatic Brain Injury: Mechanisms and Therapeutics I

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Topic: C.10. Trauma

Title: Neuropathology and behavioral changes of inflicted traumatic brain injury in developing mice

Authors: *G. WANG[1,2], Y. ZHANG[3], X. YANG[1], F. LI[4], M. CURRIE[2], T. MORIARTY[3], Z. GAO[5], R. WU[2], C. B. SHIELDS[3], J. CAI[2]
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Abstract: Animal models of abusive head trauma (AHT), especially for rotational-acceleration traumatic brain injury (TBI), are essential for testing novel hypotheses, pathological mechanisms and therapeutic interventions. Unfortunately the modeling of this unique TBI was not well developed. In this study, we introduced a new mouse model to generate rotational-acceleration brain injury. Eight postnatal day12 (P12) C57/BL6 mouse pups were culled from each litter and divided into two groups for exposure either to inflicted insult or to sham treatment as control. The animal was placed into an anesthesia box for 3 minutes that filled with 3% isoflurane that vaporized by 100% oxygen. The insulted mouse lay on a horizontal plate in a prone position and its head was firmly fixed by a rubber band in a head holder. The head holder was rotated when hit by a piston. The velocity of rotational acceleration and angle were adjusted by pneumatic pressure and moving distance of piston. One time rotational acceleration procedure was profiled as 60 psi for 60 times with ±35° rotational angle. The recovery of righting reflex was measured followed by whole brain dissection or behavioral examinations till P42 including rotarod, open field, elevated plus maze and Y maze tests. The recovery of righting reflex indicated that the animals insulted by repeated rotations needed more time to recover from the unconscious state. The subarachnoidal hemorrhage and BBB leak were found in the injured pups. Furthermore, the rotarod performance was significant lower than sham mice at P21 but completely recovered at late stages. However, the exploring function tested by Y maze dramatically declined at P40, not early stages. These findings collectively suggest that the inflicted traumatic brain injury may result in locomotor and cognitive deficits in developing mouse brain.

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Poster

522. Traumatic Brain Injury: Mechanisms and Therapeutics I

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Topic: C.10. Trauma

Title: Pairing vagus nerve stimulation with rehabilitative training enhances functional recovery after traumatic brain injury

Authors: *D. PRUITT, A. SCHMID, C. CHOUA, L. KIM, J. TRIEU, C. ABE, K. FLANAGAN, M. KILGARD, R. L. RENNAKER, II
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Abstract: Traumatic Brain Injury (TBI) is one of the largest health problems in the United States, and affects nearly two million people every year. The effects of TBI, including weakness and loss of coordination, can be observed years after the initial injury. We have developed a method by which we drive cortical plasticity through stimulation of the vagus nerve during rehabilitative therapy to enhance recovery from TBI. We trained rats to perform the isometric pull task - a task that measures volitional pull strength. After animals were proficient at the task they received a controlled-cortical impact (CCI) in the forelimb area of left motor cortex, and were then randomized into two treatment groups. The first group of animals received vagus nerve stimulation (VNS) paired with rehabilitative therapy, while another group received rehabilitative therapy alone. Following CCI, volitional forelimb strength in all animals was significantly reduced. Animals that received VNS paired with rehabilitative therapy over a period of five weeks achieved a significant but not a full recovery of both forelimb strength and success rate on the isometric pull task, while animals that received only rehabilitative training did not significantly recover forelimb strength. Our findings indicate that pairing VNS with rehabilitative therapy enhances functional recovery, and further research is warranted to investigate how VNS may transfer to clinical settings.

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Poster

522. Traumatic Brain Injury: Mechanisms and Therapeutics I

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CSTS

Title: Mitochondrial gene expression profiles and metabolic pathways in the amygdala associated with exaggerated fear in an animal model of PTSD

Authors: *H. LI[1], X. LI[2], S. SMERIN[1], L. ZHANG[1], M. JIA[1], G. XING[1], J. WEN[1], Y. SU[3], D. BENEDEK[1], R. URSANO[1]
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Abstract: The metabolic mechanisms underlying the development of exaggerated fear in post-traumatic stress disorder (PTSD) are not well defined. In the present study, alteration in the expression of genes associated with mitochondrial function in the amygdala of an animal model of PTSD was determined. Amygdala tissue samples were excised from 10 nonstressed control rats and10 stressed rats, 14 days post stress treatment.. Total RNA was isolated, cDNA was synthesized, and gene expression levels were determined using a cDNA microarray. During the development of the exaggerated fear associated with PTSD, 48 genes were found to be significantly upregulated and 37 were significantly downregulated in the amygdala complex based on stringent criteria ($p<0.01$). Ingenuity Pathway Analysis (IPA) revealed up or down regulation in the amygdala complex of four signaling networks - one associated with inflammatory and apoptotic pathways, one with immune mediators and metabolism, one with transcriptional factors, and one with chromatin remodeling. Thus, informatics of a neuronal gene array allowed us to determine the expression profile of mitochondrial genes in the amygdala complex of an animal model of PTSD. The result is a further understanding of the metabolic and neuronal signaling mechanisms associated with delayed and exaggerated fear.

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Poster

522. Traumatic Brain Injury: Mechanisms and Therapeutics I

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Topic: C.10. Trauma

Title: Effects of a blueberry-enhanced diet on pre-pulse inhibition and beta amyloid after traumatic brain injury in rats

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Abstract: Although there are multiple promising avenues of treatment for the pathologies associated with traumatic brain injury (TBI), few of these single manipulations have been successful in clinical trials, indicating a need for exploration of combined therapies, especially nutritional interventions (Erdman, Oria, & Pillsbury, 2011). Circumin, a strong antioxidant derived from the spice turmeric, normalized markers of inflammation when administered 30 minutes after controlled cortical impact TBI (Laird et al., 2010). Another promising nutritional strategy involves strong antioxidant foods such as blueberries. A blueberry-enhanced diet has been shown to be cognitively enhancing in aging, Alzheimer's, ischemia, and other studies. In this set of experiments, a 2% blueberry-enhanced diet was fed to rats for 6 weeks after they received a sham or mild TBI. Although no significant differences were found in Morris water maze (MWM) tests or acoustic startle, the blueberry-enhanced diet resulted in significant improvements in pre-pulse inhibition, a measure of cognitive gating where a warning tone dampens the startle response. Also, Beta amyloid increased in the cerebrospinal fluid of rats in this study between 3 weeks and 3 months post-injury. In a second study, the effects of a single 30-minute post-TBI circumin injection and a 2% blueberry enhanced diet beginning immediately post-TBI were combined. Additional findings on measures of MWM, acoustic startle, pre-pulse inhibition, and beta amyloid will be presented and the results will be discussed in the context of TBI pathologies such as oxidative stress and inflammation.

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Poster

522. Traumatic Brain Injury: Mechanisms and Therapeutics I

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Title: Neuropathological and biochemical assessment of chimera: A novel closed-head impact model of engineered rotational acceleration

Authors: *D. R. NAMJOSHI, W. CHENG, K. MCINNES, J. FAN, A. WILKINSON, P. CRIPTON, C. WELLINGTON
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Abstract: Background: Traumatic brain injury (TBI) is a leading cause of death and disabilities worldwide. Despite promising outcomes from many preclinical studies, clinical studies so far have failed to identify effective pharmacological therapies for TBI, suggesting that the translational potential of preclinical models requires improvement. To address the challenge of generating a simple and reliable model of rodent TBI, we have developed a novel neurotrauma model called CHIMERA (Closed-Head Impact Model of Engineered Rotational Acceleration) that fully integrates biomechanical, behavioral, and neuropathological analyses. CHIMERA is distinct from existing rodent neurotrauma models in that it uses a completely non-surgical procedure to precisely deliver impacts of defined dynamic characteristics to intact animal head while allowing unconstrained head movement. Objective: In this study we characterized the acute neuropathological and biochemical outcomes of repeated TBI (rTBI) in mice using CHIMERA. Methods: Male, wild-type mice were subjected to two closed-head impacts spaced at 24 h. Microglial response was assessed by Iba-1 immunohistochemistry while axonal injury was assessed by silver staining at 48 h post-rTBI. Levels of proinflammatory cytokines, TNFα and IL-1β were measured at 6, 12 and 48 h post-rTBI using ELISA. Phosphorylation levels of endogenous tau protein were assessed in half-brains collected at 6, 12 and 48 h post-rTBI by Western blotting. Results: Injured brains showed significant widespread microglial activation with hypertrophic and bushy morphology in white matter areas at 48 h post-rTBI. Silver staining revealed axonal injury, as indicated by intense punctate and fiber-associated argyrophilic structures in white matter. High-magnification silver-stained images revealed numerous axonal varicosities, a characteristic histological feature of human axonal pathology. Protein levels of TNFα and IL-1β showed ~ 1.7- and 2-fold increase, respectively at 48 h following rTBI. Immunoblot analysis showed significantly increased (~1.5 to 3.5-fold) tau phosphorylation at all probed epitopes (pThr231, pSer396, pSer404 and pSer202), peaking at 12 h following rTBI compared to sham brains. The change in tau phosphorylation was due to a significant increase in ratio of phosphorylated tau : total tau, but not as a result of change in total tau level. Conclusion: CHIMERA is a simple and reliable model of murine TBI that replicates several aspects of human TBI such as neuroinflammation, axonal injury as well as tau hyperphosphorylation.

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Poster

522. Traumatic Brain Injury: Mechanisms and Therapeutics I

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Topic: C.10. Trauma

Title: Repetitive mild traumatic brain injury in mice with a vulnerable cholinergic system: Severe and lasting cholinergic-attentional impairments CHT+/- mice

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Abstract: Repetitive mild traumatic brain injury (rm-TBI), as sustained by athletes and military personnel, represent one of the most common types of brain injury. Because of the predominant impairments in attention, TBI has long been hypothesized to impact cortical cholinergic functioning. We hypothesized that choline transporter (CHT) heterozygosity bestows greater vulnerability to the effects of TBI because attenuated choline transport into cholinergic terminals has previously been speculated to interact with other pathological events to facilitate cholinergic neuronal membrane decline and thus cholinergic cell loss (for review see Sarter & Parikh, 2005). CHT+/+ and CHT+/- mice acquired the mouse version of the Sustained Attention Task (SAT; St.Peters et al., 2011) and then were subjected to rm-TBI using a modification of the method described by Kane et al. (2012). Mice practiced the SAT six days a week and were subjected to five TBI events separated by seven days. Rm-TBI impaired hit rates only in CHT+/- mice, reaching and then remained at chance level after the 5th TBI. Consistent with the selective effects of cholinergic lesions on the performance in this task, non-signal trial performance was unaffected by rm-TBI. Following 7 days of SAT practice after the 5th TBI, frontal cortices were harvested for saturation analyses of hemicholinium-sensitive choline uptake in frontal synaptosomes. Results indicate that in SAT-performing CHT+/- mice that experienced rm-TBI, the capacity for choline uptake was nearly completely abolished whereas choline uptake in CHT+/+ rm-TBI mice remained above levels observed in sham-treated CHT+/- mice. These results indicate a greater rm-TBI-induced loss of behaviors, such as SAT, that depend on CHT and cholinergic signaling (Parikh et al., 2013). This evidence indicates that rm-TBI has a relatively greater impact in mice with a compromised cholinergic system, suggesting the

possibility that humans with vulnerable cholinergic systems likewise experience more severe and lasting consequences of rm-TBI.

Disclosures: **A. Koshy Cherian:** None. **V. Parikh:** None. **R.D. Blakely:** None. **M. Sarter:** None.

Poster

522. Traumatic Brain Injury: Mechanisms and Therapeutics I

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 522.13/AA17

Topic: C.10. Trauma

Support: CNRM Grant G1702577

DMRDP Grant D61_I_10_J6_214

Title: Dysfunctional CA1 hippocampal networks in mice after repetitive closed-head injury

Authors: ***O. C. LOGUE**[1], N. P. CRAMER[2], X. XU[2], Z. GALDZICKI[2]
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Abstract: Traumatic brain injury (TBI) is the leading cause of death for persons under the age of 45. Military servicemembers, who have served on multiple combat deployments, and contact-sport athletes, are at particular risk of sustaining repetitive TBI (rTBI). Cognitive and behavioral deficits resulting from rTBI are well documented. Associative long-term potentiation (LTP), occurring in the CA1 hippocampal Schaffer collateral pathway, is required for both memory formation and retrieval. Utilizing our mouse model of rTBI, we hypothesize that a triple closed-head injury (3X CHI) will trigger alterations in hippocampal function. One week after 3X CHI, extracellular recordings were performed to evoke Schaffer collateral LTP in the CA1 area. Ipsilateral CA1 LTP is enhanced in mice from the 3X CHI treatment group. The intrinsic properties of CA1 neurons were evaluated with whole-cell patch-clamp recordings. 3X CHI ipsilateral CA1 neurons exhibit significant increases in action potential amplitude, maximum rise slope, and maximum decay slope, while the action potential width at half amplitude is decreased. Gap-free voltage clamp recordings of CA1 neuron postsynaptic currents were conducted to detect spontaneous excitatory (EPSCs) and inhibitory (IPSCs) and respective miniature currents (mEPSCs and mIPSCs). In the 3X CHI mice, the sEPSCs and sIPSCs of ipsilateral CA1 neurons have an increased frequency of events but decreased peak amplitudes. Conversely, 3X CHI alters

the action potential-independent miniature postsynaptic currents in a different manner. The mEPSCs of ipsilateral CA1 neurons display both an increased frequency of events and larger peak amplitudes. Moreover, the effect of 3X CHI on mIPSCs is opposite to that of the sIPSCs. That is, the frequency of events in the mIPSCs is decreased, while the peak amplitudes are increased. These results are consistent with our hypothesis that repetitive closed-head injury may affect CA1 hippocampal function by promoting an imbalance between excitatory and inhibitory synaptic inputs.

Disclosures: O.C. Logue: None. **N.P. Cramer:** None. **X. Xu:** None. **Z. Galdzicki:** None.

Poster

522. Traumatic Brain Injury: Mechanisms and Therapeutics I

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Title: Traumatic brain injury increases chronic Tau phosphorylation in 3xTgAD mice

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Abstract: Introduction : Traumatic brain injury (TBI) is a major environmental risk factor for Alzheimer's disease (AD) and is the leading cause of disability in North America for persons under 40 years of age. Intraneuronal neurofibrillary tangles, composed of abnormally hyperphosphorylated Tau protein is one of the characteristic features of AD. Tau pathology can be found in post-mortem brain many years after a single severe or repetitive mild TBI. Since Tau

hyperphosphorylation is suggested to play a major role in AD pathogenesis, induction of Tau hyperphosphorylation after injury may play a role TBI pathology. There are reports of acute phospho-Tau accumulation after experimental TBI using a controlled cortical impact (CCI) model. Here, we examined tau and phospho-Tau accumulation in a transgenic AD mouse model 2 and 4 weeks post-CCI to determine if these acute changes can persist chronically after injury in the mouse. Methods : 9 months old 3xTgAD mice were subjected to CCI (Leica ImpactOne, 5.25m/s, 2mm depth) and were euthanized at 2 or 4 weeks post-injury (n=10/timepoint). Tau phosphorylation (at the AT270, CP13, Thr205, AT8, Tau-1, Ser199, AT180, MC-6, PHF-1 and Ser422 epitopes), APP cleavage, Tau kinases activation and phosphatases expression were examined in the ipsilateral and contralateral striatum by western blot analysis. Results : An increase in Tau phosphorylation at the Thr205 residue was observed in the ipsilateral striatum of injured mice and was associated with Tau hyperphosphorylation at the AT8 and Ser422 epitopes 4 weeks post-injury. In this side, a sustained PKA activation was observed concomitantly with an increase of the activated form of Akt at 4 weeks. In the contralateral striatum, TBI induced a transient increase in Tau phosphorylation at the AT180 and Ser422 epitopes. This Tau hyperphosphorylation was associated with a temporary activation of Akt 2 weeks after CCI. The injured mice did not show any changes in either tau phosphatase levels or APP cleavage, as compared with sham mice. Conclusion : This work provide an extensive characterization of Tau phosphorylation in the CCI mouse model. We show that CCI results in different patterns of Tau hyperphosphorylation and Tau kinases activation in the striatum of 3xTgAD mice. Our data suggest that the hyperhosphorylation of Tau found in the ipsilateral striatum could be linked to sustained PKA activation, while transient Tau hyperphosphorylation observed in the contralateral side could be related to Akt activation. Moreover, these results provide further evidence for the independent relationship between Tau hyperphosphorylation and APP processing in the TBI context.

Disclosures: **A. Noel:** None. **I. Poitras:** None. **C.N. Winston:** None. **M.P. Burns:** None. **E. Planel:** None.

Poster

522. Traumatic Brain Injury: Mechanisms and Therapeutics I

Location: Halls A-C

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Topic: C.10. Trauma

Support: Department of Veterans Affairs (RR&D Merit Review Award #B1097-I)

National Institutes of Health (NINDS T32-NS043126)

Penn's University Research Foundation

Title: Acute neuronal plasmalemmal disruptions in peri-vascular domains are exacerbated following repetitive closed head rotational acceleration traumatic brain injury in swine

Authors: E. KUO[1], C. J. MIETUS[1], K. D. BROWNE[1], J. P. HARRIS[1], L. A. STRUZYNA[1], J. A. WOLF[1], J. E. DUDA[2], *D. CULLEN[1]
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Abstract: Repetitive traumatic brain injury (TBI) may result in a progressive neurodegenerative disease referred to as chronic traumatic encephalopathy (CTE). A curious neuropathological feature of CTE is a preferential distribution of cells exhibiting intracellular tau accumulations around mid-size blood vessels. The cause of these patterns is unknown, but one possible explanation is that micro-structural discontinuities at vasculature-brain tissue interfaces result in stress concentrations that exacerbate cellular damage during traumatic loading. Therefore, the objective of the current study was to determine if perivascular neural cells exhibited elevated immediate plasmalemmal damage and evolving degeneration in a large animal model of non-impact inertial TBI. Swine were subjected to sham conditions or rapid closed-head rotational acceleration using the HYGE pneumatic actuator, either a single injury or two injuries separated by 15 minutes or 7 days. To assess plasmalemmal compromise, Lucifer Yellow (LY), a small (457 Da) cell impermeant dye, was administered into the lateral ventricles to diffuse throughout the interstitium prior to injury. Animals were sacrificed within 15 minutes (those with LY injections), 8 hours, or 7 days post-injury (n=21 total). We found that closed-head inertial TBI induced acute plasmalemmal permeability in neural cells, virtually exclusively in neurons. In particular, LY+ cells were concentrated around blood vessels in the cerebral cortex, sub-cortical white matter and thalamus/midbrain following single or repetitive TBI (each $p<0.05$ vs. sham). Maximal perivascular cell permeability was observed following repetitive TBI separated by 7 days ($p<0.01$). This exacerbated cell permeability following delayed repetitive TBI corresponded with dramatic increases in astrocyte reactivity around blood vessels, suggesting that local astogliotic changes may create additional stress concentrations. Remarkably, approximately 11% of blood vessels were surrounded by LY+ cells following delayed repetitive TBI, versus 6% following same-day repetitive and 4% following single TBI (each $p<0.05$ vs. sham). These results suggest that micro-structural discontinuities at vasculature-brain tissue interfaces contribute to exacerbated plasmalemmal damage following TBI. Ongoing analyses will assess the co-occurrence of plasmalemmal damage with the emergence of tau pathology and evolving neurodegeneration. These results increase our understanding of the links between the physical and physiological consequences of TBI, and may identify periods of enhanced biomechanical vulnerability in perivascular domains.

Disclosures: **E. Kuo:** None. **D. Cullen:** None. **K.D. Browne:** None. **C.J. Mietus:** None. **J.P. Harris:** None. **L.A. Struzyna:** None. **J.A. Wolf:** None. **J.E. Duda:** None.

Poster

522. Traumatic Brain Injury: Mechanisms and Therapeutics I

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Topic: C.10. Trauma

Support: Grant-in-Aid for Scientific Research (B) 23300133

Smoking Research Foundation

Title: STAT3 signaling in perilesional nestin-expressing reactive astrocyte is required for cortical recovery after closed-head injury

Authors: ***M. MORITA**[1], A. WATANABE[2]
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Abstract: We have developed a novel closed-head injury model, photo-injury, and found that nestin-expressing reactive astrocyte accumulates perilesional recovering region (Suzuki et al, 2012). In order to address the roles of the perilesional reactive astrocyte in wound healing, STAT3, which is crucial for astrocyte activation, has been ablated in nestin-expressing cells by using Nestin-Cre/STAT3$^{flox/flox}$ mouse. Mice after more than seven days post-injury showed significantly less accumulation of perilesional reactive astrocytes, and larger lesion size in the STAT3-ablated group than in wild type group. The importance of STAT3 signaling was further confirmed by ablating SOCS3, which suppress cytokine mediated STAT3 activation, in the nestin-expressing reactive astrocyte by using Nestin-Cre/SOCS3$^{flox/flox}$ mouse. The SOCS3-ablated groups showed significantly smaller lesion size after more than seven days post-injury, as well as significantly larger region covered by perilesional reactive astrocyte, than corresponding wild type group. Our results indicates that cytokine-mediated STAT3 activation in perilesionl astrocytes leads to the accumulation of nestin-expressing reactive astrocyte, and then promote wound healing following closed-head injury.

Disclosures: **M. Morita:** None. **A. Watanabe:** None.

Poster

522. Traumatic Brain Injury: Mechanisms and Therapeutics I

Location: Halls A-C

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Program#/Poster#: 522.17/AA21

Topic: C.10. Trauma

Support: VA Merit

DoD

Title: Long-term cognitive impairments and pathological alterations in a rat model of mild traumatic brain injury

Authors: *J. SHI, Y. LI, S. G. MASSA
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Abstract: Mild traumatic brain injury (mTBI) accounts for the majority of all traumatic brain injuries and long-term consequences of mTBI have become increasingly recognized in individuals engaged in certain sports or in military operations. Many mTBI patients suffer chronic neurobehavioral impairments, however the underlying mechanisms are not clear. Recent studies have suggested that loss or dysfunction of neurons and neuronal precursors, activation of microglia, and proliferation of astrocytes in the hippocampus may contribute to memory deficits after traumatic brain injury. In order to expedite preclinical research and therapy development, there is a need for animal models that reflect the long-term cognitive and pathological features seen in patients. In the present study, we developed and characterized a rat model of mTBI, induced by controlled cortical impact (CCI) over the left frontal hemisphere with an electromagnetic stereotaxic impact device. Varying several impact parameters, we evaluated animals in several behavioral tests to define a mild TBI injury level without motor impairment. Animals receiving mTBI showed a significant impairments in spatial learning and memory as well as depression-like behavior. Moreover, a robust astrogliosis and activated microglia were observed upon histopathological examinations several months following TBI. These findings are consistent with the deficits and pathology associated with mTBI in humans and suggest this model may be of value in the evaluation of potential therapeutic approaches.

Disclosures: J. Shi: None. **Y. Li:** None. **S.G. Massa:** None.

Poster

522. Traumatic Brain Injury: Mechanisms and Therapeutics I

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 522.18/AA22

Topic: C.10. Trauma

Support: NIH RO1 NS079061

VA RR&D B1127-I

Title: Impaired synaptic vesicle docking is a novel contributor to reduced neurotransmission in a rat model of traumatic brain injury

Authors: *S. W. CARLSON, H. Q. YAN, C. E. DIXON
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Abstract: Traumatic brain injury (TBI) impairs neuronal function and can culminate in lasting cognitive impairment. While impaired acetylcholine release has been well established after experimental TBI, little is known about the mechanisms underlying this consequence. We hypothesized that alterations in synaptic vesicle distribution and reduced vesicular docking at the pre-synaptic membrane contribute to impaired neurotransmission. To examine the ultrastructural distribution of synaptic vesicles, anesthetized adult male Sprague-Dawley rats received 2.7mm controlled cortical impact (CCI) or sham injury (n=6/group) and the brains were processed for transmission electron microscopy at 1 week post-injury. In each animal, 20 randomly selected synaptic nerve terminals from the molecular layer of the hippocampus were imaged at 100k magnification. Synaptic vesicle distribution was assessed by measuring the distance of each vesicle from the active zone for all terminals. CCI resulted in a significant reduction in vesicle frequency within 200nm of the active zone ($p<0.01$ compared to sham, repeated one-way ANOVA). Recent reports highlight that reduced vesicular density within 100nm of the active zone impairs vesicular docking and blunts neurotransmitter release. In a normal synapse, vesicular docking and neurotransmitter release requires formation of the SNARE complex. To examine the effect of TBI on the SNARE complex, rats received CCI or sham injury and were sacrificed at 6hr, 1d, 1, 2, or 4 weeks post-injury (n=6/injury/time). Immunoblotting of unboiled hippocampal homogenates showed that SNARE complex formation, identified by SNAP-25 and syntaxin immunoreactivity, was reduced by at least 48% at 1 week ($p<0.05$) and 2 weeks ($p<0.01$) after CCI. Neurotransmitter release deficits have been well characterized at 1 and 2 weeks post-injury, suggesting that changes in synaptic vesicle docking contributes to impaired neurotransmission. In this study, we provide the first evidence that TBI alters synaptic vesicle

distribution using quantitative ultrastructural analysis of electron micrographs. Our findings suggest that reductions in the standing pool of readily releasable vesicles and impaired SNARE complex formation are two novel mechanisms that contribute to the impaired neurotransmission after TBI.

Disclosures: S.W. Carlson: None. **H.Q. Yan:** None. **C.E. Dixon:** None.

Poster

522. Traumatic Brain Injury: Mechanisms and Therapeutics I

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 522.19/AA23

Topic: C.10. Trauma

Title: Nicotinamide treatment in a bilateral frontal model of traumatic brain injury: Effects on fear conditioning and memory

Authors: *S. F. BENNETT, B. E. ELMORE, M. R. HOANE
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Abstract: In the United States 1.7 million people experience a traumatic brain injury (TBI) annually, and cognitive impairments are the leading cause of TBI-related disability. Despite the frequency of this major public health issue, there are currently no FDA approved pharmacological treatments available. Previous research has shown that a derivative of the B3 vitamin, nicotinamide (NAM), has therapeutic potential as neuroprotectant in various TBI models. The purpose of this study was to evaluate cognitive functioning and learning following NAM treatment in a bilateral frontal controlled cortical impact (CCI) model in 30 male Sprague-Dawley rats. It was hypothesized that NAM would improve recovery of function in a retrograde amnesia Morris water maze (MWM) paradigm and a passive avoidance assessment. Four days prior to surgery animals were tested in the MWM paradigm for four trials per day to assess baseline learning. On the day of surgery animals were assigned to a sham (uninjured), vehicle (0.9% saline), or NAM treatment condition (150 mg/kg). Injections were administered intraperitoneally at four hours post injury, and every 12 hours for 72 hours. The animals were tested for retention in the MWM on day 11 and tested in the passive avoidance task on days 12 and 13. Animals were perfused on day 13 and standard histological procedures were conducted to assess lesion volume. The results of this study found no therapeutic benefit from treatment in the utilized tasks. An injury effect was detected though, and these findings may contribute to the

repertoire of behavioral assessments used in TBI pharmaceutical evaluations and provide further evidence of the efficacy of NAM treatments.

Disclosures: S.F. Bennett: None. **B.E. Elmore:** None. **M.R. Hoane:** None.

Poster

522. Traumatic Brain Injury: Mechanisms and Therapeutics I

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 522.20/AA24

Topic: C.10. Trauma

Title: Effect of age on amount and distribution of diffuse axonal injury after rotational trauma

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Abstract: Traumatic brain injuries (TBI) are a major public health problem in term of suffering and cost for society. About 40% of the TBI patients admitted to hospitals are non-focal injuries, usually referred to as distributed brain injuries (DBI). Studies have hypothesized that the resulting strains in the brain tissue are the primary cause of neurological deficiencies following DBI. These strains commonly appear when the skull is accelerated and the brain mass, due to its inertia, lags behind or continues its motion relative the skull. It has been suggested that the severity of the injury correlates with the amplitude of the angular acceleration, or with the resulting angular velocity. Among DBI, diffuse axonal injury (DAI) is common and regularly results in unconsciousness or death. Past studies have suggested DAI injury criteria and thresholds that can be used with crash test dummies and mathematical models of the human. However, these past studies have been performed with rather young animals. In addition, some studies have shown that brain properties change as we grow older; it is likely that this have an effect on the risk of DAI following a rotational head injury. Therefore, the aim of this study is to investigate the distribution of axonal injuries in the brain following sagittal plane rotation trauma and to determine if the injury threshold changes when the subjects grow older. In this study rats were exposed to sagittal plane rotational acceleration head trauma and the outcome studied using Amyloid Precursor Protein to detect axonal injuries. For relatively young animals, DAI were found in and along the border of the corpus callosum and in the brainstem when rotational acceleration exceeded 1.1 Mrad/s2. Slightly older animals required higher accelerations to exhibit similar injury levels and the injury patterns were different. We hypothesise that the lower

injury scores for the older subjects could be due to differences in tolerance to tissue strains or, as indicated in the literature, that the differences were due to changes in the constitutive properties of the brain tissue. The latter suggests, in combination with the observed differences between older and younger individuals, that additional studies on brain tissue properties, and studies on rotational acceleration induced DAI, should be carried out using even younger and older animals than used in this study. In conclusion, a previous study showed that the onset of diffuse axonal injuries started to appear at 10 krad/s2 with a duration of 4 ms, when data were scaled for humans, whereas new data indicate that this onset is slightly higher for occupants that are approximately 15 years older.

Disclosures: J. Davidsson: None. **M. Angeria:** None. **M.G. Risling:** None.

Poster

522. Traumatic Brain Injury: Mechanisms and Therapeutics I

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 522.21/BB1

Topic: C.10. Trauma

Title: Mild blast-related traumatic brain injury disrupts cortical and hippocampal dendritic circuitry in a mouse model: Implications for military personnel

Authors: *S. K. FOLEY[1,2], A. YAZBACK[3], P. MULLEN[2], J. LE[2], J. HERNANDEZ[2], D. PHAM[2], K. GREENE[2], S. KHALIL[2], N. KHALIL[2], A. GOSWAMI[2], V. RUBOVITCH[4], C. G. PICK[4], R. F. MERVIS[1,5]
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Abstract: Traumatic brain injury (TBI) affects 10-20% of the veterans from the Iraq and Afghanistan wars, designating it as the signature injury of the conflict. 80% of those brain injuries are categorized as mild. Improvised explosive devices (IEDs) are often a major source of TBI to both combatants and civilians. Cognitive and emotional disorders are often a sequelae of such brain damage. The nature of blast-related TBI is unique and the neuropathology is poorly understood. Here we sought to characterize neuronal damage in a mouse model of blast TBI (bTBI) involving low-level blast pressure effects to the brain but without systemic injuries. Anesthetized 7 week-old mice were exposed to a single explosion which generated a pressure

wave of 5.5 psi (lbs/sq in) and sacrificed 72 hrs later. Using the Golgi impregnation technique, the dendritic arbor of cortical and hippocampal neurons were microscopically evaluated for alterations in dendritic branching which would reflect the extent of neuronal damage. From coded slides, dendritic branching of randomly selected Layer V and VI pyramids from the parietal and frontal cortices, along with hippocampal CA1s, were quantitatively evaluated. All neuronal populations were affected: CA1s lost 17% of their dendritic domains, cortical pyramids of the parietal cortex lost up to 30%, and cortical pyramids of the frontal cortex lost approximately 19%. Additional studies will determine if this damage is transient or long-lasting, but this is the first evidence to demonstrate that even mild blast-related TBI results in disruption of dendritic circuitry. This may serve as an anatomical basis for subsequent cognitive dysfunction and/or post-traumatic stress disorders. The implications of these findings to military who have served overseas and have been exposed to IEDs are ominous.

Disclosures: **S.K. Foley:** None. **A. Yazback:** None. **P. Mullen:** None. **J. Le:** None. **J. Hernandez:** None. **D. Pham:** None. **K. Greene:** None. **S. Khalil:** None. **N. Khalil:** None. **A. Goswami:** None. **V. Rubovitch:** None. **C.G. Pick:** None. **R.F. Mervis:** None.

Poster

522. Traumatic Brain Injury: Mechanisms and Therapeutics I

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 522.22/BB2

Topic: C.10. Trauma

Support: JO and JR Wicking Trust

Title: The effect of focal brain injury on beta–amyloid plaque deposition, inflammation and synapses in the APP/PS1 mouse model of Alzheimer's disease

Authors: *J. COLLINS, A. E. KING, A. WOODHOUSE, M. T. K. KIRKCALDIE, J. C. VICKERS
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Abstract: Purpose: Traumatic brain injury has been proposed to increase the risk of Alzheimer’s disease (AD), however, the effect of such brain damage on the onset and progression of beta-amyloid plaque pathology is not well understood. This study utilized an *in vivo* model of focal brain injury to examine how structural cortical injury may alter the onset and progression of beta-amyloid plaque deposition as well as inflammatory and synaptic changes, in

a transgenic model of AD. **Methods:** The APPswe,PSEN1dE9 (APP/PS1) transgenic AD model was used for this study. Under isoflurane anaesthesia 3- and 9-month-old transgenic (Tg) and wild type (Wt) mice were subjected to a focal brain injury involving insertion of a needle into the somatosensory neocortex, or sham surgery (3-4 mice per group), and examined at 24hrs and 7d post-injury (PI). Sections were stained with thioflavine-S (Thio-S) or immunolabelled with the antibody MOAB-2, which specifically reacts with beta-amyloid but not APP. Microglial and synaptic responses were examined by quantification of the % area occupied by Iba-1 immunopositive microglia at the injury site and the number of synaptophysin-labelled puncta near and distant from the injury site, respectively. **Results:** Focal brain injury did not induce Thio-S stained or MOAB-2-labeled plaques at either 24hrs or 7d PI in 3-month-old Tg or Wt mice. Thio-S and MOAB-2 plaque load was not significantly ($p>0.05$) altered by brain injury in 9-month-old Tg mice compared to sham animals at 24hrs PI (0.09±0.02% vs 0.1±0.04% for Thio-S, 3.24±0.68% vs 2.8±061% for MOAB-2) or 7d PI (0.14±0.07% vs 0.09±0.02% for Thio-S, 3.00±0.52% vs 2.88±0.39% for MOAB-2). At 7 days PI, there was a significant ($p<0.001$) increase in the percent cortical area occupied by Iba-1 positive microglia in injured (24.72±1.41%) compared to sham mice (10.01±0.30%). However, there was no difference in this response between Tg and Wt mice ($p>0.05$). At 24hrs PI, there was a significant reduction ($p<0.01$) in the number of synaptophysin puncta near the injury site (1001±80) as compared to sites distant to the injury (1519±49) and sham mice (1533±50). At 7d PI, there was no significant difference ($p>0.05$) in the number of puncta near (1420±53) versus distant (1527±76) to the injury site or compared to sham mice (1571±41). Again, there was no significant effect of genotype on this response ($p>0.05$). **Conclusion:** These results indicate that focal brain injury and the associated microglial response do not alter beta-amyloid plaque deposition in the APP/PS1 mouse model. Furthermore the current study demonstrates that the brains of both Wt and Tg mice are capable of substantial synaptic remodeling post-injury.

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Poster

522. Traumatic Brain Injury: Mechanisms and Therapeutics I

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 522.23/BB3

Topic: C.10. Trauma

Title: Expansion of peripheral lymphocytes following a fluid percussion traumatic brain injury in mice

Authors: *L. A. SHAPIRO[1,2,3], R. TOBIN[1], S. MUKHERJEE[1], J. KAIN[1], M. NEWELL-ROGERS[1,3]
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Abstract: Each year, traumatic Brain Injury (TBI) impacts millions of people worldwide. Despite the increased resources dedicated to understanding the complex series of physiological events that follow a TBI, effective diagnostic and treatment options are lacking. The majority of research on TBI has focused on the role of the central nervous system (CNS) in mediating disease manifestation. This "CNS-centric" approach has yielded a number of putative mechanisms involved in TBI pathogenesis. These include mechanisms of neuronal damage, dysfunction and death, loss of myelin, astrocyte and microglial contributions, neuroinflammation, endothelial cell alterations and blood brain barrier (BBB) breakdown. There is little doubt that most, if not all of these components are involved in the post-TBI phenotypes. However, accumulating evidence strongly supports peripheral contributions to the TBI conditions. It is well known that following TBI, numerous types of peripheral immune cells are mobilized and infiltrate into the CNS. Less understood is how TBI affects peripheral immune cell expansion and activation. For this study, we hypothesized that a fluid percussion TBI would induce rapid expansion and activation of B and T cells in the periphery, and that subsets of these cells would be observed in the CNS. Using flow cytometry and immunohistochemistry, our results demonstrate expansion of B and T cells in the periphery by 24 hours after injury. By 72 hours after injury, subsets of these cells are observed in the CNS. The appearance of activated T cells in both the periphery and in the CNS is highly suggestive of a switch from innate to adaptive immunity following TBI. Follow-up studies are warranted to test the putative mechanisms of these cell types on TBI-related pathology.

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Poster

522. Traumatic Brain Injury: Mechanisms and Therapeutics I

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 522.24/BB4

Topic: C.10. Trauma

Support: Emerging Technology Funds (State of Texas)

Institute for Regenerative Medicine Funds

Department of Veterans Affairs

Title: Shock waves of a single blast can cause longstanding impairments in pattern separation and other cognitive function, mood and hippocampus neurogenesis, and white matter injury

Authors: *A. K. SHETTY[1,2], V. MISHRA[1,2], B. HATTIANGADY[1,2], A. B. ROBBINS[3], M. R. MORENO[3], D. J. PROCKOP[1], R. JONES[4], A. OBENAUS[5], B. SHUAI[1,2], M. KODALI[1,2], X. RAO[1,2]

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Abstract: Mild traumatic brain injury (mTBI) can occur from an exposure to blast shock waves (BSWs). Blasts are believed to be responsible for two-thirds of the combat related mTBI occurred in Operation Iraqi Freedom and Operation Enduring Freedom. While MRI scans and other imaging studies do not typically reveal obvious brain injury at interim post-exposure time points, blast-related mTBI can lead to memory and mood dysfunction several years after the incident. Animal studies have documented several immediate changes after an exposure to shock waves of a single blast or repeated blasts, with some studies implying that cognitive impairments recover spontaneously over time. In this study, using C57BL6 mice, we ascertained long-standing effects of an exposure to shock waves of a single blast on cognitive and mood function, hippocampus neurogenesis and white matter integrity. For inducing BSW mediated mTBI, we wrapped each mouse in a flexible Kevlar after anesthesia and placed inside a Schedule 80 PVC container designed to leave only the head exposed. The container was then fixed to the distal end of the shock tube apparatus. The head was exposed once to BSWs (11.79 ± 0.10 psi) through restrained rupture of a Mylar membrane, which separated the blast tube from a high-pressure chamber. Another cohort of age-matched mice served as sham controls, which were anesthetized and placed near the shock tube to receive only the sound. Six months following the exposure, animals were examined using different behavioral tests. Mice exposed to BSWs displayed inability for pattern separation function in a behavioral test that examined their ability for discriminating distinct familiar objects placed on a different floor pattern. These animals also showed memory impairments in water maze, object-in-place and novel object recognition tests and mood dysfunction in modified novelty suppressed feeding and forced swim tests. Imaging of fixed brains *ex vivo* through diffusion tensor imaging revealed significant corpus callosum white matter alterations in a subset of mice exposed to BSWs compared to sham controls. Radial diffusivity (a marker of myelin degradation) was increased 68% bilaterally but no changes in axial (axonal injury) or mean diffusivity (global tissue measures) were observed. Relative

anisotropy (asymmetry of water mobility) was decreased 42%. Moreover, measurement of doublecortin+ newly born neurons in the dentate gyrus revealed ~33% decrease in neurogenesis. These results suggest that 6-8 months after exposure to BSWs there are significant cognitive and mood impairments associated with declined hippocampus neurogenesis and ongoing myelin degradation in the corpus callosum.

Disclosures: **A.K. Shetty:** None. **V. Mishra:** None. **B. Hattiangady:** None. **A.B. Robbins:** None. **M.R. Moreno:** None. **D.J. Prockop:** None. **R. Jones:** None. **A. Obenaus:** None. **B. Shuai:** None. **M. Kodali:** None. **X. Rao:** None.

Poster

522. Traumatic Brain Injury: Mechanisms and Therapeutics I

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 522.25/BB5

Topic: C.10. Trauma

Support: Physicians' Services Incorporated Foundation

Title: Machine learning classifiers discriminate concussed athletes from non-concussed subjects based on diffusion tensor imaging metrics of the uncinate fasciculus

Authors: P. DUFORT[1,4], R. GOSWAMI[2], M. C. TARTAGLIA[4,5,6], R. GREEN[4,7,3], A. P. CRAWLEY[4,1,8], C. H. TATOR[4,9,2], R. WENNBERG[4,6], M. KEIGHTLEY[4,11,10], *K. D. DAVIS[2,4,9]
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Abstract: **Introduction:** Concussions can result in a range of cognitive, psychiatric and physical symptoms and associated brain pathology. We recently reported that impulsivity correlated with white matter metrics of the uncinate fasciculus (UF), based on diffusion tensor imaging (DTI), in retired professional athletes (Goswami et al., 2014). However, it is not known whether DTI of the UF can be predictive of concussion history in single subjects. Here we determined if a machine learning classifier could be trained to discriminate concussed retired athletes from

healthy controls on the basis of DTI of the UF. **Methods:** DTI scans of 19 retired Canadian Football League players (50 ±12yo) with a history of concussions and 17 age-matched controls without concussion (46 ±10yo) were obtained using 3T MRI. Data were pre-processed using the standard FSL DTI pipeline and TBSS was used to generate spatial maps of the fractional anisotropy (FA), mean diffusivity (MD), axial diffusivity (AD), and radial diffusivity (RD) projected onto a common white matter skeleton. Whole-brain skeletons were restricted to the left and right UF by transforming probabilistic tractography-based segmentations of each subject's UF into standard space, averaging, thresholding and taking their intersection with the TBSS skeletons. Eight support vector machine (SVM) classifiers (4 DTI metrics x 2 hemispheres) were trained to discriminate athletes from controls based on voxels from each hemisphere's uncinate tract. Performance was optimized over algorithm meta-parameters by grid search. Leave-one-out cross validation computed accuracy and random label permutation tests assessed statistical significance. **Results:** Classifiers based on MD and RD measured at voxels of the right UF achieved statistical significance ($p=0.018$ and $p=0.04$, respectively), with an optimal operating point sensitivity/specificity of 84/82% for the former and 79/82% for the latter. The areas under the ROC curves were 0.81 and 0.80, respectively. The spatial pattern of classifier weights revealed MD and RD that were higher in concussed athletes than in controls at the orbitofrontal end of the UF, but lower in athletes compared to controls at the temporal end of the UF. **Conclusion:** These data provide evidence for the use of machine learning with DTI to assess spatial locations of white matter abnormalities in multiply-concussed athletes that may not be identifiable with standard DTI approaches. Furthermore, this approach has potential utility in the general assessment and diagnosis of brain abnormalities following concussion.

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Poster

522. Traumatic Brain Injury: Mechanisms and Therapeutics I

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 522.26/BB6

Topic: C.10. Trauma

Title: The study of correlation between TBI severity and neurofilament level in CSF

Authors: *K. SUMIYOSHI, Y. TAKASATO
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Abstract: Introduction Various biomarkers have reported to detect the severity of brain damage. The application of biomarkers is suitable for the assessment of TBI (traumatic brain injury) severity. However, this method do not detect the locational information of the injury. In this study, we examined a correlation between the neurofilament level in CSF and the ultimate severity of brain damage after TBI, and ask whether the neurofilament level enables to predict long-term consciousness state in TBI patients. Methods The patients who could not obey simple command within 3 days after head injury were examined. Those with severe local damage to the speech area, thalamus or brain stem were excluded. The CSF level of phosphorylated neurofilament H (pNF-H) were measured using ELISA at several time points from Days 1 to 70. The changes of brain parenchymal volume (BPV) and the state of consciousness were also observed. Results Five patients were observed (4 males, 1 female; age 19-73). Three of those recovered consciousness and obeyed simple command within 2 months after injury (early-recovered group), whereas, the other two still showed disturbed consciousness one year after injury (VS group). The pNF-H level showed different temporal pattern between these groups. Maximum level of pNF-H was found right after injury in early-recovered group, whereas, that in VS group was observed a few weeks after injury. The difference in reduction rate of BPV was not significant a month after injury between the groups, however the persistent reduction was also found in VS group after Day 30. Conclusion This preliminary study raises the possibility that pNF-H level in CSF enables to predict final brain damage in TBI patients. By combining this quantitative information with positional information from radiological examination, early prediction can be expected about long-term outcome of consciousness state.

Disclosures: K. Sumiyoshi: None. **Y. Takasato:** None.

Poster

522. Traumatic Brain Injury: Mechanisms and Therapeutics I

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 522.27/BB7

Topic: C.10. Trauma

Title: Neural mechanisms of recovery after mild traumatic brain injury: an fMRI study

Authors: *G. R. WYLIE[1,3,4], H. AZMI[5], C. OGEDEGBE[5], E. DOBRYAKOVA[2,3], G. VOELBEL[6], P. DAVE[5], J. FELDMAN[5]

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Abstract: Working memory (WM) refers to our ability to maintain and manipulate information 'on-line' for a short period of time, and WM is frequently affected by mild traumatic brain injury (mTBI). Two constituent processes of working memory are capacity and manipulation. While a distributed network of brain regions has been shown to be involved in information processing associated with increased capacity and manipulation in both HCs and mTBIs, these two processes are frequently confounded in working memory paradigms. In order to better understand which aspects of working memory are affected in mTBI, we used a novel WM paradigm that allowed us to independently manipulate capacity and manipulation: the CapMan paradigm. Moreover, in order to assess changes in these constituent WM processes over time, we assessed working memory performance and brain activation at two time points: within 10 days after the injury and 4 months (+/- 2 two weeks) after the injury. 11 healthy controls and 7 individuals with mTBI participated in the current study. fMRI data was acquired with a 3T Siemens Trio scanner. The paradigm was presented in the scanner in a blocked design using E-Prime software. There were four task blocks, each lasting 64 seconds and each composed of four trials/conditions (high capacity and manipulation: CAP-MAN; high capacity, low manipulation: CAP-man; low capacity, high manipulation: cap-MAN; low capacity and manipulation: cap-man). fMRI data was analyzed with a 2x2x2x2 mixed ANOVA (Group: mTBI vs. HC; Session; time 1 vs. time 2; Capacity: high vs. low demand; Manipulation: high vs. low demand). All group-level statistical maps were thresholded at $p < 0.05$ (corrected for multiple comparisons). The analysis resulted in main effects of Group, Capacity, and Manipulation. We also observed a Group X Session and Group X Load interaction. Two cerebellar regions showed a Group X Session interaction. Cerebellum is reported to play a role in working memory and is thought to be responsible for inner vocalization/phonological storage (e.g. Marvel & Desmond, 2010). Four regions showed a Group X Load interaction: right lentiform nucleus, left superior temporal gyrus/inferior frontal gyrus, left posterior insula, right cerebellar tonsil. The significance of these effects and interactions will be discussed.

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Poster

522. Traumatic Brain Injury: Mechanisms and Therapeutics I

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Topic: C.10. Trauma

Support: VA Office of Public Health/WRIISC funds

Title: Age related atrophy of white matter tracts in Veterans with TBI and PTSD

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Abstract: OBJECTIVES: Diffusion Tensor Imaging (DTI) fiber tractography has been used to assess the relationship between age and microstructural changes in white matter tracts (WMT). Such studies typically include healthy adults and commonly report measures of white matter integrity such as Fractional Anisotropy (FA). Our objective was to investigate WMT changes in a Veteran population with multiple health complaints including mild to moderate Traumatic Brain Injury (TBI) (68%) and/or Post Traumatic Stress Disorder (PTSD) (78%). METHODS: Participants were recruited from the War Related Illness and Injury Study Center, CA (WRIISC CA). Ninety-one participants (Control n=16, PTSD n=17, TBI n=11, PTSD+TBI n=47) were given a high-resolution T1 anatomical scan and a 30 direction DTI scan. DTI data were analyzed with custom software for the creation of tensor maps and white matter tractography that provided common DTI indices of FA for sixteen fibers of interest. RESULTS: Compared to controls, all three patient groups revealed a distinct pattern of aging (Figure 2).We focused on three fiber tracts that are implicated in trauma in both TBI & PTSD populations. There was an Age x TBI Interaction for Left Inferior Frontal Occipital Fasiculus (LIF), $F(1, 90) = 5.02$, $p < 0.05$. Results differed from previous work in healthy aging as bilateral cingulum and uncinate FA in our "control" population did not show a linear negative correlation with age (almost zero). In contrast, the correlations in both these tracts were very evident in the TBI population (R2 range = -0.3 to -0.6). Bilateral uncinate and cingulum had a negative correlation in both PTSD & TBI/PTSD population (R2 range = -0.1 to -0.5). RIF/LIF were also highly negatively correlated with age in all four groups (with only RIF in controls) whereas in previous research in civilian populations, R2 ranges are reported to be ~ 0.19. The only significant interaction was found in the TBI group where TBI (mild & moderate) appeared to amplify the effect of age on LIF. CONCLUSIONS: Aging effects related to TBI and PTSD were found. These effects were more pronounced in Veterans with one or more events of mild or moderate TBI, regardless of PTSD status andwere found in long association fibers (e.g., LIF), which are known to be particularly vulnerable to TBI. Veterans with PTSD, regardless of TBI, revealed compromised fiber integrity

in both cingulum and uncinate fibers - previously implicated in PTSD. This study demonstrates how DTI can illuminate the complex pathological substrates of WM changes with aging even with clinical scans that are initially read as “unremarkable”.

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Poster

523. Spinal Cord Injury: Therapeutic Strategies II

Location: Halls A-C

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Topic: C.10. Trauma

Support: Swedish Medical Research council

Soderbergs foundation

Strat Regen KI.

Title: Transplantation of adult subventricular zone-derived neural stem/progenitor cells to immune competent rats improves locomotor function after spinal cord injury

Authors: ***S. SANKAVARAM**, A. FROSTELL, J. GRIPENLAND, M. SVENSSON, L. BRUNDIN
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Abstract: Spinal cord injuries (SCI) are caused by trauma and leads to devastating and irreversible consequences like loss of sensory and motor functions below the injury. Adult NSCs have capacity to differentiate into cells of the CNS and constitute a potential source for therapy for SCI. Here we transplanted NSCs isolated from the SVZs of ubiquitin:EGFP transgenic Lewis rats into SCI of immune competent wild type litter mates 10 days after injury. Spinal contusion injury was made on the dorsal side of thoracic T11 level using IH impactor. A total of 230,000 neurospheres (≤40μm) in 5μl of medium were transplanted in two injection sites of the spinal cord at the epicenter of the injury. We assessed recovery of rats after NSC cell transplant with BBB score and kinematic apparatus. The majority of the NSCs survived after 15weeks of transplantation and differentiated into oligodendrocytes (APC), astrocytes (GFAP) and neurons

(Tuj-1). The NSCs filled the cyst and migrated into entire injured area. We identified regional differences in the differentiation of NSCs within the injured spinal cord. Transplanted NSCs differentiated into more neurons (Tuj-1) in dorsal funiculus compared to central region. Majority of the cells differentiated into functional oligodendrocytes (MBP) and some migrated into the white matter of the spinal cord. The NSC transplanted animals performed significantly better than the control animals after 28 days of post transplantation. We conclude that transplanted NSCs from SVZ will improve recovery after SCI by integration and differentiation into oligodendrocytes, neurons and astrocytes and the degree of improvement depends on the number of cells and survival of the cells with in the host tissue. *Funding: Swedish Medical Research council; Soderbergs foundation; Strat Regen KI.*

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Poster

523. Spinal Cord Injury: Therapeutic Strategies II

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Topic: C.10. Trauma

Support: NIH Grant MH051699

NIH Grant NS082092 to JC

Title: Activation of lysophosphatidic acid receptor 1 (LPA1) contributes to pathophysiology of spinal cord injury

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Abstract: Lysophosphatidic acid (LPA) is an extracellular lipid mediator with many physiological functions, which signals through 6 known G protein-coupled receptors (LPA1-6). A wide range of LPA effects have been identified in the CNS, including neural progenitor cell physiology, astrocyte and microglia activation, neuronal cell death, and axonal retraction. *In vivo* studies also show that LPA is involved in the etiology of fetal hydrocephalus, as well as the development of neuropathic pain after sciatic nerve injury and cerebral ischemia. Here we demonstrate for the first time that LPA acting via LPA1 contributes to secondary damage after spinal cord injury (SCI). Our data revealed that LPA levels increase ~10 fold in the contused spinal cord parenchyma. To assess the potential contribution of increased LPA levels in the spinal cord tissue, we injected 5 nmoles of LPA into the dorsal column of an intact spinal cord. The injection of LPA led to an inflammatory response and demyelination, suggesting that the increased levels of LPA observed in the contused spinal cord may contribute to tissue damage. We then assessed the expression of LPA receptors in the spinal cord, and found that LPA1 is the form expressed at higher constitutive levels. Intraspinal injection of LPA into the intact spinal cord of LPA1-null mice resulted in reduced myelin loss, although demyelination was not completely abrogated. Similar results were observed after injecting LPA and AM095 (a potent and selective LPA1 antagonist) into the spinal cord, suggesting that the demyelinating effects of LPA are partially mediated via LPA1. To study the mechanisms underlying the demyelinating response triggered by LPA through LPA1 signaling we exposed oligodendrocyte primary cultures to 1μM LPA for 24 hours. Survival of oligodendrocytes was reduced ~20% upon LPA stimulation; however, cell death was unchanged by the administration of AM095. Conditioned media of primary cultured microglial cells stimulated with LPA led to marked reduction in oligodendrocyte survival (~85%). However, cell survival was enhanced ~3 fold when microglial LPA1 was blocked with AM095, suggesting that the detrimental actions of LPA1 are mediated by microglial cells. We finally assessed whether LPA-LPA1 signaling contribute to secondary damage following spinal cord injury. Oral administration of AM095 resulted in reduced locomotor deficits and myelin loss after SCI. Overall, these results provide clear evidence that the increase in LPA levels that occurs after SCI contribute to demyelination, and that such detrimental effects are mediated, in part, by microglial activation via LPA1 signaling.

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Poster

523. Spinal Cord Injury: Therapeutic Strategies II

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Support: NINDS grant R01NS079702

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Title: Overexpression of the astrocyte glutamate transporter, GLT1, using AAV exacerbates phrenic motor neuron degeneration and diaphragm dysfunction following cervical contusion spinal cord injury

Authors: *A. C. LEPORE[1], K. LI[1], C. NICAISE[2], D. SANNIE[1], T. J. HALA[1], E. JAVED[1], J. L. PARKER[1], R. PUTATUNDA[1], K. A. REGAN[1], V. SUAIN[2], J.-P. BRION[2], F. RHODERICK[3], M. C. WRIGHT[4], D. J. POULSEN[3]
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Abstract: A major portion of spinal cord injury (SCI) cases affect mid-cervical levels, the location of the phrenic motor neuron (PhMN) pool that innervates the diaphragm. While initial trauma is uncontrollable, a valuable opportunity exists in the hours-to-days following SCI for preventing PhMN loss and consequent respiratory dysfunction that occurs during secondary degeneration. One of the primary causes of secondary injury is excitotoxic cell death due to dysregulation of extracellular glutamate homeostasis. GLT1, mainly expressed by astrocytes, is responsible for the vast majority of functional uptake of extracellular glutamate in the CNS, particularly in spinal cord. We found that, in BAC-GLT1-eGFP reporter mice following unilateral mid-cervical (C4) contusion SCI, numbers of GLT1-expressing astrocytes in ventral horn and total intraspinal GLT1 protein expression were reduced early after injury and the decrease persisted for at least 6 weeks. We employed intraspinal delivery of adeno-associated virus type 8 (AAV8)-Gfa2 vector to rat cervical spinal cord ventral horn for targeting focal astrocyte GLT1 overexpression in areas of PhMN loss. Intraspinal delivery of AAV8-Gfa2-GLT1 resulted in transduction primarily of GFAP+ astrocytes that persisted for at least 6 weeks post-injury, as well as increased intraspinal GLT1 protein expression. Surprisingly, we found that astrocyte-targeted GLT1 overexpression increased lesion size, PhMN loss, phrenic nerve axonal degeneration, and diaphragm neuromuscular junction denervation, and resulted in reduced functional diaphragm innervation as assessed by phrenic nerve-diaphragm compound muscle action potential (CMAP) recordings. These results demonstrate that GLT1 overexpression via intraspinal AAV-Gfa2-GLT1 delivery exacerbates neuronal damage and increases respiratory impairment following cervical SCI.

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Poster

523. Spinal Cord Injury: Therapeutic Strategies II

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Topic: C.10. Trauma

Support: Craig Nielsen Foundation grant #190140 to A.C.L

NINDS grant #1R01NS079702 to A.C.L

Title: Transplantation of human iPS cell-derived astrocytes can be used as a therapeutic approach for delivering the glutamate transporter, GLT1, to injured cervical spinal cord

Authors: *A. FALNIKAR[1], K. LI[1], E. JAVED[1], T. J. HALA[1], J.-P. RICHARD[2], N. J. MARAGAKIS[2], A. C. LEPORE[1]
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Abstract: Transplantation-based replacement of lost and/or dysfunctional astrocytes is a promising therapeutic approach for traumatic spinal cord injury (SCI) that has not been extensively explored, despite the integral roles played by astrocytes in the intact, injured and degenerative CNS. Induced pluripotent stem (iPS) cells are a clinically-relevant source of pluripotent cells generated from adult somatic cell types, avoiding ethical issues of embryonic stem cells. This technology also allows for homogeneous derivation of mature cell types in large quantities, potentially in an autologous fashion. Despite the promise of this approach, the iPS cell transplantation field is in its infancy with respect to evaluating *in vivo* graft integration and therapeutic usefulness in relevant SCI models. Astrocytes express the major CNS glutamate transporter, GLT1, which is responsible for the vast majority of functional glutamate uptake and plays a central role in regulating extracellular glutamate homeostasis in spinal cord. Following SCI, astrocyte loss and/or altered GLT1 expression/function can result in further susceptibility to glutamate excitotoxicity. Given these observations of GLT1 dysfunction, we evaluated intraspinal transplantation of hiPS cell-derived astrocytes into ventral horn following cervical contusion SCI as a novel therapeutic strategy for reconstituting GLT1 function. Transplant-

derived cells robustly survived for at least 5 weeks post-injection and efficiently differentiated into astrocytes in injured spinal cord of both immune suppressed mice and rats. However, the majority of transplant-derived astrocytes did not express GLT1, particularly at early time points post-injection. To enhance the ability of transplants to modulate extracellular glutamate levels, we engineered hiPS cell-derived astrocytes *in vitro* with lentivirus to overexpress GLT1. Overexpression resulted in significantly increased GLT1 protein and functional GLT1-mediated glutamate uptake levels following astrocyte differentiation *in vitro*. Following transplantation of GLT1-overexpressing cells into cervical contusion SCI, nearly all hiPS cell-derived astrocytes expressed high levels of GLT1 *in vivo*. Our findings demonstrate that hiPSA transplantation is a therapeutically-powerful and clinically-relevant approach for targeting astrocyte dysfunction in both SCI and other CNS diseases associated with astrocyte pathogenesis. Ongoing studies are aimed at determining therapeutic efficacy of these transplants for protecting respiratory motor neurons and preserving diaphragm function following cervical SCI.

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Poster

523. Spinal Cord Injury: Therapeutic Strategies II

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Topic: C.10. Trauma

Support: NINDS grant R01NS079702

Craig H. Neilsen Foundation grant 190140

Title: Transplantation of glial progenitor-derived astrocytes engineered to overexpress the glutamate transporter, GLT1, preserves functional diaphragm innervation following cervical contusion spinal cord injury

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Abstract: Approximately half of human traumatic spinal cord injury (SCI) cases affect cervical regions, resulting in significant and often chronic respiratory compromise. The majority of these injuries affect mid-cervical levels, the location of the important pool of phrenic motor neurons (PMNs) that innervates the diaphragm. A valuable opportunity exists in the hours-to-days following SCI for preventing PMN loss and consequent respiratory dysfunction that occurs during secondary degeneration. One of the primary causes of secondary injury is excitotoxic cell death due to dysregulation of extracellular glutamate homeostasis. Astrocytes express the major glutamate transporter, GLT1, which is responsible for the vast majority of glutamate clearance in the CNS. We find that in rodent models of unilateral mid-cervical (C4) contusion SCI, numbers of GLT1-expressing astrocytes in ventral horn and total intraspinal GLT1 protein expression are reduced soon after injury and persist for at least 6 weeks. Given these observations of GLT1 dysfunction, we aim to evaluate intraspinal transplantation of Glial-Restricted Precursors (GRPs) - a class of lineage-restricted astrocyte progenitors - into ventral horn following cervical contusion as a novel therapeutic strategy for reconstituting GLT1 function, preventing excitotoxicity, and consequently protecting PMNs. Following transplantation of GRPs derived from transgenic BAC-GLT1-eGFP promoter reporter mice, only a small percentage of transplant-derived astrocytes express GLT1 in injured spinal cord, particularly at early time points post-engraftment. To enhance the ability of transplants to modulate extracellular glutamate levels, we engineered GRPs *in vitro* with AAV8 vectors to overexpress GLT1 only in astrocytes using the astrocyte-specific GFA2 promoter. Overexpression resulted in significantly increased GLT1 protein expression and functional GLT1-mediated glutamate uptake following astrocyte differentiation *in vitro* and was restricted to only GFAP-expressing astrocytes. Following transplantation of GLT1-overexpressing cells into C4 contusion SCI, nearly all GRP-derived astrocytes expressed high levels of GLT1 *in vivo*. Compared to medium-only control and unmodified GRPs, GLT1-overexpressing transplants reduced lesion size, diaphragm neuromuscular junction denervation by PMNs, and diaphragm dysfunction. Our findings demonstrate that transplantation-based replacement of GLT1 is a promising therapeutic approach for SCI.

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Poster

523. Spinal Cord Injury: Therapeutic Strategies II

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

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Topic: C.10. Trauma

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Title: K+ channel blocker 4-aminopyridine-3-methanol restores motor function and alleviates neuropathic pain following spinal cord injury

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Abstract: Spinal cord injury (SCI) is a severely disabling neurotrauma that leads to the loss of sensory and motor function and currently affects approximately 273,000 people in the United States. Primary trauma typically sustained through contusion, compression, or laceration is exacerbated by secondary pathophysiological injury mechanisms which can significantly reduce vital sensory and motor function. Demyelination through both physical and chemical injury exposes and activates underlying juxtaparanodal K+ channels, which results in conduction block clinically observed as mobility loss in SCI victims. Functional restorative therapeutic strategies seek to reverse this pathology through application of K+ channel blockers (such as 4-AP or AMPYRA) has shown promised result. As such, 4-AP has been approved by FDA to treat multiple sclerosis (MS). In our recent investigation, we have shown that a 4-AP derivative, 4-aminopyridine-3-methanol (4-AP-3-MeOH), has a 10-fold increased potency and appears to be a new and alternative promising K+ channel blocker to treat not only MS but also SCI. Therefore, we sought to characterize and compare functional restoration of 4-AP-3-MeOH and 4-AP following SCI in both *in vitro* and *in vivo* preparation. Double sucrose gap electrophysiological recordings of complete rat spinal cord sections were employed to obtain functional data. In addition, behavioral assessments for motor function and neuropathic pain, using rotarod and mechanical allodynia tests, were also conducted. From our experiments, we showed that application of either 4-AP or 4-AP-3-MeOH significantly increased compound action potential (CAP) amplitude following mechanical and acrolein-mediated injury *in vitro*. Furthermore, motor function is significantly enhanced and neuropathic pain significantly alleviated with application of 4-AP-3-MeOH at 5 mg/kg *in vivo*. At lower concentrations, little improvement in motor function was noted for both chemicals, while 4-AP resulted in fatalities at 5 mg/kg. These results further indicate the potential for 4-AP-3-MeOH as a viable alternative for 4-AP which can overcome adverse side effects at higher and more effective dosage.

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Poster

523. Spinal Cord Injury: Therapeutic Strategies II

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Topic: C.10. Trauma

Title: Intercostal nerve-lumbar dorsal root anastomosis promotes axonal regeneration beyond a spinal cord injury

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Abstract: To promote axonal regeneration beyond a spinal cord injury (SCI), a nerve bypass strategy was conducted in adult female Sprague Dawley (SD) rats (n=24). After a right sided spinal cord hemisection was performed at the T13 level, the T11 and T12 intercostal nerves and the L2 and L3 dorsal roots were identified, isolated, and cut at their distal ends. The proximal stumps of the T11 and T12 intercostal nerves were then anastomosed to the proximal stumps of the L2 and L3 dorsal roots, respectively, on each side. At 18 weeks post-surgery, a second surgery was performed and the animals received a complete spinal transection (TX) at the previously hemisected site. Animals then received anterograde tracing of biotinylated-dextran amine (BDA, n=9), electrophysiological recordings (n=12), or retrograde tracing of Fluoro-Gold (FG) (n=5). Our results showed that: (1) the bypass promoted a significant improvement in hindlimb locomotor function assessed by Basso, Beattie, and Bresnahan (BBB) open field and contact placing scores at 6 months post-TX; (2) axons in the bypass penetrated through the dorsal root entry zoon (DREZ) of the L2-3 spinal segments, reinnervated the gray matter, and formed new synapses on host neurons; (3) regenerated axons were originated from motoneurons of the T11-12 segments above the TX; and (4) new functional circuit was established across the lesion gap, evidenced by electrophysiological recordings. Thus, our results provide strong evidence for a novel bypass strategy that leads to anatomical regeneration and functional recovery in a complete spinal cord transection model. Further investigation will be required to dissect out the mechanisms underlying plasticity and specificity of reinnervation in this model.

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Poster

523. Spinal Cord Injury: Therapeutic Strategies II

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Topic: C.10. Trauma

Support: Neilsen Foundation Training Grant

Title: Microtubule stabilization therapy promotes breathing outcomes after cervical spinal cord injury

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Abstract: More than half of all spinal cord injuries (SCI) are at the cervical level. These injuries can result in the disruption of the respiratory motor pathways leading to the innervation of the diaphragm, resulting in the need for mechanical ventilation. To examine the neuronal circuitry involved in cervical SCI and potential interventions, our laboratory utilizes a C2 lateral hemisection. This model induces paralysis of the hemi-diaphragm ipsilateral to the hemisection. By leaving one side of the diaphragm still active, this model allows us to examine the breathing circuitry affected and spared by cervical SCI without the need for mechanical ventilation of experimental subjects. A recent investigation has shown that the microtubule stabilizer paclitaxel (Taxol) can improve outcomes in thoracic injury. In the thoracic injury model, paclitaxel has been shown to reduce the perineuronal (PNN) net and increase serotonin (5-HT) after injury. However, its application in cervical injury is untested. Taken together, our central hypothesis is that microtubule stabilization will promote respiratory motor recovery after cervical spinal cord injury by 1) decreasing inhibition by reducing the PNN and 2) increasing 5-HT expression and sprouting after cervical injury. Here we examined the role of paclitaxel on the recovery of breathing after a C2 spinal hemisection. Subjects received various doses of paclitaxel for 6 weeks after injury. For recovery ventilation and diaphragmatic electromyographical (EMG) activity was assessed. Preliminary data indicate that cervical SCI subjects that receive Taxol have improved respiratory motor function compared to vehicle treated animals.

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Poster

523. Spinal Cord Injury: Therapeutic Strategies II

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Program#/Poster#: 523.09/BB17

Topic: C.10. Trauma

Title: Modulation of the pten/mtor pathway rescues respiratory motor function after cervical contusion injury

Authors: *D. V. GUTIERREZ, W. J. ALILAIN
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Abstract: Cervical spinal cord injuries (SCI) can disrupt the descending bulbospinal fibers originating from the medullary rostral ventral respiratory group (rVRG) and which innervate phrenic motor neurons (PMNs; located C3-C6). Injuries at this level are quite significant and often result in diaphragm paralysis leading to mechanical ventilation to support breathing and survival. These patients have a diminished quality of life. Given that current regenerative therapies do not completely restore motor function, gaining a more comprehensive understanding of the cellular mechanisms that influence plasticity and recovery in the injured spinal cord is crucial to the development of novel treatment interventions. An ideal strategy would target intrinsic factors known to initiate synaptic plasticity, promote an enhanced regenerative environment and augment the sprouting of spared pathways. One key signaling component in axonal regeneration is phosphatase and tensin homolog (PTEN). Deletion of PTEN can robustly enhance axonal regeneration through the upregulation of mTOR and phosphorylated S6. Furthermore, deletion of PTEN and upregulation of mTOR leads to enhanced neurotransmission. The present study seeks to determine the functional outcome of pharmacologically modulating PTEN activity after a high cervical lesion. Specifically, adult rodents underwent a C3/4 unilateral contusion model of SCI and were treated with bisperoxovanadium (bpV[pic]) to inhibit PTEN. Animals were then exposed to a delayed (3 weeks post contusion) contralateral C2 hemisection to directly investigate treatment efficacy on spared and injured pathways. We hypothesize that inhibiting PTEN after cervical contusion will enhance the regeneration and sprouting of injured rVRG axons, improve neurotransmission, and augment phrenic motor neuron dendritic arborization. Futhermore, this plasticity will support the rescue of respiratory motor function. Excitingly, data obtained from diaphragm electromyogram (EMG) recordings revealed that bpV[pic] treatment significantly influenced the length of breathing time and frequency post-hemisection. Additionally, bpV[pic] therapy bilaterally enhanced the overall breathing amplitude. Improvements in breathing output were not present in saline treated animals. The

results obtained thus far suggest that bpV[pic] mediated modulation of the PTEN/mTOR pathway rescues the ability to breathe after cervical SCI by making injured pathways more efficacious and repairing the injured SC.

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Poster

523. Spinal Cord Injury: Therapeutic Strategies II

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Topic: C.10. Trauma

Support: International Spinal Research Trust

Craig H Neilsen Foundation

MetroHealth Medical Centre

Title: Extensive recovery of respiratory motor function at chronic and super-chronic time points following cervical spinal cord injury

Authors: *P. M. WARREN[1], P. M. MACFARLANE[2], J. SILVER[3], W. J. ALILAIN[1]
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Abstract: Treatments to restore respiratory function following chronic cervical spinal cord injury (SCI) have not been extensively studied. We provide evidence that a pharmacological agent and rehabilitative training may provide the key for recovery of diaphragm activity following chronic trauma. The ablation of respiratory function is caused by disruption of motoneuron pathways, formation of the chondroitin sulphate proteoglycan (CSPG) rich astroglial scar, and a reduction in interneuron, motoneuron and synaptic density. Following acute cervical SCI, CSPG breakdown by application of chrondroitiase ABC (ChABC) can restore functional diaphragm activity while intermittent hypoxia (IH) training increases respiratory drive and synaptic strength. We now provide evidence for the recovery of robust functional respiratory motor activity at both chronic (3 month) and super-chronic (1.5 year) time points following LC2H through a combination of IH training and ChABC. We used diaphragmatic electromyography (diaEMG) and phrenic nerve recordings to demonstrate that a single

application of ChABC (0.005U) can recover extensive respiratory motor function following chronic and super-chronic SCI. Control treated animals showed no endogenous recovery of diaphragm function. While having limited effect upon diaEMG patterns, IH training alone was shown to enhance maximal phrenic nerve activity. However, the combined treatment of IH and ChABC was shown to substantially enhance diaEMG and maximal phrenic nerve activity beyond that demonstrated by either group alone. Interestingly, in a subpopulation of animals the muscle activity in this combination group can become unstructured, degrading patterned activity on the lesioned side. This tonic/chaotic activity is governed by a serotonergic (5-HT) mechanism and suggests considerable remodeling of spinal cord circuitry below the level of the lesion at chronic stages. Indeed, ChABC and IH treated animals which recover normal breathing patterns following treatment can be made chaotic by giving exogenous 5-HT, while those that are already chaotic can be normalized by blocking certain 5-HT receptors. These data demonstrate the significant restoration of diaphragm function and nerve activity at chronic and super-chronic time points following cervical SCI due to matrix modification, induction of plasticity and facilitation of drive. Yet, the potential emergence of chaos is indicative of the complications inherent in repairing the chronically injured spinal cord and suggest the need for tight mechanistic and environmental control.

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Poster

523. Spinal Cord Injury: Therapeutic Strategies II

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Topic: C.10. Trauma

Title: The neuroprotective effects of co-ultraPEALut in a mouse model of spinal cord injury

Authors: I. PATERNITI[1], D. IMPELLIZZERI[1], R. CRUPI[1], G. BRUSCHETTA[1], E. ESPOSITO[1], S. CUZZOCREA[1], *J. V. PRIESTLEY[2]
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Abstract: Traumatic injuries to the spinal cord frequently cause permanent neurological disabilities and yet there is no effective therapeutic option to improve functional recovery. Spinal cord injury (SCI) is well-known to induce the formation of reactive astrocytes and the infiltration

of immune cells in the area of the lesion site, but whether SCI also induces the production of new neurons *in vivo* remains controversial. Neurogenesis has been described in various regions of the central nervous system. Significant spontaneous neuroplasticity occurs over the weeks and months following brain or spinal cord trauma leading to some functional recovery. Moreover, studies have shown that spinal neurogenesis occurs to a limited extent after SCI, but that it could be stimulated by experimental intervention. In that regard, in a recent study, we have demonstrated that treatment with a new composite, a formulation including palmitoylethanolamide (PEA) and the antioxidant compound luteolin (Lut), subjected to an ultramicronization process, co-ultraPEALut, significantly reduced inflammatory secondary damage associated with SCI. Thus, the aim of this study was to investigate the neuroprotective effect of co-ultraPEALut in the injury-induced neurogenesis in a mouse model of SCI. Materials and Methods: SCI was induced in mice through spinal cord compression by the application of vascular clips (force of 24 g) to the dura via a four-level T5 to T8 laminectomy. The animals were sacrificed and the spinal cord were collected. Results: Chronic exogenous administration of co-ultraPEALut increased bromodeoxyuridine (BrdU) and doublecortin immunoreactive cells in the spinal cord of SCI subjected mice. This neuronal development was correlated with synaptic plasticity, identified using the Golgi impregnation method to quantify dendritic spines in spinal cord. In addition, co-ultraPEALut treatment also increased the expression of different neurotrophic factors such as brain-derived neurotrophic factor (BDNF), glial cell-derived neurotrophic factor (GDNF), nerve growth factor (NGF) and neurotrophin-3 (NT-3). Conclusions: The results indicate that co-ultraPEALut could have a role on birth, survival, and differentiation of new neurons and maturation of spines in the spinal cord and could be a therapeutic target in traumatic diseases.

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Poster

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Title: Development of combinatorial, biomaterial-mediated gene therapies for spinal cord tissue regeneration

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Abstract: The local environment after spinal cord injury (SCI) lacks cues support axon growth, cell survival, and remyelination and exhibits an abundance of cues that inhibit these processes. Development of clinically effective strategies to restore tissue function after SCI will require consideration of multiple aspects of this inhibitory environment. The goal of this research is to develop a multifaceted gene therapy for spinal cord injury repair that delivers vectors encoding for growth factor cues that enhance cell survival, reduce inflammation, prevent glial scar formation and promote axonal growth and remyelination. For these studies, porous biomaterials fabricated from poly(lactide-co-glycolide) (PLG) were used as a platform for tandem, localized delivery of lentiviral vectors encoding for multiple regenerative factors, including sonic hedgehog (SHH), neurotrophin-3 (NT-3), chondroitinase and interleukin-10. Previously, we have reported that porous bridges with a defined channel architecture that significantly encourage axons to regenerate across the injury site and can be used to deliver lentiviral vectors. This research builds upon the success of these bridges by adding a gene delivery component to enable localized, sustained expression of multiple factors designed to simultaneously address different barriers to spinal cord regeneration. Moreover, these factors were selected to target various barriers to spinal cord regeneration. First, we report that delivery of lentivirus encoding for interleukin-10 (IL10) significantly reduces the presence of specific inflammatory cells thought to be detrimental to repair. In addition, we demonstrate that tandem delivery of sonic hedgehog (SHH) and neurotrophin-3 (NT-3) resulted in significant increases in the number of regenerated axons through the bridge platforms and myelination of these axons by oligodendrocytes. Although PLG biomaterials are useful as an investigational platform for identifying clinically relevant gene therapy targets *in vivo*, they require that sections of spinal cord be removed prior to implantation and do not adequately resemble the native tissue. As a clinically viable alternative, we are also investigating the use of hydrogel biomaterials based on hyaluronic acid, which is abundant in the extracellular matrix of the uninjured spinal cord and down-regulates the inflammatory response after injury for their ability to mediate SCI repair.

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Poster

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Title: Agmatine modulates the phenotype of macrophage after spinal cord injury

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Abstract: Agmatine is a decarboxylated arginine by arginine decarboxylase (ADC). Agmatine has been known as a neuroprotective agent. It has been reported that agmatine work in many kinds of CNS injuries as a NMDA receptor blocker or a competitive nitric oxide synthase (NOS) inhibitor. In spinal cord injury, agmatine showed reduction of neuropathic pain, improvement of locomotor function, and neuroprotection. Macrophage is a key cellular component in neuroinflammation, a major cause of impairment after spinal cord injury. Macrophage has subtypes, M1 and M2 macrophages. M1 macrophage induces pro-inflammatory response but M2 inspires anti-inflammatory response. In this study, it is clarified whether neuroprotective effect of agmatine is related with the modulation of macrophage subdivision after spinal cord injury. Spinal cord injury was induced in SD rats with 25.0 gCm contusion by MASCIS. Animals received agmatine (100mg/kg/day, IP) for 1 week after spinal cord injury. The proportion of M1 and M2 macrophages in the epicenter of injury are confirmed with immunohistochemistry. iNOS +/CD68+ cells were counted as M1 macrophages and CD206+/CD68+ cells as M2 macrophages. The treatment of agmatine increased CD206 expression. These results suggested that agmatine reduce impairment after spinal cord injury through modulating the phenotype of macrophage from M1 to M2 macrophage.

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Poster

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TPRM CIHR

Title: Promoting human induced pluripotent stem cell-derived oligodendrocyte precursor cell survival after transplantation into the injured spinal cord with hydrogels

Authors: T. FUEHRMANN[1], B. BALLARIN[1], I. E. DONAGHUE[1], R. TAM[1], B. COLES[1], D. VAN DER KOOY[1], C. MORSHEAD[1], A. NAGY[3], C. TATOR[4], *M. S. SHOICHET[2]
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Abstract: Introduction: Traumatic spinal cord injury (SCI) is a devastating condition, leading to severe, and often permanent, motor, sensory and autonomic disturbances, associated with a poor prognosis. The lack of functional improvements is not primarily due to an inherent lack of capacity for regeneration, but rather to the overall balance of growth promoting and growth inhibitory molecules. Since remyelination of spared, demyelinated axons after spinal cord injury has the potential to preserve functional pathways and improve function we are investigating the hypothesis that implanted human induced pluripotent stem cell-derived oligodendrocyte precursor cells (hOPCs), a potentially autologous source, will promote functional tissue repair in an experimental animal model of SCI. However, cell replacement strategies rely on the long term

survival and integration of the cells upon transplantation, which is limited with current methods. Therefore, we designed a minimally invasive injectable cell-delivery system comprised of hyaluronan and methylcellulose (HAMC) aimed to enhance the survival and integration of grafted cells, which may lead to greater beneficial effects. **Methods:** Human iPS cells were differentiated into oligodendrocyte precursor cells using purmorphamine, T3, NT-3 and PDGF. T2 vertebra of adult female rats was exposed and compressed with a modified aneurism clip (24g) for 1 minute. One week post operation, animals received transplants of hOPCs with and without HAMC at 4 sites rostral and caudal to the lesion (total volume 8 µl; 10.000 cells / µl). **Results:** Human induced pluripotent stem cells were differentiated into oligodendrocyte precursor cells (hOPCs) as shown by their expression of OLIG2, NKX2.2, SOX10, and PDGF-receptor alpha using RT-PCR, flow cytometry and immunocytochemistry. Surviving cells were found at the injury/injection site 7 days after transplantation (14 days after injury), but only when transplanted in HAMC. The surviving hOPCs expressed SOX10, a marker for oligodendrocytes and oligodendrocyte precursor cells. No surviving cells were found in animals receiving hOPCs in media. **Conclusion:** These data indicate the feasibility of our approach to promote graft cell survival with hydrogels. Cell survival is being correlated with functional repair in ongoing studies using a modified hydrogel and greater cell numbers.

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Poster

523. Spinal Cord Injury: Therapeutic Strategies II

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Title: The effects of the combination treatment of keratan sulfate digestion and rehabilitation in rat spinal cord injury

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Abstract: Various treatments have been used to overcome the neurological dysfunctions and inhibitory mechanisms resulting from spinal cord injury (SCI). Single-agent treatments used in previous studies did not result in recovery after SCI in most cases. Recently, a number of reports have suggested that combination therapies are more effective. The combination of Chondroitinase ABC (ChABC) and rehabilitation is an effective therapy that promotes plasticity and functional recovery. Our previous study showed that Keratanase II (K-II), a keratan sulfate (KS) digestion enzyme, and ChABC exert comparable effects on SCI. No studies have examined the combination treatment of K-II and rehabilitation. The purpose of this study was to evaluate the effects of this combination treatment on SCI. First, we examined the localization of KS in adult Sprague Dawley (SD) rats. BCD4-positive KS surrounded neurons and colocalized with components of the perineuronal nets (PNN). Second, to establish how K-II affects the PNN, we examined sections treated with K-II and confirmed the disappearance of KS that was colocalized with the PNN. Next, SD rats received a SCI at C3/4 and treatment with K-II, C-ABC, or saline with or without forearm training. Their recovery from SCI was evaluated by behavioral and histological assessments. In each of the treated groups, animals receiving training had a significantly improved recovery rate from SCI compared to those without training (K-II + training: 66.5%, K-II + no training: 38.9%, chABC + training: 65.2%, chABC + no training: 36.5%, saline + training: 53.4%, saline + no training: 26.0%). However, the combination of K-II or chABC with training did not result in significantly better recovery rates for fine motor skills compared to combination of saline and training. Histologically, 5-HT/GAP43 positive fibers rostral to the lesion site were counted as 5HT/GAP43-positive pixels. In each treatment group, rats that received training had significantly larger positive pixel areas than those with no training. For both 5HT and GAP43, rats receiving K-II or ChABC with training showed pixel areas that were twice that of rats receiving saline with training. These results suggest that combination of K-II or ChABC with training promotes more axon sprouting/regeneration compared to training only. These results suggest that combination of K-II or ChABC with training promotes more axon sprouting/regeneration compared to training only. These robust regrown axons did not contribute to the behavioral changes. Hence, other experimental conditions such as the timing of K-II treatment or evaluation needs consideration.

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Poster

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Topic: C.10. Trauma

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Title: Enhanced axon regeneration with combined exercise and peripheral nerve grafts after acute and chronic spinal cord injury

Authors: *C. C. THEISEN, V. NINAN, C. SZE, S. AUSTIN, J. D. HOULE
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Abstract: Exercise (Ex) of rats after spinal cord injury (SCI) leads to increased levels of intraspinal neurotrophic factors and increased levels of cFos and phospho-S6 in interneurons of thoracic and lumbar spinal cord. Intraspinal peripheral nerve grafts (PNGs) provide a hospitable environment for axon regeneration through or around a lesion site. Here we provide evidence that Ex increases the number and distribution of propriospinal neurons that regenerate their ascending or descending axon into a graft. Adult female Sprague Dawley rats received a thoracic level 12 complete transection injury and either acute PNGs at the time of injury or delayed PNGs 6 weeks after SCI. Groups of rats received 4-5 weeks of cycling exercise, which began 5 days after SCI for the acute PNG group and either 5 days or 5 weeks after SCI for the chronic PNG groups. Control rats received PNGs but no exercise. Five weeks after grafting, the distal ends of PNGs were exposed to True Blue (TB) to retrogradely label neurons that regenerated their axon into the graft. One week later, Ex and control rats were perfused and the brain, spinal cord and lumbar dorsal root ganglia (DRGs) were harvested and sectioned in serial order. In rats receiving acute PNGs, the number and location of TB+ neurons relative to the lesion site was recorded. In acute PNG Ex rats there were significantly more (> 2 fold increase) TB+ neurons close to (within 5mm of) the injury/graft sites, a 3-fold increase in TB+ neurons in the thoracic cord, and a 9-fold increase in TB+ neurons in the lumbar cord caudal to the injury. There was no significant effect of Ex on regeneration by peripheral sensory neurons (DRGs). The effect of Ex on regeneration by chronically injured neurons is under study. This demonstration of exercise-dependent plasticity and regeneration by propriospinal or interneurons provides evidence of a reproducible, non-invasive therapeutic treatment that may be useful in promoting structural and functional recovery after SCI. Whether Ex leads to enhanced axonal outgrowth beyond the PNGs remains to be demonstrated.

Disclosures: C.C. Theisen: None. **V. Ninan:** None. **C. Sze:** None. **S. Austin:** None. **J.D. Houle:** None.

Poster

523. Spinal Cord Injury: Therapeutic Strategies II

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 523.17/BB25

Topic: C.10. Trauma

Support: NRF 2012051429, Republic of Korea

Title: Poly(phosphazene) hydrogel can bridge cystic cavities in contusive spinal cord injury model by inducing extracellular matrix remodeling

Authors: A. H. LE[1,2], D. H. HWANG[1,2], S. C. SONG[4], *B. G. KIM[3,1,2]
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Abstract: Cavity formation after spinal cord injury (SCI) is one of the major obstacles for axonal regeneration since injured axons fail to reach caudal tissue in the absence of physical and mechanical support from extracellular matrix (ECM). Implanting artificial scaffolds has been considered as a promising approach, but successful bridging with scaffolding biomaterials has not been convincingly demonstrated in contusive SCI model, which closely mimics human SCI. Unpredictable and irregular geometry of lesion cavities formed in this model would necessitate the use of injectable hydrogel for this purpose. In the present study, we injected temperature sensitive poly(phosphazene) hydrogel, with a sol-gel transition behavior at 37°C, into the lesion epicenter in contusive rat SCI model at 2 week after injury. The hydrogel injection almost completely prevented cavity formation. In animals with the hydrogel injection, the lesion epicenter was replaced by fibronectin-enriched ECM by 4 weeks after the injection. The fibronectin positive ECM was surrounded by GFAP positive glial scars with an interface laden with chondroitin sulfate proteoglycans. Injection of hydrogel mixed with Taxol, which was previously reported to selectively suppress fibrotic scars, resulted in the failure of bridging cavities, suggesting a role of ECM produced by fibroblasts in the hydrogel effects. Interestingly, zymography showed upregulation of MMP-9 activity in animals with the hydrogel injection, and MMP-9 was highly expressed at the center of the fibronectin enriched ECM. The cellular source for the MMP-9 immunoreactivity was CD11b positive macrophages. Our study convincingly demonstrates that the poly(phosphazene) hydrogel successfully bridges lesion cavities in contusive SCI model. Modulation of the molecular environment in the newly generated ECM may lead to successful axonal regeneration through the hydrogel-induced bridge.

Disclosures: A.H. Le: None. **D.H. Hwang:** None. **S.C. Song:** None. **B.G. Kim:** None.

Poster

523. Spinal Cord Injury: Therapeutic Strategies II

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 523.18/BB26

Topic: C.10. Trauma

Support: Neurotrauma Research Program (WA)

Title: Enbrel treatment promotes transplanted donor human mesenchymal precursor cell survival following spinal cord injury

Authors: S. J. LOVETT[1], A. R. HARVEY[1], G. W. PLANT[2], *S. I. HODGETTS[1]
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Abstract: Immediately following spinal cord injury (SCI), pro-inflammatory cytokines such as tumour necrosis factor alpha (TNFα) and interleukins 1β and 6 are released from damaged cells and resident cells of the spinal cord. This cytokine release initiates the inflammatory response to injury and is responsible for the extensive and persistent spread of secondary damage following SCI. The activation and infiltration of immune cells into the spinal cord contributes to secondary degeneration with an increase in apoptosis of neurons and glia, demyelination of intact axons and cyst formation at the injury site. We have previously shown marked improvements in functional (locomotor) and morphological (tissue sparing, cyst size) outcomes in host tissue following transplantation of adult human mesenchymal precursor cells (hMPCs) into the contused spinal cord. However, donor hMPCs do not survive beyond 4 weeks post transplantation, most likely due to the host immune response. Improving donor hMPC survival (past 4 weeks) may promote further beneficial morphological and functional (locomotor) effects of hMPC therapy. Modulating pro-inflammatory cytokine levels immediately following SCI may attenuate the immune response, reduce immune cell activation and promote neuroprotective effects following injury. By combining the TNFα antagonist Enbrel with hMPC transplantation our aim is to reduce the activity of TNFα immediately following SCI thereby reducing the amount of secondary degeneration and promoting prolonged survival of transplanted hMPCs. Initial data analysis shows that Enbrel treatment improved donor cell survival in all Enbrel + hMPC treated animals, with hMPCs present in the spinal cord at 4 weeks post-transplantation (c.f. two thirds of hMPC only treated animals). Although transplanted hMPC survival was increased, there were no statistically significant improvements in functional recovery at 5 weeks post-injury for any treatment groups. Also, combined Enbrel + hMPC treatment did not further reduce cyst size compared to hMPC transplantation alone. Extended time points are currently being evaluated to assess the effects of Enbrel treatment on long-term donor hMPC survival. Enbrel may have a

moderate impact on SCI repair alone, but may be useful in combinatorial clinical applications to enhance donor stem cell survival in transplantation based therapies.

Disclosures: S.J. Lovett: None. **S.I. Hodgetts:** None. **A.R. Harvey:** None. **G.W. Plant:** None.

Poster

523. Spinal Cord Injury: Therapeutic Strategies II

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 523.19/BB27

Topic: C.10. Trauma

Support: Craig H. Neilsen Foundation 260771

NIH/NINDS 2P30NS051220

Title: Treatment with ketone bodies preserves mitochondrial function and reduces oxidative stress following contusion spinal cord injury

Authors: *S. P. PATEL[1], J. L. VANROOYEN[2], N. P. VISAVADIYA[1], T. L. SMITH[1], P. G. SULLIVAN[3], A. G. RABCHEVSKY[2]
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Abstract: The prophylactic ketogenic diet (KD) has been shown to be a neuroprotective strategy clinically, as well as experimentally, for several neurological disorders and traumatic brain injuries, which all show that mitochondrial dysfunction is a pivotal therapeutic target. The KD is comprised of high fat and low carbohydrates to mimic the effects of fasting which increases serum levels of ketone bodies, notably β-hydroxybutyrate (BHB), acetoacetate, and acetone due to the breakdown of fatty acids in mitochondria. Accordingly, we hypothesized that administration of BHB will maintain mitochondrial function, increase tissue sparing and improve hindlimb functional recovery after contusion spinal cord injury (SCI). The current study assessed the effects of BHB treatment on mitochondrial bioenergetics and markers of oxidative stress following contusion SCI. Injured rats (250 kdyn at L1/L2 spinal level) were administered Vehicle (saline) or BHB (0.415 or 0.83 or 1.66 mmols/kg body weight, i.p.) 15 min post-injury followed by immediate insertion of an osmotic mini-pump (s.c.) to deliver Vehicle or BHB (0.415 or 0.83 or 1.66 mmols/kg body weight/day.) for 24hrs. A Sham group received only a T12

laminectomy. At 24hr post-injury, Ficoll purified mitochondria were isolated from spinal cord tissue (1.5cm centered on injury site) and assessed for mitochondrial oxygen consumption rate (OCR) using Seahorse Bioscience XFe24 extracellular flux analyzer. Levels of oxidative stress markers including Protein Carbonyls (PC), 4-Hydroxynonenal (4-HNE), and 3-nitrotyrosine (3-NT) were assessed using slot blot. Results showed significantly decreased OCR in Vehicle-treated injured group compared to Sham. Treatment with 0.83mmol BHB significantly preserved mitochondria OCR near Sham levels, whereas 0.415 and 1.66 mmol BHB dosages were not effective. Levels of PC, 4-HNE and 3-NT were significantly increased after SCI. Treatment with BHB reduced PC levels but not 4-HNE or 3-NT. On-going experiments are assessing the effects of BHB treatment on activity of key mitochondrial enzyme complexes and glutathione levels at 24 hr post-injury, as well as long-term tissue sparing and recovery of hindlimb function following SCI.

Disclosures: S.P. Patel: None. **J.L. VanRooyen:** None. **N.P. Visavadiya:** None. **T.L. Smith:** None. **P.G. Sullivan:** None. **A.G. Rabchevsky:** None.

Poster

523. Spinal Cord Injury: Therapeutic Strategies II

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Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

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Topic: C.10. Trauma

Support: Ohio Third Frontier Grant (OTFBP 10-833)

NINDS NS25713

Title: Intravenous multipotent adult progenitor cell treatment for acute spinal cord injury: promoting recovery through immune modulation

Authors: *M. A. DEPAUL[1], S. A. BUSCH[2], M. PALMER[2], J. A. HAMILTON[2], R. CUTRONE2[2], B. LANG[1], A. E. TING[2], R. J. DEANS[2], R. W. MAYS[2], J. SILVER[1]
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Abstract: Adult bone marrow derived stem cells are known to have immunomodulatory capabilities, but their potential to alter inflammatory processes and promote regeneration after spinal cord injury (SCI) has not been thoroughly studied. In this study, we sought to determine

the optimal window of administration, dosing, and the biodistribution of human multipotent adult progenitor cells (MAPCs) in a contusion model (250 kdyn Infinite Horizon impactor injury at T8). MAPCs are the research grade variant of the clinical grade MultiStem® product currently in clinical evaluation for treatment of ischemic stroke. Rats received saline or $4x10^6$ cells via iv injection immediately following or 1 day post injury (DPI). We performed locomotor testing through 10 weeks post injury (WPI) and found significant and sustained improvements in BBB scores, subscores, and the Catwalk regularity index. We monitored urination every two weeks using metabolic cages and found the void volume significantly reduced 10 WPI in cell treated animals. At the study endpoint, rats underwent urodynamic assessment. Bursting activity of the external urethral sphincter was seen in correlation with a void in several treated animals, whereas in untreated animals bursting was rare, sporadic, and uncoordinated. Treated animals urinated at a smaller bladder volume, had less residual volume, showed improved return to baseline pressure following a void, and had a decrease in bladder weight. Increasing the dose to $8x10^6$ cells showed no increase in locomotor recovery, but did improve bladder function. Cell distribution was determined using CryoViz technology by iv infusing Qdot-labeled MAPCs into either SCI or laminectomy control animals 1 DPI. Lungs, liver, spleen, and spine were collected 24 or 48 hours after treatment. MAPCs were found in the lungs, liver, and spleen at 24 hours, amounting to <5% of administered cells, and cell numbers decreased at 48 hours. Normalizing cell counts to tissue weight showed a preferential homing to the spleen, while few cells were found in the spinal column. Microarray analysis of the lesion, blood, and spleen suggests MAPCs alter many injury-induced pathways including those involved in recruitment, activation and migration of immune cells. In support of this data, we found a decrease of $ED1^+$ macrophages at the lesion site 4 DPI in treated animals. These data suggest that MAPCs, when administered iv in an acute model of SCI, are more likely to exert benefit through peripheral organ systems than via homing and direct interaction with the site of injury.

Disclosures: **M.A. Depaul:** None. **S.A. Busch:** A. Employment/Salary (full or part-time):; Athersys Inc. **M. Palmer:** A. Employment/Salary (full or part-time):; Athersys Inc. **J.A. Hamilton:** A. Employment/Salary (full or part-time):; Athersys Inc. **R. Cutrone2:** A. Employment/Salary (full or part-time):; Athersys Inc.. **B. Lang:** None. **A.E. Ting:** A. Employment/Salary (full or part-time):; Athersys Inc. **R.J. Deans:** A. Employment/Salary (full or part-time):; Athersys Inc. **R.W. Mays:** A. Employment/Salary (full or part-time):; Athersys Inc.. **J. Silver:** None.

Poster

523. Spinal Cord Injury: Therapeutic Strategies II

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

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Topic: C.10. Trauma

Support: Nathalie Rose Barr Studentship from the International Spinal Research Trust.

Title: Promoting neuroplasticity after spinal cord injury by over-expressing polysialic acid

Authors: *L. ADAMS, Y. ZHANG, P. K. YIP, A. T. MICHAEL-TITUS, J. V. PRIESTLEY, X. BO
Barts and The London Sch. of Med. and Dent., Ctr. For Neurosci. and Trauma, London, United Kingdom

Abstract: Some neuronal populations are surrounded by a dense extracellular matrix structure known as the perineuronal net (PNN). Formed during the late stages of postnatal development, the PNN contains a high proportion of chondroitin sulphate proteoglycans (CSPGs), molecules that exert a strong inhibitory effect on neuroplasticity. CSPGs are up-regulated following spinal cord injury and the PNN is believed to be partly responsible for the poor behavioural recovery observed in this condition. Unpublished data from our laboratory suggests there is an inverse relationship between the PNN and polysialic acid (PSA), a sugar molecule found almost exclusively bound to the neural cell adhesion molecule. We have noted that the development of the PNN coincides with the down-regulation of PSA, from which we propose that PSA may restrict the formation of the PNN *in vivo*. The aim of the present study is to further examine the relationship between PSA and the PNN and to investigate the use of PSA as a plasticity-promoting therapy for spinal cord injury. Adult rats were injected with a lentiviral vector carrying the cDNA for the polysialyltransferase gene (LV/PST) into the sensorimotor cortex. In a separate experiment, adult rats received an injection of LV/PST into the cervical spinal cord. Strong PSA immunolabelling could be detected surrounding the injection site 2, 4 and 6 weeks post-injection in animals injected with LV/PST. Preliminary histological analysis shows that Wisteria floribunda agglutinin lectin (WFA)-PNN immunolabelling is reduced or absent around neurons transduced by LV/PST. We believe this is due to a reduction in WFA binding to its site within the PNN. To see whether this treatment can cause any behavioural improvements following spinal cord injury, adult rats were given a C5 hemisection lesion. Rats received two injections of LV/PST or the control virus LV/GFP rostral and caudal to the lesion between C4 and C7 and forelimb function was monitored for six weeks. Sprouting of corticospinal axons to or across the lesion site and the formation of new neural circuits with the surviving interneurons or spinal motor neurons, along with improved functional recovery, will be examined.

Disclosures: L. Adams: None. **Y. Zhang:** None. **P.K. Yip:** None. **A.T. Michael-Titus:** None. **J.V. Priestley:** None. **X. Bo:** None.

Poster

523. Spinal Cord Injury: Therapeutic Strategies II

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Topic: C.10. Trauma

Support: NSF 1159774 to CES

Mission Connect JWG, ZZK, CES

Title: Local and sustained delivery of growth factor mediates spinal learning after injury

Authors: *Z. Z. KHAING[1], J. H. PARK[2], J. W. GRAU[3], K. H. LEE[3], A. NIEMERSKI[3], C. E. SCHMIDT[1]
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Abstract: Damaged axons in the adult mammalian central nervous system (CNS), including those of the spinal cord, do not regenerate spontaneously. This is the result of both intrinsic and extrinsic inhibitory factors that limit the regeneration of adult neurons. Even after the removal of extrinsic inhibitory components, regeneration of adult neurons is still limited. Therefore, additional factors that can further enhance the intrinsic plasticity of the adult neurons are need. To this end, we developed a highly versatile biomaterial platform for sustained, localized delivery of brain-derived neurotrophic factor (BDNF). Using injectable hydrogel in combination with microparticles, we intend to deliver BDNF in order to improve neuronal regeneration. We hypothesize that BDNF from the hydrogels will release first, followed by BDNF release from microparticles. A physical blend of hyaluronic acid (HA) and methylcellulose (MC) was used to produce a thermally gelling hydrogel. We determined that HAMC hydrogels (1.5% HA, 7% MC) degraded by 7 days (n=5 gels at each time points) and their compressive modulus is within the range to that of adult rodent spinal cord tissue (~800 Pa). We loaded HAMC hydrogels with BDNF and determined the therapeutic dose released using ELISAs and found that over 70% of the encapsulated BDNF was by 12 hrs *ex vivo*. Currently, these hydrogels are being further refined using rheological analyses, degradation and BDNF release studies. For our secondary release mechanism, a FDA approved, degradable synthetic polymer poly-lactic-co-glycolic acid (PLGA) was used. To promote sustained delivery, microparticles with a range of sizes (5 - 40 μm) have been produced. Moreover, we applied BDNF, a growth factor with known beneficial effects following spinal cord injury (SCI), locally to the injured spinal cord using HAMC hydrogels. We found spinal mediated learning in HAMC loaded with BDNF while the learning

was not observed in animals that received HAMC gels alone. Our initial degradation of BDNF-loaded PLGA particles studies suggest that the microparticles can lasts for up to 28 days *in vitro*. We are currently optimizing *in vitro* and *in vivo* growth factor release profiles using this hydrogel microparticle composites. If appropriate release rates are observed, we will perform longer-term local delivery of BDNF for spinal learning after SCI. Our data indicate that an injectable HAMC hydrogel provides a flexible platform for loading bioactive molecules for sustained, localized delivery. Composite systems like these can be used for sustained and localized delivery of therapeutics to the brain and spinal cord.

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Poster

523. Spinal Cord Injury: Therapeutic Strategies II

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Topic: C.10. Trauma

Support: MSCRFII-0159-00

MSCRFII-0091-00

R21HD057487-01A1

MSCRFII-0109-00

Title: The feasibility of using human induced pluripotent stem cells derived oligodendrocyte progenitors in treatment of spinal cord injury

Authors: *P. MOHAMMAD GHARIBANI[1], A. ALL[1], C. KERR[2]
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Abstract: Pluripotent stem cells can expand indefinitely and differentiate into any other cell type such as neurons and glial cells. Induced pluripotent stem (iPS) cells, a type of pluripotent stem cell that can be generated directly from adult cells, are highly similar to natural pluripotent stem cells and since iPS can be derived directly from adult tissues they can be made in a patient-matched manner for autologous transplants without the risk of immune rejection. In this study, three different reprogramming strategies were used to generate Oligodendrocyte Progenitor cells

(OPs) from three different iPS cell lines (BC1, A1-4 and MR3). These cells were also used in transplants for moderate spinal cord injury (SCI) during the acute stage. Interestingly, in our *in vitro* studies, iPS derived OPs (iPS-OPs) from all three cell lines yielded 40-60% of total cells. Moreover, all three cell lines generated a similar number of early neural and glial progenitors. BC1-iPS derived OPs (using episomal, non-integrating plasmid approach) expressed more mature OP markers than early OP markers. Our *in vivo* studies showed that engrafted OPs survived for several months and almost 70% of them differentiated into mature oligodendrocytes. Notably, not only transplanted OPs yielded a significant increase in the number of myelinated axons, but also led to almost 5 fold reduction of cavity size and glial scarring in the contusion site. In addition, no tumor formation or any abnormal growth was seen in the site of injection. In conclusion, our results demonstrated that iPS derived OPs can be a candidate for replacing oligodendrocyte populations and axon remyelination following spinal cord injury.

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Poster

523. Spinal Cord Injury: Therapeutic Strategies II

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 523.24/BB32

Topic: C.10. Trauma

Title: Intracranial xenograft model as a validation system to assess tumorigenicity of NS/PCs for transplantation therapy

Authors: *K. HORI[1,2], J. KOHYAMA[2], K. MATSUBAYASHI[1,2], A. IWANAMI[1], H. OKANO[2], Y. TOYAMA[1], M. NAKAMURA[1]
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Abstract: [Purpose] Transplantation of neural stem/progenitor cells (NS/PCs) is now considered to be a promising treatment for various central nervous system disorders including spinal cord injury, Alzheimer's disease, Parkinson's disease, and brain infarction. In countries where fetal NS/PCs are not allowed to use due to ethical issues, iPS cells are especially a potential cell source of NS/PCs for cell therapy. To apply these cells to clinic, the risk of tumorigenicity must be eliminated. The purpose of this study is to establish an *in vivo*-validation system to assess tumorigenicity of transplanted NS/PCs efficiently. [Method] Human NS/PCs induced from adult

dermal fibroblast-derived hiPS cells were labeled with Lenti-ffLuc (Venus fused to firefly luciferase) vector. Two kinds of xenograft models were examined in this study: intracranial and spinal xenograft models. For intracranial xenograft models, 1x10^6 cells were injected into each side of the striatum of NOG mice or NOD/SCID mice without injury. For spinal xenograft models, 5x10^5 cells were transplanted into the spinal cord of NOD/SCID mice 9 days after contusion injury. Survival of transplanted cells was evaluated using bioluminescence-imaging system in both models. For histological analysis, mice were intracardially perfused 6~30 weeks after transplantation. [Result] The transplanted cells could be detected by bioluminescence-imaging throughout this study, both in intracranial and spinal xenograft model. Histological examinations revealed that the transplanted NS/PCs proliferated at the injected site, differentiated into neurons predominantly, and extended neuronal process to broad areas. In some cases of brain xenograft model, extramedullary proliferation of transplanted cells was found. [Conclusion] The transplanted cells survived and proliferated in both models. Compared with spinal xenograft model, intracranial xenograft model would be better to detect tumorigenicity of NS/PCs for transplantation therapy, since it allows us to evaluate a larger number of cells in each animal by the reproducible procedure. Further study exploring the character of transplanted cells around injection site is needed.

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Poster

523. Spinal Cord Injury: Therapeutic Strategies II

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Topic: C.10. Trauma

Support: NIH 2P30 NS051220

Kentucky Spinal Cord & Head Injury Trust grant #10-10

Title: Gabapentin management of autonomic dysreflexia: Effects on systemic inflammation

Authors: *A. G. RABCHEVSKY[1], K. C. ELDAHAN[1], J. L. VANROOYEN[1], C. Y. WANG[2], T. L. SMITH[2], D. H. COX[2], S. P. PATEL[1]
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Abstract: Autonomic dysreflexia (AD) is an abnormal hypertensive syndrome often triggered by unperceived pain that affects individuals with a spinal cord injury (SCI) above the sixth thoracic (T6) level; the vast majority of cases. We are currently investigating whether treatment with a widely-used drug for chronic neuropathic pain, gabapentin (GBP, Neurontin), after complete T4 SCI in rats abrogates the inflammatory responses in dorsal root ganglia (DRG) that contribute to maladaptive intraspinal plasticity underlying the temporal development of AD. DRG sprouting responses to injury invariably involve macrophage activation, 'inflammatory' cytokine production and upregulation of 'sprouting' transcription factors. Critically, administration of GBP significantly attenuates both noxious colorectal distension (CRD)-induced AD and induced tail spasticity weeks after complete T4 SCI, and daily GBP administration reduces spontaneous AD events detected using an algorithm based on blood pressure telemetry data. The current studies, therefore, were designed to test the hypothesis that prolonged, repeated CRD is correlated with increased 'inflammatory cytokine' and 'sprouting factor" gene expression in the L6/S1 and T13/L1 DRGs, their corresponding spinal cord segments, and the colon, bladder and visceral organs they innervate. We further hypothesized that since GBP treatment effectively reduces the severity of AD, then this will be correlated with blunted injury-induced gene expression in central and peripheral tissues. One week after abdominal aorta probe implantation, adult female Wistar rats underwent complete T4 SCI and 21 days later were subjected to prolonged, intermittent CRD for 1.5 hr (30 sec on, 60 sec off) before all sampled tissues were processed by qRT-PCR for mRNA expression of stress-induced transcription factors (e.g. ATF-3) and inflammatory cytokines (e.g. IL-1b, CCL2), with or without accompanying GBP. In addition, ELISA was used to examine IL-1b protein expression in innervated pelvic visceral tissues, with or without GBP treatment. Preliminary results show that chronic T4 SCI alone increased the expression of ATF-3 and inflammatory cytokines in neural tissues. Moreover, CRD further elevated IL-1b protein expression in peripheral tissues that was also reversed by GBP. Since AD has recently been reported to result in chronic immunosuppression, these studies are critical to establish whether GBP has immunomodulatory effects that are associated with its alleviation of AD. Such an effect may allow individuals with SCI to be weaned off other drugs that they are also taking for AD, spasticity and/or immunotherapy (e.g. polypharmacy).

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Poster

523. Spinal Cord Injury: Therapeutic Strategies II

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Program#/Poster#: 523.26/CC1

Topic: C.10. Trauma

Support: The Burke Foundation

Title: Using transcription factors to promote the survival of transplanted cells for spinal cord injury repair

Authors: *K. A. SCORPIO, J. L. BROWN, B. T. DAVID, R. R. RATAN, C. E. HILL
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Abstract: Cellular transplants offer a multifaceted approach for spinal cord injury (SCI) repair. Despite significant progress in understanding their clinical utility, a common feature of all cell transplants, which may have detrimental effects on the use of transplants clinically, is the early death of transplanted cells.The precise cause of transplanted cell death remains elusive and strategies that significantly enhance transplant survival have not yet been developed. To date, strategies to prevent transplanted cell death have focused on either blocking induction of cell death or blocking cell death signaling once started. Although there has been some success with these strategies, the presence of multiple cell death inducers and complex cross-talk between cell death pathways once activated have meant that the effects of the aforementioned strategies are modest. An alternative strategy is to counteract acute cell death signaling via direct activation of pro-survival pathways. One method to elevate pro-survival pathways in cells is to activate transcription factors involved in endogenous adaptive responses to stress. Among the transcription factors implicated in adaptive cellular responses to stress are the hypoxia inducible factors (HIFs). In the current study we examine the effect of enhancing HIF activity in Schwann cells following transplantation into the injured spinal cord of rats. We demonstrate that transplanted cells normally have low levels of HIF transcriptional activity and that enhancing HIF activity through either over-expression or pharmacological manipulation results in improved survival of transplanted cells. This suggests that transcription factor activation may be a beneficial strategy for promoting the survival of transplanted cells.

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Poster

523. Spinal Cord Injury: Therapeutic Strategies II

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Title: Chronic L1 overexpression causes widespread changes in expression of molecules engaged in neuronal plasticity Adcy1, GAP-43 and synaptophysin, but not in ADAM10 - L1 shedding metalloprotease in rats with complete spinal cord transection

Authors: *R. K. PLATEK[1], A. WIECKOWSKA[1], K. GRYCZ[1], J. CZARKOWSKA-BAUCH[1], S. KÜGLER[2], M. SKUP[1]
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Abstract: Recovery from spinal cord injury (SCI) requires neuronal remodeling which is regulated by cell adhesion molecules (CAMs) and inhibited by chondroitin sulfate proteoglycans (CSPGs). Among approaches targeting restoration of function after SCI a promising one is to use L1 cell adhesion molecule (L1-CAM), found to promote axon growth, guidance, fasciculation and myelination during development and after injury of the nervous system. Our aim was to evaluate the effectiveness of L1 in (1) promoting regeneration of the corticospinal tract (CST) and (2) altering markers of synaptic plasticity [synaptophysin (Syn), adenylate cyclase1 (Adcy1), growth-associated protein 43 (GAP43)] and CSPG phosphacan expression in the chronic phase after complete spinal cord transection in an adult rat. To overexpress L1, adeno-associated viral vector serotype 5 encoding L1 (AAV5-L1) was injected into the first lumbar spinal segment after complete spinal cord transection at Th10/Th11. Two groups of spinal AAV5-L1 and AAV5-EGFP rats were used (for histology 4 rats/group; for biochemistry 7 AAV5-L1, 5 AAV5-EGFP rats). Five weeks after transection AAV5-L1 rats showed reduced retraction/increased outgrowth of DiI-labeled CST axons in 2 mm segment rostrally to the lesion border, as compared to the rats receiving AAV5-EGFP ($p<0.05$). AAV5-L1 rats showed increased levels of Syn, Adcy1 and GAP43 transcripts below the transection (BT) and of Adcy1 above (AT), as compared to AAV5-EGFP rats, which showed widespread decrease of Syn and Adcy1 after transection. On the contrary, significantly elevated phosphacan immunostaining BT in AAV5-EGFP rats was reduced (2-fold, $p<0.05$) in AAV5-L1 rats. AAV5-L1 transduced neurons and astrocytes in segments below transection. It lead to 80-and 60-fold increase of L1 mRNA level at thoracic segments BT (Th<L; $p<0.05$) and in L1-2 segments ($p<0.05$), respectively, as compared to intact control. While AAV5-L1 caused an increase of endogenous L1 protein AT, transgenic L1 was found only BT. This raised a question whether overexpression of L1 molecule BT may stimulate the production of soluble L1 forms, which might reach segments AT and interact with transected

CST. To examine it, an expression of metalloprotease ADAM10 responsible for L1 membrane shedding was evaluated. AAV5-L1 rats upregulated ADAM10 mRNA at Th<L and L1-L2, where overexpression of L1 occured but was not followed by increased ADAM10 protein. We conclude that both increased endogenous L1 above and transgenic L1 below transection contribute to structural remodeling of spinal network by decreasing phosphacan expression and upregulating molecules indispensible for axonal growth.

Disclosures: R.K. Platek: None. **A. Wieckowska:** None. **K. Grycz:** None. **J. Czarkowska-Bauch:** None. **M. Skup:** None. **S. Kügler:** None.

Poster

523. Spinal Cord Injury: Therapeutic Strategies II

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 523.28/CC3

Topic: C.10. Trauma

Support: Veterans Administration

NIH S042291

NIH EB014986

the Adelson Medical Research Foundation

Title: Neural progenitor cells overcome extrinsic inhibitors and extend axons in chronically injured spinal cord

Authors: *K. KADOYA[1], K. NGUYEN[1], M. TUSZYNSKI[1,2]
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Abstract: Environment of chronic stage of spinal cord injury (SCI) is more refractory to adult axonal growth, at least due to additional obstacles, including myelin debris and developed scar formation at lesion sites consisting of reactive astrocytes and chondroitin sulfate glycosaminoglycans (CSPGs). At the previous SFN meeting, we reported a great axonal extension from 6 months delayed NPC grafts (chronic) placed in T3 complete transection sites. But, there was a significant reduction in their number of axons, compared to 2 weeks delayed NPC grafts (subacute) placed in the same lesion. Because size of graft/lesion, presence of fibrous tissue separating the graft, and the extent of atrophy of host spinal cord were different, the

undiluted effect of chronically injured spinal cord on axonal growth from NPCs remains to be clarified. To ask this question, in the present study, GFP expressing NPCs were grafted into sites of rat C4 dorsal column injury at a time point 2 weeks (subacute) or 6 months (chronic) after the initial injury. Six weeks later, lesion sites were filled with mature neurons and glia without fibrous tissue, and there was no difference of graft/lesion size in both groups. GFP-labeled, graft-derived axons emerged from the lesion site in high numbers and extended over long distances of up to 2cm. Of note, there was no significant difference in the number of emerging GFP axons in host white matter when quantified 3mm caudal and rostral to grafts. In both grafted groups, GFAP and CSPG expression around the lesion site was attenuated compared to control lesioned, non-grafted groups. In another group of subjects, we examined whether degenerating white matter in chronic SCI inhibits NPC-derived axon outgrowth. Lesions were placed in the C5 dorsal columns, and GFP expressing NPCs were micro-grafted subacutely or chronically into dorsal column sensory tract at the C4 level, where axons were undergoing Wallerian degeneration after injury. While numerous myelin debris were present from 1 week to 6 weeks after injury, clearance of much debris had occurred by 6 months. Extending axons from NPCs in this degenerating tract were in equal numbers in both groups at a point 3 mm rostral to grafts, when examined 6 weeks later, indicating that NPCs can extend axons robustly in degenerating white matter at the both of chronic and subacute stage of SCI. Collectively, these findings indicate that NPCs exhibit a remarkable ability to extend axons over chronic scar formation around injury site and through degenerating white matter.

Disclosures: **K. Kadoya:** None. **K. Nguyen:** None. **M. Tuszynski:** None.

Poster

523. Spinal Cord Injury: Therapeutic Strategies II

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 523.29/CC4

Topic: C.10. Trauma

Support: NIH-NS 025713

Title: Histology and mechanisms underpinning dose response injections of a PTPRS modulator following spinal cord injury

Authors: ***A. TRAN**[1], B. T. LANG[1], J. M. CREGG[1], M. A. DEPAUL[1], Y. SHEN[2], J. SILVER[1]
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Abstract: A lack of restorative medical treatments has prompted a greater drive towards understanding molecular mechanisms following spinal cord injury (SCI) in order to develop new therapies that promote regeneration and restore function. Following SCI-induced gliosis, upregulation of inhibitory components of the extracellular matrix such as chondroitin-sulphate proteoglycans (CSPGs) result in signaling by membrane-bound receptor Protein Tyrosine Phosphatase Sigma (PTPRS). Our lab has designed a small peptide modulator of PTPRS (Intracellular Sigma Peptide, ISP), which we have previously shown to restore coordinated walking and micturition following a T8 contusive injury. This peptide is capable of pulling down rat and mouse PTPRS from brain and spinal cord lysates, in addition to binding to recombinant human intracellular PTPRS. No interaction was seen between ISP and either LAR or NOGO-Receptor. Here, we also tested whether locomotor and urinary behaviors could be further restored by increasing the daily subcutaneous dose of ISP following moderate to severe spinal cord injury (T8 Infinite Horizon Contusion at 250kDyne). Interestingly, locomotor recovery, as measured by the BBB scale and gridwalk test was not ISP dose-dependent, as each treatment group showed a similar number of responding animals. However, improvements in micturition, as measured by void frequency at 12 weeks post SCI, was remarkably ISP dose-dependent. Indeed, all animals became responders showing the greatest average improvement at the 4x (44ug/day) dose. We monitored anatomical plasticity by bi-lateral injections of the retrograde label Fast-Blue caudal to the lesion. Behavioral improvements correlated with an increased number of Fast-Blue positive neurons in various regions rostral to the lesion in ISP-treated rats compared to controls. The activation of PTPRS elicits a cascade of intracellular pathways that may cause axonal growth cones to become dystrophic resulting in a failure of neuronal regeneration; however, the exact mechanisms underlying regeneration is still unknown. Using *in vitro* dorsal root ganglion (DRG) cultures, we are beginning to elucidate how ISP induces such remarkable behavioral outcomes. In this way, we are beginning to show how PTPRS signaling is implicated in SCI.

Disclosures: **A. Tran:** None. **B.T. Lang:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patent Holder of ISP. **J.M. Cregg:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patent Holder of ISP. **M.A. DePaul:** None. **J. Silver:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patent Holder of ISP. **Y. Shen:** None.

Poster

523. Spinal Cord Injury: Therapeutic Strategies II

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 523.30/CC5

Topic: C.10. Trauma

Support: NIH Grant NINDS R01-NS08111

Title: Breathing easier: The benefits of neural progenitor transplantation after cervical spinal cord injury

Authors: ***V. SPRUANCE**[1], K. M. NEGRON[1], D. SANCHEZ[2], T. BEZDUDNAYA[1], B. OSTEEN[2], P. REIER[2], M. A. LANE[1], T. J. WHELAN[1]
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Abstract: Breathing deficits and related complications remain the leading causes of mortality following cervical spinal cord injury (SCI). Considering over half of all SCIs occur at the cervical level, therapeutic approaches to improve respiratory function are desperately needed. Enhancing endogenous spinal plasticity will likely yield the greatest improvements in long-term respiratory recovery, rather than relying on long distance regeneration of respiratory circuits. The goal of the present study is to promote repair of the injured spinal cord using a cell transplantation strategy, and harness ongoing respiratory neuroplasticity, to improve phrenic motor function and recovery of diaphragm activity. Recent studies have revealed that a population of pre-phrenic interneurons may play an integral role in plasticity related to recovery of diaphragm function, the major muscle of inspiration. Therefore, we hypothesized that transplantation of interneuronal rich progenitor cells derived from fetal spinal cord tissue may provide an anatomical substrate for the formation of new, relay pathways that will improve diaphragm function. Adult, female Sprague-Dawley rats received lateralized C3/C4 contusion injuries using the Infinite Horizons Impactor Device (intended impact force: 200 kilodynes). One week following injury, FSC tissue suspension obtained from E13.5 day rats was transplanted into the injury cavity. Animals were allowed to recover for one month before receiving injections of pseudorabies virus (a retrograde, transynaptic tracer, PRV) to the diaphragm or transplant, and were sacrificed 72 hours later. Respiratory function was assessed using terminal diaphragm EMG recordings or phrenic nerve recordings. To assess temporal change in function, a subset of animals was implanted with telemetric diaphragm electrodes and underwent weekly plethysmograph and EMG recordings. Electrophysiological recordings were made under normoxic and hypoxic (10% oxygen) conditions to assess function during eupneic and challenged breathing. Immunohistochemical analysis of PRV demonstrated anatomical connectivity between host and transplanted tissue, while EMG and plethysmograph studies reveal improved breathing abilities during both normal conditions as well as respiratory challenge. Ongoing experiments will combine transplantation with optogenetic and rehabilitative

techniques to further enhance synaptic integration between host and transplant, endogenous respiratory plasticity and optimize long-term recovery of breathing.

Disclosures: **V. Spruance:** None. **K.M. Negron:** None. **D. Sanchez:** None. **T. Bezdudnaya:** None. **B. Osteen:** None. **P. Reier:** None. **M.A. Lane:** None. **T.J. Whelan:** None.

Poster

524. Schizophrenia: Dopamine and Antipsychotic Drugs

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 524.01/CC6

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: NIH Grant MH090963

Title: Cell-type specific analysis of antipsychotic drug action in D1 and D2 neurons of the striatum

Authors: ***R. C. SCHWARCZ**[1], S. CHENG[2], P. GREENGARD[2], A. C. NAIRN[3]
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Abstract: Antipsychotic drugs (APDs) have been the primary treatment for the positive symptoms of Schizophrenia for over fifty years. APDs are classified as either typical or atypical drugs. Although both drug classes were found to block dopamine D2 receptors to some extent, the downstream mechanisms of therapeutic action remain unclear. Furthermore, APDs usually produce potentially irreversible side effects (including movement and metabolic disorders), indicating the need for a more targeted approach. In this study, we will examine cell-type specific effects of APDs on downstream signaling elements in the two primary cell types of the striatum: D1 and D2-type neurons. We hypothesize that phosphorylation at residue Serine-97 (S97) of the striatal enriched protein DARPP-32, a mediator of dopamine action, will be decreased by APD exposure. Dephosphorylation of the S97 residue results in the nuclear retention of DARPP-32, which can promote changes in histone modifications and potentially result in long term gene expression changes. We are employing a line of BAC transgenic mice with differentially tagged flag and myc-tag DARPP-32, expressed under the control of the D1 or D2 receptor promoter, respectively. We have treated these animals (n=10) with both typical and atypical antipsychotics, differentially immunoprecipitated flag/myc-DARPP-32 (D1/D2), and used immunoblotting to assess S97 phosphorylation in total, D1, and D2 extracts. We have also

used immunohistochemistry to correlate our results with DARPP-32 cellular localization. Our results indicate that APDs can induce significant cell-type specific alterations, which are sometimes masked in overall striatal extracts. This may help provide insight into potential common mechanisms of APD-induced signaling regulation, and possible targets for future therapeutics.

Disclosures: R.C. Schwarcz: None. **S. Cheng:** None. **P. Greengard:** None. **A.C. Nairn:** None.

Poster

524. Schizophrenia: Dopamine and Antipsychotic Drugs

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 524.02/CC7

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: K23MH079498

R01MH059852

Title: Molecular changes in odorant signaling in olfactory neuroepithelial cells from patients with schizophrenia

Authors: *K. BORGMANN-WINTER[1,2], H.-Y. WANG[3], R. RAY[4], B. WILLIS[1], B. TURETSKY[1], C.-G. HAHN[1]
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Abstract: Schizophrenia is associated with pervasive deficits in olfactory function, including odor detection and discrimination. Little is known, however about the molecular underpinnings of pathophysiologic deficits in olfactory function in schizophrenia. Turetsky and colleagues have demonstrated that patients with schizophrenia exhibit increased electrolfactogram (EOG) measures induced by odorants while they show decreased olfactory evoked potentials. In this study we examined human olfactory neuroepithelial (ON) cells derived from 18 patients with schizophrenia and their matched controls for odorant and neurotransmitter mediated G protein coupling. .To assess ligand induced G protein activation, we examined membrane fractions from ON cells, incubated with odorants and S35-GTPgS. Incorporation of S35-GTPgS into G proteins

were assessed by immunoprecipitation of membrane fractions for specific subclasses of G proteins and measurement of S35 incorporation. In ON cells of patients and controls, we found a significant decrease in the activation of Gs/olf (students t test; t = 2.264, df=32 and p = 0.03) and of Go in the SCZ group (two tailed students t test; t=3.348 df=32 and p = 0.002). A subgroup of the same matched pairs were also examined for G protein activation in response to odorant Mix B. We observed similar decreases in Gs/Golf as well as Go. To test whether these changes were mediated by the expression of key molecules of G protein coupling, we examined mRNA and protein expression of ACIII, PKCgamma, Gs and Golf in a subset of 7 subjects and their matched controls. No between group differences were seen at the mRNA level, but ACIII protein expression was decreased in patients with schizophrenia (students paired t test; t = 3.077, df=7 and p = 0.02). Together, these suggest that increased odorant signaling found in EOG studies may not be the direct result of decreased G protein coupling but rather reflect increased function of G protein activation or ACIII. Alterations in G protein coupling could be either a reflection of pervasive deficits across various receptors or specific to receptors. We examined G protein activation in response to dopamine (DA) or 5HT in OE cells of SCZ patients and controls. We found significant increases in activation of Gs/olf and Gq/11 mediated by dopamine (DA) or 5HT in OE cells of SCZ patients. Divergent changes in G protein activation between odorant- and DA/5HT- stimulation may suggest altered G protein coupling in a receptor specific manner in schizophrenia. Future studies should test if increased DA induced G protein coupling could be a molecular substrate for increased activation of D2R signaling as implicated for schizophrenia.

Disclosures: **K. Borgmann-Winter:** A. Employment/Salary (full or part-time):; University of Pennsylvania, Children's Hospital of Philadelphia. **H. Wang:** A. Employment/Salary (full or part-time):; City University of New York. **R. Ray:** A. Employment/Salary (full or part-time):; University of Pennsylvania. **B. Willis:** A. Employment/Salary (full or part-time):; University of Pennsylvania. **B. Turetsky:** A. Employment/Salary (full or part-time):; University of Pennsylvania. **C. Hahn:** A. Employment/Salary (full or part-time):; University of Pennsylvania.

Poster

524. Schizophrenia: Dopamine and Antipsychotic Drugs

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 524.03/CC8

Topic: C.15. Schizophrenia and Bi-polar Disorder

Title: Dissociation kinetics of [14C]F17464 binding at human dopamine D2S and D3 receptor subtypes

Authors: *J.-C. MARTEL, N. DANTY, A.-M. ORMIERE, G. PULOU, P. SOKOLOFF
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Abstract: Receptor-ligand dissociation rate seems to be a more critical parameter for the kinetics of brain receptor occupancy by antipsychotic drugs than changes in blood concentration. For instance, perospirone, which displays a high affinity (Ki = 0.58 nM) at dopamine hD2 receptor, has a short plasma half-life (1.9 h), but a long-lasting hD2 receptor occupancy with an occupation half-life of approximately 24 h. While development of D3-selective antipsychotics is still being actively investigated, the available clinical reports suggest that optimal hD3 receptor occupation has not yet been achieved in clinical studies, thus highlighting the importance of a better control over this parameter in future clinical studies. F17464 is a D3-selective antipsychotic candidate, having 46-71 fold higher affinity at hD3 versus the hD2 receptor subtype isoforms, suggesting that it should dissociate much faster from hD2 than from hD3. In order to verify this property of F17464 and better predict its brain dopamine receptor occupancy kinetics, the dissociation kinetics of [^{14}C]F17464 was evaluated at 37°C on hD2S and hD3 receptor subtypes. Binding assays on dopamine hD2S and hD3 receptors were performed on membranes from Chinese hamster ovary cells expressing these receptors at high levels (hD2S: 24 pmol/mg proteins and hD3: 4.1 pmol/mg proteins). [^{14}C]F17464 (final concentration of 32 nM (hD2S) or 6 nM (hD3) in 2 mL final volume) was incubated with 1600 µL of the appropriate membrane preparation which was added to start the kinetics. Incubations were performed for times ranging from 2 to 360 minutes, and nemonapride (10 µM) was added after an initial 1 hr incubation to initiate dissociation. At the end of the set incubation time, each tube was rapidly filtered under vacuum through GF/B filters. While [^{14}C]F17464 dissociated rapidly from hD2S (K_{OFF} : 0.51 min^{-1}; $t_{1/2}$: 1.4 min), a dissociation constant close to that of clozapine at hD2, it dissociated much more slowly from hD3 (K_{OFF} : 0.0064 min^{-1}; $t_{1/2}$: 110 min). This study demonstrates that [^{14}C]F17464 binds relatively tightly to hD3 receptors, while having a much looser attachment to hD2S receptors, in agreement with its hD3 receptor selectivity. Such tight attachment of F17464 to hD3 predicts a long-lasting hD3 occupancy *in vivo*.

Disclosures: **J. Martel:** A. Employment/Salary (full or part-time):; Pierre Fabre Médicaments. **N. Danty:** A. Employment/Salary (full or part-time):; Pierre Fabre Médicaments. **A. Ormiere:** A. Employment/Salary (full or part-time):; Pierre Fabre Médicaments. **G. Pulou:** A. Employment/Salary (full or part-time):; Pierre Fabre Médicaments. **P. Sokoloff:** A. Employment/Salary (full or part-time):; Pierre Fabre Médicaments.

Poster

524. Schizophrenia: Dopamine and Antipsychotic Drugs

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 524.04/CC9

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: Elsa-Neumann PhD scholarship

Title: Chronic treatment with the D2 receptor partial agonist 2-bromoterguride does not induce changes in body weight and body fat composition in rats

Authors: *R. T. FRANKE[1], H. H. PERTZ[2], H. FINK[3], J. BROSDA[3]
[1]Free Univ. Berlin, Berlin, Germany; [2]Dept. of Biology, Chemistry, and Pharmacy, FU Berlin, Inst. of Pharm., Berlin, Germany; [3]Sch. of Vet. Medicine, FU Berlin, Inst. of Pharmacol. and Toxicology, Berlin, Germany

Abstract: Introduction: Schizophrenia is a chronic mental illness. Current pharmacological therapy has limited efficacy and is related with severe side effects, e.g. motor adverse effects, weight gain and changes in body fat composition. Partial agonism at dopamine D2 receptors represents a sophisticated option for effective antipsychotic treatment with diminished risk for side effects. Our previous *in vitro* and *in vivo* data revealed that 2-bromoterguride (2-BT) is a dopamine D2 partial agonist that shows promising antipsychotic characteristics in acute treatment [1]. Methods: We examined chronic effects of 2-BT with the same doses as used before (0.1 and 0.3 mg/kg; 21 days; twice daily) [1] on food and water intake, body weight and fat tissues in female Sprague Dawley rats. In addition, we investigated the influence of chronic 2-BT on spontaneous behavior in the open field box and cataleptic behavior in the bar and grid test. The atypical antipsychotic olanzapine (2 mg/kg) was used as a positive control. Results: In contrast to olanzapine, chronic 2-BT administration did not induce changes in food and water intake, body weight and body fat composition. Similar to acute conditions, 2-BT induced no cataleptic behavior but decreased spontaneous locomotion. Conclusions: Apparently, chronic 2-BT treatment does not result in accelerated weight gain or altered body fat composition. Both doses of 2-BT seem to be slightly sedative without inducing motor adverse effects. The present study confirms our previous observations [1] that the D2 receptor partial agonist 2 BT could be a promising candidate for the treatment of schizophrenia with minor side effects. Reference: [1] F. Jantschak, J. Brosda, R.T. Franke, H. Fink, D. Möller, H. Hübner, P. Gmeiner, H.H. Pertz (2013). Pharmacological profile of 2-bromoterguride at human dopamine D2, porcine serotonin 5 hydoxhydroxytryptamine 2A, and α2C-adrenergic receptors, and its antipsychotic-like effects in rats. J Pharmacol Exp Ther 347:57-68.

Disclosures: **R.T. Franke:** None. **H.H. Pertz:** None. **H. Fink:** None. **J. Brosda:** None.

Poster

524. Schizophrenia: Dopamine and Antipsychotic Drugs

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 524.05/CC10

Topic: C.15. Schizophrenia and Bi-polar Disorder

Title: Modulation of dopaminergic signalling in the striatum by phosphodiesterase 10A (PDE10A) inhibitors

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Abstract: Phosphodiesterase 10A (PDE10A) is a dominant phosphodiesterase in striatal medium spiny neurons (MSN) that hydrolyse the second messengers cAMP and cGMP. Inhibition of PDE10A leads to increased cyclic nucleotide levels in the striatum and is expected to negatively modulate dopamine D2 receptor signalling in the indirect pathway MSN and positive modulate dopamine D1 receptor signalling in the direct pathway MSN. Preclinical *in vivo* evidence suggests antipsychotic as well as pro-cognitive activity for PDE10A inhibitors. We present comparative behavioural data for structurally unrelated PDE10A inhibitors along with PDE10A occupancy assessed by *in vivo* binding. In agreement with previously published studies, PDE10A inhibitors exhibited a profile very similar to D2 antagonists in assays such as phencyclidine-induced hyperactivity and conditioned-avoidance response suggesting a predominant effect on indirect pathway MSNs. However, in models where dopamine is elevated, such as amphetamine-induced hyperactivity, D2 antagonist-like effects only dominated at low PDE10A occupancy, while prominent D1 agonist-like effects became apparent at higher occupancy. The mechanism underlying those observations was further investigated by examining induction of cFOS expression in D1 and D2 expressing MSNs at different levels of PDE10A inhibition as a measure of activation of those neurons. These studies supported predominant activation of D2-expressing MSNs at low occupancy and activation of all MSNs at high PDE10A occupancy.

Disclosures: J. Nielsen: A. Employment/Salary (full or part-time):; Lundbeck. **B. Steiniger Brach:** A. Employment/Salary (full or part-time):; Lundbeck. **P.H. Larsen:** A. Employment/Salary (full or part-time):; Lundbeck.

Poster

524. Schizophrenia: Dopamine and Antipsychotic Drugs

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 524.06/CC11

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: Tourette Syndrome Association (TSA)

COST (Action CM1103)

NIH (Grant HD070611)

Regione Autonoma della Sardegna (RAS)

Fondazione Banco di Sardegna

Title: Inhibition of 5alpha-reductase enzyme restores gating deficits elicited by D1 and D3, but not D2 activation in rats

Authors: R. FRAU[1], L. MOSHER[2], V. BINI[1], A. PARDU[1], R. PES[1], S. FANNI[1], M. BORTOLATO[2], *P. DEVOTO[1]
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Abstract: Although multiple lines of evidence support a role for neurosteroids in the modulation of dopamine (DA) neurotransmission, the neurobiological underpinnings of this link remain poorly understood. Accordingly, we previously found that inhibition of 5alpha-reductase (5AR), a key neurosteroidogenic enzyme, normalized the behavioral outcomes elicited by non-selective DA receptor agonists in rats, including stereotyped behaviors and sensorimotor gating deficits, as measured by the prepulse inhibition (PPI) of the acoustic startle reflex. Furthermore, we recently showed that in C57BL/6 mice the 5AR blockade attenuated the behavioral responses ensuing D1-, but not D2-like receptor activations. Notably, all these antidopaminergic effects were not associated to extrapyramidal manifestations. Here, to further delineate the specific influences of the 5AR in the DAergic regulation of PPI, we tested the impact of the 5AR inhibitor finasteride (FIN) on the PPI-disruptive effects of D1, D2 and D3 DA agonists in Sprague-Dawley (SD) and Long-Evans (LE) rats. We found that the mixed D1/D2 agonist apomorphine (0.5 mg/kg, SC) elicited PPI disruptions in both strain, and these deficits were efficiently reversed by FIN (100 mg/kg, IP) in SD, but not in LE rats. On the other hand, the D1 receptor agonists SKF-38,393 (10 mg/kg, SC), SKF-82,958 (1 mg/kg, SC) and SKF-83,959 (1 mg/kg, SC) had no effects on

PPI in SD rats. Nevertheless, the full D1 agonist SKF-82,958 (1 mg/kg, SC) was able to reduce PPI parameters on LE rats, and these impairments were normalized by FIN (100 mg/kg, IP). The D2/D3 agonists quinpirole (O.6 mg/kg, SC) and the selective D2 agonist sumanirole (3 mg/kg, SC) disrupted PPI in both strain, but FIN did not revert these effects. Of note, the pretreatment with FIN (100 mg/kg, IP) significantly restored the PPI deficits induced by the selective D3 agonist PD 128907 (0.1 mg/kg, SC) in SD rats, whereas this latter compound failed to disrupt PPI in LE rats. Finally, to ascertain whether FIN may regulate D3 receptors by pre- or -post synaptic mechanisms, microdialysis studies performed in the nucleus accumbens of SD rats revealed that the pretreatment with FIN fully counteracted the reductions of DA release produced by PD injections. These results indicate that 5AR plays a crucial role in the dopaminergic regulation of gating through multiple mechanisms, based on pre- and post- synaptic actions via D3 and D1 receptors, respectively. Furthermore, these findings strengthen our previous evidence supporting 5AR in the therapy of neuropsychiatric disorders and emphasize D3 receptors as a novel intriguing molecular target underlying FIN mechanisms.

Disclosures: R. Frau: None. **L. Mosher:** None. **P. Devoto:** None. **V. Bini:** None. **A. Pardu:** None. **R. Pes:** None. **S. Fanni:** None. **M. Bortolato:** None.

Poster

524. Schizophrenia: Dopamine and Antipsychotic Drugs

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 524.07/CC12

Topic: C.15. Schizophrenia and Bi-polar Disorder

Title: Chronic treatment with antipsychotic drugs disrupt histone H3 homeostasis in forebrain of rats

Authors: *I. PODDAR, C. M. HERNANDEZ, A. V. TERRY, Jr.
Pharmacol. and Toxicology, Georgia Regents Univ., Augusta, GA

Abstract: Cognitive dysfunction is considered one of the most debilitating symptoms of schizophrenia, however, our current understanding of how the primary treatments of this disease, first and second generation antipsychotics (FGAs and SGAs, respectively) affect cognition remains inadequate. This lack of knowledge extends to how antipsychotics (AP) affect the neurobiological substrates of cognition as well as responses to drugs that have been developed to improve cognition in schizophrenia. Our previous work in animals has established that both representative FGAs and SGAs can negatively affect attention, spatial learning, and memory if

administered chronically, however, the mechanism for these adverse effects have not been clearly elucidated. Here we tested the hypothesis that AP-related epigenetic modifications were the basis for the cognitive impairments. Male Wistar rats were treated with FGAs chlorpromazine (10.0 mg/kg/day), haloperidol (2.0 mg/kg/day) or SGAs risperidone (2.5 mg/kg/day), olanzapine (10.0 mg/kg mg/kg/day) in drinking water for 90 days and then sacrificed. Oral antipsychotic dosing was based on: 1) previous rodent studies in our laboratory in which time dependent behavioral and neurochemical effects were detected; 2) plasma drug levels were achieved that approximated those often associated with AP effects in humans 3) the doses selected were expected to achieve comparable and therapeutically relevant dopamine D2 receptor occupancy values *in vivo*. We evaluated the expression of post-translational histone H3 modifications with known associations to changes in gene expression that are a prerequisite for memory consolidation, specifically histone H3 lysine acetylation (K14) and methylation (K9) by western blot using epitope-specific antibodies. Our results indicate that both FGAs and SGAs were associated with significant changes in histone H3 homeostasis in regions important for learning and memory. Such epigenetic alterations may provide an explanation for AP-related cognitive impairments previously observed in animals as well as recent clinical failures of pro-cognitive agents in patients chronically treated with antipsychotics.

Disclosures: I. Poddar: None. **C.M. Hernandez:** None. **A.V. Terry:** None.

Poster

524. Schizophrenia: Dopamine and Antipsychotic Drugs

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 524.08/CC13

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: Grant-in-Aid for Young Scientists (B) (Grant Number 23791344) from the Japan Society for the Promotion of Science

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Title: Effects of the -141C Ins/Del polymorphism in the dopamine D2 receptor gene on the dopamine system in the striatum in schizophrenia

Authors: *J. MATSUMOTO[1], Y. KUNII[1], I. MIURA[1], M. HINO[1], A. WADA[1], S.-I. NIWA[2], H. NAWA[3], M. SAKAI[3], T. SOMEYA[4], H. TAKAHASHI[5], A. KAKITA[5], H. YABE[1]
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Abstract: (Background) The administration of antipsychotics which attenuates mainly dopamine systems have been believed to be crucial. The both systems of dopamine and glutamate are also considered to be related to the pathophysiology of schizophrenia(SCZ). Since dopamine- and cAMP-regulated phosphoprotein of molecular weight 32 kDa (DARPP-32) and Calcineurin (CaN) are the regulatory molecules of these two systems, it has been strongly suggested that these molecules might play the central roles in the etiology of SCZ. Thus in this study, we analyzed the associations of the expression of DARPP-32 and CaN with dopamine D2 receptor gene (DRD2) polymorphism, -141C Ins/Del in the postmortem striatum from patient with SCZ. (Materials and methods) We used immunoblotting techniques to examine the expression levels of two major DARPP-32 isoforms, full-length (FL-DARPP) and truncated (t-DARPP), and of CaN in the striatum of postmortem tissue samples from patients with SCZ and from normal control individuals. We also determined the -141C Ins/Del polymorphism in the DRD2 of brain samples by polymerase chain reaction and assessed whether there was any significant correlation between the expression levels of either protein and the -141C Ins/Del polymorphism in the DRD2. 12 postmortem brain samples from patients with SCZ were obtained from the Fukushima Brain Bank at the Department of Neuropsychiatry, Fukushima Medical University and 12 normal control postmortem brain samples were obtained from autopsy cases at the Brain Research Institute, University of Niigata. This study was approved by the Ethics Committee of Fukushima Medical University and complied with the Declaration of Helsinki. (Results) We found that the expression of t-DARPP in the SCZ with Del allele was significantly decreased compared to that with Ins/Ins genotype in Putamen ($p=0.042$) and the expression of CaN in the SCZ with Del allele was marginally significantly decreased compared to that with Ins/Ins in the Caudate ($p=0.0504$). Also, in the control subjects the expression of FL-DARPP and t-DARPP in the Caudate was significantly associated with -141C Ins/Del polymorphism in the DRD2 ($p=0.040$, 0.001, respectively). (Discussion) In this study we found that the striatal expression of DARPP and CaN was decreased in Del allele carriers. One previous study indicates that SCZ with Del allele noncarriers might be better responders of antipsychotics for the anxiety and the depression than that with Del allele carriers. Therefore, these results may reflect potential molecular mechanisms based on the antipsychotics resistant to anxiety and depression in SCZ.

Disclosures: **J. Matsumoto:** None. **Y. Kunii:** None. **I. Miura:** None. **M. Hino:** None. **A. Wada:** None. **S. Niwa:** None. **H. Nawa:** None. **M. Sakai:** None. **T. Someya:** None. **H. Takahashi:** None. **A. Kakita:** None. **H. Yabe:** None.

Poster

524. Schizophrenia: Dopamine and Antipsychotic Drugs

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Program#/Poster#: 524.09/CC14

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: MH093967

R01DA025890

Title: Cacna1c haploinsufficiency leads to altered mesolimbic dopamine system function

Authors: ***C. TERRILLION**, M. ARAD, D. T. DAO, R. CACHOPE, J. F. CHEER, T. D. GOULD
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Abstract: CACNA1C codes for the L-type calcium channel Cav1.2, and has been associated with clinical diagnoses of bipolar disorder, schizophrenia, and depression. L-type calcium channels are associated with normal function of the mesolimbic dopamine (ML-DA) system, dysregulation of which is linked to these disorders. We hypothesized that decreased levels of Cav1.2 leads to decreased ML-DA system function, resulting in attenuation of a subset of DA mediated behaviors. Cacna1c heterozygous (HET) and wild-type (WT) mice were tested in several behaviors following stimulant challenge, including acute locomotor response, sensitization, conditioned place preference (CPP), and stereotypic behavior. Using fast-scan cyclic voltammetry (FSCV), subsecond DA release and reuptake in the nucleus accumbens of HET and WT mice was measured following stimulation of the ventral tegmental area. Recordings were taken at a series of stimulation amplitudes and after GBR12909 administration. Western blot was used to determine levels of dopamine transporter (DAT) protein. HET mice manifested significantly reduced hyperlocomotion following acute administration of psychostimulants specific to DAT (amphetamine, cocaine, and GBR12909) but not to glutamate (MK-801), as well as delayed sensitization. There was no effect of genotype on stereotypic behavior or CPP. FSCV revealed that HET mice had significantly more rapid DA reuptake following GBR12909 administration compared to WT mice. There was no effect of genotype on

DAT protein levels. Cacna1c haploinsufficiency was associated with attenuation of selective DA dependent behaviors. FSCV revealed that Cav1.2 has a role in presynaptic ML-DA system function, including a likely role in regulating DAT function. However, this is not due to total levels of DAT protein suggesting that DAT activity is regulated through an alternative mechanism. Current experiments include using conditional Cacna1c knockout and transgenic lines to examine the specific role of Cav1.2 in the nucleus accumbens and ventral tegmental area.

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Poster

524. Schizophrenia: Dopamine and Antipsychotic Drugs

Location: Halls A-C

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Program#/Poster#: 524.10/CC15

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: NSERC Discovery Program 385732-2012

Graham Boeckh Foundation

Title: Effects of chronic treatment with typical and atypical antipsychotics on mouse brain volume in genetic deletion models of D2-like dopamine receptors

Authors: *E. GUMA[1], J. ROCCHETTI[1], B. COURCOT[2], A. MATHIEU[2], G. DAL BO[1], P. PATEL[1], B. GIROS[1]
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Abstract: Longitudinal MRI studies have consistently shown that schizophrenic exhibit brain volume reductions over the course of the illness. However, these patients are often treated with antipsychotic (AP) medication. Thus, it is difficult to determine whether the changes are related to the disorder, to the effects of the medication or both. The use of animal models allows for a more ethical assessment of the effect of AP treatment on brain volume, without interference of the pathology. Naïve C57BL/6J mice received daily intraperitoneal (i.p.) injections of saline or haloperidol (HAL) (0.5mg/kg/day) for 9 weeks. Animals were scanned using a Bruker 7T small MRI scanner before starting treatment, then at 3, 6 and 9 weeks to assess volumetric changes in the whole brain and predefined subregions, such as the prefrontal cortex, hippocampus, and

striatum. In accordance with previous studies done in rats, this treatment lead to a decrease in pre-frontal cortex volume. With access to D2 and D3 dopamine (DA) receptor knockout transgenic mice, we aimed to assess the role that D2-like DA receptors may play in the brain volume changes associated with AP use. Thus, D3-KO, D2-KO mice and their wild-type littermates were treated with daily i.p. injections of saline, HAL (typical AP) (1mg/kg/day), or clozapine (atypical AP) (5 mg/kg/day) for 9 weeks. Again, animals were scanned before starting the treatment, and then at 3, 6, and 9 weeks to track brain volume changes. Furthermore, to understand the cellular basis of volume changes, stereological analysis of NeuN+ neuronal populations, Iba1+ microglia, and GFAP+ astrocytes was performed in the prefrontal cortex. Studying the effect of a typical and atypical AP medication on the brain volume in D2KO and D3KO mice will allow us to better explain if the decrease in volume of the prefrontal cortex observed in wild-type animals after HAL medication is primarily due to the activity of D2-like receptors, highly targeted by typical AP medication, or whether these changes involve alternate - dopaminergic or non dopaminergic - pathways. In conclusion, this study helps to elucidate the region-specific structural effects of antipsychotics on mouse brain and the pathways implicated in these changes, which could allow for improvements in the design of treatments for psychotic disorders.

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Poster

524. Schizophrenia: Dopamine and Antipsychotic Drugs

Location: Halls A-C

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Program#/Poster#: 524.11/CC16

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: Ministry of Science and Technology Grant, Taiwan: NSC101-2320-B-182-040-MY3

Title: Exploration of the role of dopamine D4 receptor in methamphetamine-induced psychosis

Authors: *I.-M. LIAO[1], J.-C. CHEN[2]
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Abstract: Chronic methamphetamine (METH) use not only leads to addiction, but also gives rise to the development of psychotic symptoms, including delusion, hallucination, and

disorganized speech, in humans. Preclinical and clinical studies have addressed the significance of dopamine system in METH-induced psychosis; however, most studies were dopamine D2 receptor-oriented due to its putative role as the main drug target of typical antipsychotics. In spite of the fact that many studies attempt to address the potential of dopamine D4 receptor (D4R) as a novel molecular target of psychosis treatment, it remains unclear whether D4R signalling is critically participated in psychosis due to inconsistent results. Therefore, the purpose of this study is to investigate and delineate the role of D4R in METH psychosis development. To this aim, we used pharmacological manipulations and behavioral assessments to evaluate if D4R is involved in the development of METH psychosis. Male mice from six to eight weeks were used in this study. Selective D4R antagonist (also an atypical antipsychotic) clozapine (1, 2.5, 5 mg/kg, i.p.) was pre-treated before daily METH (2 mg/kg, i.p.) administration for 7 consecutive days. Horizontal locomotor activity and stereotypy were evaluated on the first and last day for 1 hour after METH treatment. Results showed that clozapine (1, 2.5, 5 mg/kg/day, i.p.) pre-treatment dose-dependently inhibited METH-induced behavioral sensitization (by 25%, 50%, 80%, respectively), suggesting that D4R may be critically involved during the sensitization process. The molecular mechanism underlying D4R-mediated METH sensitization antagonism is currently under investigation to elucidate the action of D4R, as well as D4R-associated protein(s), in METH-induced psychosis.

Disclosures: I. Liao: None. **J. Chen:** None.

Poster

524. Schizophrenia: Dopamine and Antipsychotic Drugs

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Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: Johnson & Johnson Translational Innovation Fellow

Title: Forebrain Specific Ankyrin G knockout Mouse displays mania like behavior, rescued by anti-psychiatric drugs and shows deficit in GABAergic synapse formation and ion channel activity

Authors: *S. ZHU[1], J. KIM[2], X. WANG[1], V. BENNETT[5], M. PLETNIKOV[1], S. BROWN[2], C. ROSS[1,2,3,4]
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[3]Pharmacol. and Mol. Sci., [4]Neurol. and Neurosurg., the Johns Hopkins Univ., Baltimore, MD; [5]Cell Biology, Biochemistry, and Neurobio., Duke Univ., Durham, NC

Abstract: The ANK3 (Ankyrin G) locus is a risk factor for schizophrenia and bipolar disorder. In the CNS, Ankyrin G localizes to the axon initial segment (AIS) and Node of Ranvier, and tethers sodium and potassium channels. In local cortical circuits, chandelier cells are fast spiking interneurons that send axon-axonic inhibitory synapses to the pyramidal cell AIS, forming GAT-1 and GAD67 positive cartridge-like structures wrapping around the AIS. We established a forebrain-specific Ankyrin G conditional knockout (KO) mouse model, in which knockout is restricted to adulthood, characterized its behavior, examined the effects of psychiatric medications and explored the molecular mechanisms using double label immunofluorescence and electrophysiology. The forebrain conditional Ankyrin G knockout model displays increased motor activity, increased exploratory activity, and less anxiety-like behavior, reminiscent of affective disorder. Interestingly, c-fos (a marker of neuronal activity) expression is significantly increased in Ankyrin G KO mice. Application of several anti-mania agents such as Clozapine, Lithium and Valproic Acid ameliorated the hyperactivity. Voltage gated sodium channel and potassium are missing from AIS and Node of Ranvier. Intriguingly, neurons that lost Ankyrin G expression have altered excitability. Chandelier interneuron cartridge structures were diminished in the Ankyrin G KO mice. This forebrain-specific Ankyrin G conditional knockout mouse model may be useful as potential model of mania, and for development of tools to explore the mechanisms of psychiatric drugs, and to develop new therapeutic strategies for bipolar and schizophrenia.

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Poster

524. Schizophrenia: Dopamine and Antipsychotic Drugs

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 524.13/CC18

Topic: C.15. Schizophrenia and Bi-polar Disorder

Title: The association between atypical antipsychotic drugs with P-glycoprotein in the mice brain

Authors: *T. WATANABE, K. OSADA, T. HAGA, A. MUTO, Y. OGAWA, A. TAGUCHI, 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 antipsychotic medications. The antipsychotic drugs such as aripiprazole and risperidone are the substrates for P-glycoprotein. On the other hand, Clozapine is a therapeutic drug of treatment-resistant schizophrenia, but is not the substrate of P-glycoprotein. There may be association between treatment of treatment-resistant schizophrenia and 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 atypical antipsychotic drugs once daily for six weeks. To quantify the amount of mRNA in mice brain, we performed real-time PCR (StepOne Real-Time PCR System)by using TaqMan Fast Universal PCR Master Mix (life technologies). A PCR reaction mixture of 10µl containing 5µl of TaqMan Fast Universal PCR Master Mix, 4.5µl of cDNA and 0.5µl TaqMan Gene Expression Assays.

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Poster

524. Schizophrenia: Dopamine and Antipsychotic Drugs

Location: Halls A-C

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Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: DGAPA-PAPIIT IN 302512-3

Title: Nicotine effect on a model of hyperactivation of the dopaminergic system using a temporal bisection task

Authors: *I. GONZALEZ RIVERA[1], D. B. PAZ-TREJO[2,3], O. ZAMORA-AREVALO[1], O. GALICIA[4], H. SANCHEZ-CASTILLO[2,3]
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Abstract: Schizophrenia is a disorder that involves many biochemical abnormalities. Patients with schizophrenia have a high prevalence of smoking. Nicotine is theoretically able to reduce some of the symptoms of schizophrenia. However, there is controversy regarding the role of nicotine in the disorder. The main goal of this investigation was to examine the effect of nicotine administration on a model of hyperactivation of the dopaminergic system, using a temporal bisection task. We use 8 male Wistar rats tested in a temporal bisection task discriminating durations of 2 or 8 seconds and intermediate duration (2.5, 3.17, 4, 5 and 6.4 s) with repeated administration of methylphenidate (3.0 mg/kg) for seven days and thereafter they were tested with repeated administration of methylphenidate (3.0 mg/kg) and nicotine (1.5 mg/kg) administered simultaneously for 7 days. We obtain the percentage of responses for the long stimulus and compare the responses on the control condition against the responses with each drug condition. Also, we compare psychometric measures for bisection task: bisection point, limen and Weber fraction. There were not statically significant differences in bisection point, Limen or Weber fraction. However, we observed a displacement of the sigmoidal curve and a decrement of the responses with the administration of methylphenidate (3.0 mg/kg). We also observed that the sigmoidal curve of repeated administration of nicotine and methylphenidate together is very similar to the curve of the control condition. The results support the fact that the cognitive effects of nicotine dependent on a variety of factors, including the underlying neural systems used for tasks and interaction with other drugs and systems. It is proposed that the evaluation of the processing of temporal information can serve as a tool in the understanding and assessment of the cognitive deficits of schizophrenia.

Disclosures: I. Gonzalez Rivera: None. **D.B. Paz-Trejo:** None. **O. Zamora-Arevalo:** None. **H. Sanchez-Castillo:** None. **O. Galicia:** None.

Poster

524. Schizophrenia: Dopamine and Antipsychotic Drugs

Location: Halls A-C

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Program#/Poster#: 524.15/CC20

Topic: C.15. Schizophrenia and Bi-polar Disorder

Title: Redox and methylation effects of D4 dopamine receptor expression and activation

Authors: *R. C. DETH[1], N. HODGSON[1], Y. LI[1], M. WALY[2]
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Abstract: We previously described the ability of the D4 dopamine receptor (D4R) to promote methylation of the phospholipid phosphatidylethanolamine, using methyl groups provided by the folate and vitamin B12-dependent enzyme methionine synthase (MS). Genetic variants of the D4R, resulting in proline-rich repeats in the third cytoplasmic loop, have been linked to different personality traits, drug abuse liability and to longevity. Using stably transfected CHO cells expressing D4Rs with 2, 4 or 7 repeats, we compared the ability of dopamine (DA; 10 μM) to induce phospholipid methylation (PLM), activate MS, affect the levels of redox and methylation pathway metabolites and alter global DNA methylation. The D4.7R was <50% as effective as D4.2R or D4.7R in supporting DA-stimulated PLM. Lower activity of the D4.7R may relate to its linkage to ADHD. Expression of each D4R was associated with a similar 90% decrease in basal MS activity. DA treatment increased MS by 90, 140 and 180% for D4.2R, D4.4R and D4.7R, respectively, while the D4R antagonist L-745870 decreased basal activity by ~65% for all three D4Rs. This indicates that D4Rs compete for MS activity and this competition is sensitive to receptor conformation. Expression of each D4R decreased basal GSH levels, but addition of DA increased levels by 49, 45 and 37% for D4.2, D4.4 and D4.7 receptors. Thus D4R expression endows cells with redox responsiveness to DA. DA increased the methylation index (SAM to SAH ratio) by 5.1, 8.6 and 4.6-fold for D4.2, D4.4 and D4.7 receptors, while global DNA methylation was increased 2.3, 2.0 and 2.0-fold, respectively. All responses were blocked by L-745870. These results demonstrate that D4R expression also confers DA responsiveness to methylation pathways, including DNA methylation. Since epigenetic mechanisms are linked to memory formation, D4Rs may provide a mechanism by which attended information can be remembered.

Disclosures: R.C. Deth: None. **N. Hodgson:** None. **Y. Li:** None. **M. Waly:** None.

Poster

524. Schizophrenia: Dopamine and Antipsychotic Drugs

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Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: Unrestricted Grant from Lundbeck Italy

Title: Homer transcripts topography is modulated in cortex and striatum by minocyline or memantine when added to haloperidol: Implication for treatment resistant psychosis

Authors: E. F. BUONAGURO[1], C. TOMASETTI[1], F. MARMO[1], C. SARAPPA[1], A. ERAMO[2], F. IASEVOLI[1], *A. DE BARTOLOMEIS[1]
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Abstract: Compounds targeting the N-methyl-D-aspartate (NMDA) receptor, such as memantine and minocycline, have been proposed as add-on therapies in treatment-resistant schizophrenia. NMDA receptors are the core of the post-synaptic density (PSD), a protein mesh deputed to glutamatergic signaling and implicated in dopamine-glutamate interplay. PSD genes (e.g. Homer1a and Homer1b genes of the Homer1 family) are induced by dopaminergic compounds including antipsychotics. PSD genes modulation could be a marker of glutamate/dopamine neurotransmission. Herein, we evaluated the expression of Homer1a and Homer1b genes as a marker of dopamine-glutamate interplay in different translational models utilizing both dopaminergic and glutamatergic compounds, either alone or in combination. Expression of Homer1a and Homer1b genes was assessed in cortical and striatal regions by means of *in situ* hybridization. The relative ratio of Homer1a/Homer1b expression was calculated in all paradigms. Data were processed by ANOVA and Tukey's post-hoc test. Acute NMDA receptor modulation paradigm. Sprague-Dawley rats were treated by vehicle; ketamine (25mg/kg and 50mg/kg); memantine 5mg/kg. Homer1a expression was significantly induced and Homer1b expression decreased by ketamine (25mg/kg) in cortical regions. Memantine shifted the Homer1a/Homer1b ratio toward Homer1b in the same brain regions. Chronic haloperidol/acute memantine add-on paradigm. Rats were chronically treated by haloperidol (0.8mg/kg) and then acutely treated by memantine 5mg/kg or vehicle before sacrifice. Acute memantine administration significantly increased Homer1a mRNA expression compared to acute vehicle in the cortex. No significant differences where found in Homer1a and Homer1b gene expression in both acute vehicle and memantine administered-rats. Minocycline add-on paradigm. Minocycline (45mg/kg) alone or in combination with haloperidol (0.8mg/kg) was administered to rats exposed or not to ketamine (30mg/kg). Minocycline per se did not affect gene expression. In the ketamine treated rats, Homer1a expression was significantly reduced by haloperidol+minocycline compared with vehicle in the cortex and, albeit less extensively, in the striatum. These results suggest that memantine and minocycline may have low propensity to affect glutamatergic genes when administered alone. However, they modify the effects of

haloperidol on these genes when administered in add-on, contributing to provide a rationale for the clinical efficacy of these compounds.

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Poster

524. Schizophrenia: Dopamine and Antipsychotic Drugs

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Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: Science Undergraduate Research Fellowships Grant

ASPIRE I Grant

Title: Homodimerization and phosphorylation of n-terminal serines control the subcellular localization of PDE11A4

Authors: ***G. PATHAK**[1], S. HEGDE[2], S. P. WILSON[2], J. L. FISHER[2], M. P. KELLY[2]
[2]Pharmacology, Physiology, and Neurosci., [1]Univ. of South Carolina Sch. of Med., Columbia, SC

Abstract: Phosphodiesterases (PDEs) are the only known enzymes to degrade cAMP and cGMP; therefore, PDEs are also crucial for a number of physiological processes including brain function. PDE11A, the most recently identified PDE family, has 4 isoforms. We study PDE11A4, as it is the only PDE11A isoform expressed in brain and it is the only PDE in brain with expression restricted to the hippocampus, a brain region affected in many neuropsychiatric illnesses. Recently, we identified disease-relevant genetic and environmental manipulations that change expression of PDE11A4 within select subcellular compartments of hippocampal tissues. Therefore, we sought to identify the intramolecular signals that control PDE11A4 subcellular compartmentalization. The regulatory N-terminal of PDE11A4 contains 2 confirmed phosphorylation sites and 2 full GAF domains: GAF-A and GAF-B. The GAF-A domain binds cGMP and the GAF-B domain is a protein-protein binding domain that mediates homodimerization. Currently, nothing is known about what signals control the subcellular localization of PDE11A4; however, phosphorylation and protein-protein binding are known to

control compartmentalization of other PDE families. As such, we determined if phosphorylation of N-terminal residues or homodimerization at the GAF-B domain could control the compartmentalization of PDE11A4 in COS1 and HEK293T cells. In both cell types, microscopy showed wild-type PDE11A4 was localized throughout the cytosol and in punctate aggregates. Phosphomimic mutations of serines 117 and 124 drove PDE11A4 from the cytosol towards the punctate structures; whereas, the phosphomimic mutation of serine 162 shifted PDE11A4 away from the punctate structures towards the cytosol. Introduction of an isolated GAF-B domain designed to disrupt PDE11A4 homodimerization also shifted wild-type PDE11A4 from the puncta toward the cytosol and reversed the effects of phosphomimic mutations at serines 117 and 124. Importantly, this effect of the isolated GAF-B domain did not require phosphorylation of serine 162. Biochemical fractionation studies yield similar results, with phosphomimic mutations of serines 117 and 124 driving PDE11A4 from the cytosol toward the membrane and phosphomimic mutation of serine 162 and addition of the isolated GA-B domain driving PDE11A4 from the membrane toward the cytosol. These results suggest that phosphorylation of serine 162 prevents homodimerization at the GAF-B domain, whereas phosphorylation of serines 117 and 124 promotes homodimerization at the GAF-B domain, thereby controlling subcellular localization of PDE11A4.

Disclosures: G. Pathak: None. **S. Hegde:** None. **S.P. Wilson:** None. **J.L. Fisher:** None. **M.P. Kelly:** None.

Poster

524. Schizophrenia: Dopamine and Antipsychotic Drugs

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 524.18/CC23

Topic: C.15. Schizophrenia and Bi-polar Disorder

Title: Global profiling of phospoproteome using mass spectroscopy to identify phosphorylation changes upon M1 activation in striatum

Authors: *M. POPIOLEK, J. HARMS, S. XI, S. GRIMWOOD
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Abstract: Preclinical and clinical studies suggest that muscarinic receptor activation may be therapeutically beneficial in treatment of schizophrenia and Alzheimer's disease. The M1 receptor is a Gαq-coupled member of the muscarinic acetylcholine receptor family which is thought to mediate some of those beneficial effects. TBPB [1-(1'-2-methylbenzyl)-1,4'-

bipiperidin-4-yl)-1H-benzo[d]imidazole-2(3H)-one], a highly selective M1 receptor activator, has been reported to potentiate NMDA receptor currents in hippocampal pyramidal cells, increase cFos expression in prefrontal cortex and striatum, and reverse amphetamine-induced hyperlocomotion in rats. We aimed to profile biochemical effects mediated by M1 *in vivo*. With this M1-selective and brain penetrant tool we explored striatal phospho-proteome in mice through mass spectrometry. By utilizing mass spectrometry and label-free quantification, we detected 1930 phospho-peptides from 747 proteins. Out of these, 182 sites on 116 proteins showed phosphorylation changes (p value < 0.1). Expected outcomes such as increased pERK levels have been observed due to TBPB treatment. We will discuss novel phosphoprotein changes associated with M1 activation. Results obtained with the mass spectrometry were confirmed with Western blots showing wide effect of M1 activation on regulation of striatal phosphorylation.

Disclosures: **M. Popiolek:** None. **J. Harms:** None. **S. Xi:** None. **S. Grimwood:** None.

Poster

524. Schizophrenia: Dopamine and Antipsychotic Drugs

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 524.19/CC24

Topic: C.03. Parkinson's Disease

Support: NSERC

Title: Haloperidol-environment interaction modulates expression of c-Fos proteins within the regions of the basal ganglia in rats

Authors: *L. N. SCHIMMEL[1], E. HAWKEN[1], E. DUMONT[1], T. BANASIKOWSKI[2], R. BENINGER[1]
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Abstract: Repeated treatment with dopamine (DA) receptor antagonists reduces the incentive value of environmental stimuli and gradually produces motivational impairments observed as a loss of motor engagement with the environment. The neural mechanisms of context-dependent catalepsy sensitization in rats were examined with an immunohistochemical assay for c-Fos, a marker of recent neuronal activity. During the training phase (15 daily sessions), the paired group received haloperidol (0.25 mg/kg; n=9), a DAD2 receptor-preferring antagonist, 1 h prior

to a catalepsy test carried out in a specific environment by placing the rats with their forepaws resting on a horizontal bar. The unpaired group (n=9) was treated with saline before catalepsy test and then injected with haloperidol 1 h later; a third group (n=9) was treated with saline before and 1 h after catalepsy test. On the test day all animals were treated with haloperidol prior to the catalepsy test. Behavioral results showed that animals in the paired group spent significantly more time with both paws on the bar compared to the unpaired and saline controls. Our results confirm previous findings that acquisition and expression of catalepsy sensitization to haloperidol is conditional on the interaction between the drug and test environment. Rats were sacrificed immediately following this test. Immunohistochemical results paralleled our behavioral findings;we found that paired animals had significantly fewer neurons expressing c-Fos compared to unpaired and saline controls following haloperidol challenge on test day. Specifically we observed a significant impairment in c-Fos expression in the nucleus accumbens shell and ventral pallidum, regions that link motivation to actions and process incentive learning. Results implicate basal ganglia circuitry in the neuronal mechanisms modulating catalepsy sensitization.

Disclosures: L.N. Schimmel: None. **E. Hawken:** None. **E. Dumont:** None. **T. Banasikowski:** None. **R. Beninger:** None.

Poster

525. Schizophrenia: Experimental Therapeutics and Preclinical Pharmacology

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 525.01/CC25

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: BBSRC

Title: Burst firing in the mediodorsal thalamus and related cognitive circuits is modulated by metabotropic glutamate receptors

Authors: *C. S. COPELAND[1], S. A. NEALE[2], T. E. SALT[1]
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Abstract: Cognitive deficits in schizophrenia remain largely unaffected by current antipsychotic therapies. Thalamo-cortical synchronization between the prefrontal cortex (PFC) and mediodorsal thalamus (MD) is thought to play an important role in cognition: disturbed synchrony in this and other thalamo-cortical circuits may therefore account for cognitive deficits

associated with schizophrenia. As Group II metabotropic glutamate receptor (mGlu2/3) compounds have efficacy in animal models of schizophrenia, with two compounds reaching phase 2 clinical trials, we sought to investigate the action of the Group II agonist LY354740 and the mGlu2 positive allosteric modulator (PAM) LY487379 on the firing pattern of MD and somatosensory ventrobasal thalamus (VB) neurons. Extracellular single neuron recordings were made *in vivo* with multibarrel electrodes in the MD or VB of urethane-anaesthetized Wistar rats. Drugs were applied locally by iontophoresis. For MD recordings, the PFC was electrically stimulated to activate the MD, whilst for VB recordings neurons were activated by vibrissa stimulation. The Group II agonist disinhibited responses to vibrissa stimulation in the VB; a disinhibition that was potentiated upon co-application of the mGlu2 PAM (Copeland et al., 2012; J Physiol 590:937-51). Furthermore, the Group II agonist significantly reduced burst firing in the VB, an effect that was also potentiated by the mGlu2 PAM (control: 66±7% spikes in bursts; LY354740 alone: 51±9% spikes in bursts; LY354740 plus LY487379 38±9% spikes in bursts). Whilst focal application of the Group II agonist also significantly disinhibited responses to PFC stimulation in the MD (156±10% of control) and significantly reduced burst firing (control: 76±5% spikes in bursts; LY354740: 57±2% spikes in bursts), neither of these effects were potentiated upon co-application of the mGlu2 PAM. The significance of these results is two-fold. Firstly, they demonstrate that Group II mGlu receptor function across thalamic nuclei is not uniform. Secondly, compounds active exclusively at the mGlu2 receptor are unlikely to perturb cognitive deficits arising from MD malfunction, with activity at mGlu3 receptors likely more appropriate. Interestingly, genetic studies have detected polymorphisms in the mGlu3 receptor gene GRM3, but not the mGlu2 receptor gene, in schizophrenia patients (Harrison et al., 2008; J Psychopharmacol 22:308-22).

Disclosures: **C.S. Copeland:** None. **S.A. Neale:** None. **T.E. Salt:** None.

Poster

525. Schizophrenia: Experimental Therapeutics and Preclinical Pharmacology

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 525.02/CC26

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: NIH Grant MH099993

Title: Preclinical efficacy of novel 5-HT_{2c} agonists for the treatment of schizophrenia-like behaviors

Authors: C. M. SCHMERBERG[1], V. M. POGORELOV[1], P. SKIBA[1], R. M. RODRIGUIZ[1], S.-M. PARK[1], B. L. ROTH[2], A. P. KOZIKOWSKI[3], *W. C. WETSEL[1]
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Abstract: Schizophrenia is a complex, pervasive, and heterogeneous neuropsychiatric disorder characterized by positive, negative, and cognitive symptoms. First-generation antipsychotics (FGAs) typically alleviate only positive symptoms, have undesirable extrapyramidal side-effects (EPS), and act primarily through antagonism of the dopamine (DA) D2 receptor (D2R). Second generation antipsychotic drugs (SGAs) are successful also in controlling positive symptoms and some drugs show improvement in negative symptoms with few EPS. While all SGAs act through D2Rs, they exert actions also through multiple serotonin (5-HT) receptor subtypes. Thus, while modulation of D2R-mediated signaling is important, targeting it directly is not sufficient to alleviate all symptoms of schizophrenia. Since activation of 5-HT_{2C} receptors in the ventral tegmental area decreases DA release in mesolimbic areas, 5-HT_{2C}-selective agonists may reduce mesolimbic DA activity without affecting the nigrostriatal system; thereby diminishing psychotic symptoms without inducing EPS. Interestingly, 5-HT_{2C} receptors are found also in cortical and other non-dopaminergic limbic forebrain regions implicated in schizophrenia. In our studies several novel 5-HT_{2C} agonists were tested in mouse models of schizophrenia. Since Compounds 2 and 3 were similar in structure, they were not examined in the same behavioral tests. In C57BL/6 mice, compound 3 reduced amphetamine-stimulated hyperlocomotion in the open field, it restored phencyclidine-disrupted prepulse inhibition, and decreased conditioned avoidance responding. It also had very low cataleptic activity compared to haloperidol. We used NR1-knockdown (NR1-KD) mice for assessments of social and cognitive behaviors. Compared to the wild-type controls, untreated NR1-KD mice were severely deficient in social affiliation and in episodic memory. Compound 2 rescued the NR1-KD deficits in social affiliation in the sociability test and in episodic memory at 24 hr in the novel object recognition memory test. These preclinical results provide support for the 5-HT_{2C} receptor as a potential therapeutic target in the treatment of schizophrenia.

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Poster

525. Schizophrenia: Experimental Therapeutics and Preclinical Pharmacology

Location: Halls A-C

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Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: IHMRI Project Grant 00229380

SRI Grant 00271793

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Title: Effects of pharmacological blockade of Lingo-1 signaling pathways in a phencyclidine rat model for schizophrenia

Authors: *J. L. ANDREWS[1,2], R. P. SULLIVAN[3], K. A. NEWELL[1,2], X.-F. HUANG[1,2], F. FERNANDEZ-ENRIGHT[1,2,4]
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Abstract: Background and Aims: Dysfunctional myelination is one of the strongest hypotheses implicated in schizophrenia pathophysiology. Interestingly, myelination peaks during late adolescence, coinciding with the onset of schizophrenia. Lingo-1, a transmembrane signal-transducing molecule expressed on oligodendrocytes and neurons, is a potent negative regulator of oligodendrocyte differentiation, axonal growth and myelination. Since myelination and neuronal outgrowth disturbances lead to cognitive dysfunction, and considering the involvement of Lingo-1 in these processes, we have investigated the effects of pharmacological inhibition of Lingo-1 as a novel treatment for schizophrenia. Methods: Adolescent male Sprague Dawley rats (4 weeks) were injected subcutaneously with either saline vehicle or PCP (10 mg/kg, Sigma) for a total of 8 days. On the third day, rats (n=12/group) were concurrently treated for 5 days with either saline, olanzapine (Olz) (oral administration by cookie dough 1 mg/kg/day, 3 times/day) or anti-Lingo-1 functional antibody ab23631 (Abcam, UK), (intraventricular injection, with surgery for intracranial cannula implantation performed one week prior). Relative protein expression levels of Lingo-1, and myelination marker myelin basic protein (MBP) were examined within the prefrontal cortex of the treated rats. Results: Lingo-1 levels were significantly increased in PCP treated rats ($p=0.015$) and MBP was significantly reduced in PCP treated rats ($p=0.002$); both were restored to near control levels in anti-Lingo-1/Olz treated rats ($p=0.032$). Restoration of MBP levels was most likely to have been caused by the anti-Lingo-1 treatment, as rats treated with PCP/Olz had similar MBP levels as PCP/Vehicle treated rats in the prefrontal cortex. Conclusions: This is the first study to have shown that Lingo-1 and myelination levels are altered

following PCP treatment and that these levels are restored following treatment with a Lingo-1 antagonist. We suggest that Lingo-1 may be a suitable target for the development of new future therapeutic treatments for schizophrenia.

Disclosures: J.L. Andrews: None. **F. Fernandez-Enright:** None. **K.A. Newell:** None. **X. Huang:** None. **R.P. Sullivan:** None.

Poster

525. Schizophrenia: Experimental Therapeutics and Preclinical Pharmacology

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Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: CHOP Foerderer Grant

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P30HD026979

Title: D-serine treatment enhances cortical parvalbumin GABAergic neuronal development through synaptic NMDA receptors

Authors: H. LIN[1], F.-C. HSU[1], A. JACOBI[1], B. H. BAUMANN[1], D. A. COULTER[2,1], S. A. ANDERSON[2,1], *D. R. LYNCH[2,1]
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Abstract: D-serine, synthesized by the enzyme serine racemase (SR) through the conversion of L-serine to D-serine, is an endogenous coagonist which preferentially gates synaptic rather than extrasynaptic NMDA receptors(NMDARs). NMDAR hypofunction due to specific loss of D-serine has been implicated in the pathophysiology of schizophrenia and other neuropsychiatric disorders. Our previous studies have demonstrated that deletion of α7 nicotinic acetylcholine receptor (nAChR) gene in mice leads to specific loss of synaptic NMDARs and their coagonist D-serine, as well as cortical parvalbumin (PV) GABAergic deficits reminiscent of schizophrenia. Here we report that D-serine treatment enhances cortical PV GABAergic neuronal development through synaptic NMDARs in neuronal cultures and organotypic slice cultures from wild-type

(WT) and α7 nAChR knockout (α7-KO) mice. D-serine (50μM) treatment promotes GABAergic marker expression in WT and α7-KO cortical cultures, including Glutamic Acid Decarboxylase 65/67 (GAD65/67), the α1 subunit of GABAA receptors (GABAAα1), and gepherin. D-serine treatment not only increases PV levels in cortical PV GABAergic interneurons in WT neuronal and organotypic slice cultures, but also rescues cortical PV GABAergic deficits, demonstrated by increase of PV levels and perisomatic synapse formation in α7-KO in neuronal cultures and organotypic slice cultures. Moreover, D-serine treatment increases pCREB levels in cortical cultures, which can be blocked by NMDAR antagonist MK-801. Taken together, our findings suggest that D-serine treatment enhances cortical PV GABAergic development through synaptic NMDARs, thereby providing important insights into designing better treatment for schizophrenia and other interneuron-related neuropsychiatric diseases.

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Poster

525. Schizophrenia: Experimental Therapeutics and Preclinical Pharmacology

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 525.05/CC29

Topic: C.15. Schizophrenia and Bi-polar Disorder

Title: NR_{2b} antagonist CP101,606 abolishes pitch-mediated deviance detection in awake rats

Authors: *S. V. DIGAVALLI, P. CHEN, Y. YANG, Y.-W. LI, R. PIESCHL, M. K. AHLIJANIAN
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Abstract: Schizophrenia patients exhibit a decreased ability to detect change in their auditory environment as measured by auditory event related potentials such as mismatch negativity. This deficit has been linked to abnormal NMDA neurotransmission since, among other observations, non-selective channel blockers of NMDA reliably diminish deviance detection in human subjects as well as in animal models. Recent molecular and functional evidence link the NR_{2b} receptor subtype to aberrant NMDA transmission in schizophrenia. However, it is unknown if NR_{2b} receptors participate in pre-attentive deviance detection. We recorded event related potentials from the vertex of freely behaving rats in response to frequency mismatch protocols. We saw a robust increase in N1 response to deviants compared to standard as well as control stimuli indicating true deviance detection. Moreover, the increased negativity was highly sensitive to

odd-ball probability. Next, we tested the effect of a non-selective NMDA channel blocker (ketamine, 30 mg/kg) and a highly selective NR_{2b} antagonist, CP-101,606 (10 or 30 mg/kg) on deviance detection. Ketamine attenuated deviance mainly by increasing the amplitude of the standard N1. Amplitude and/or latency of several other ERP components were also markedly affected. In contrast, CP-101,606 robustly and dose-dependently inhibited the deviant's N1 amplitude and as a consequence, completely abolished deviance detection. No other ERP or components were affected. Thus, we report first evidence that NR_{2b} receptors robustly participate in processes of automatic deviance detection in a rodent model. Lastly, our model demonstrates a path forward to test specific pharmacological hypotheses using translational endpoints relevant to aberrant sensory processing in schizophrenia.

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Poster

525. Schizophrenia: Experimental Therapeutics and Preclinical Pharmacology

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 525.06/CC30

Topic: C.15. Schizophrenia and Bi-polar Disorder

Title: Neuregulin-1 treatment prevents PCP-induced parvalbumin and GAD67 changes in frontal cortex neurons

Authors: *M. ENGEL[1,2], L. OOI[1], X.-F. HUANG[1,2], E. FRANK[1,2]
[1]Fac. of Science, Med. and Hlth., Illawarra Hlth. and Med. Res. Inst., Wollongong, Australia; [2]Schizophrenia Res. Inst., Sydney, Australia

Abstract: Evidence from genetic and post-mortem studies strongly suggest that altered Neuregulin 1 (NRG1) signaling through ErbB4 is associated with the pathophysiology of schizophrenia. ErbB4 receptors are primarily located on GABAergic interneurons in schizophrenia-relevant brain areas, including the prefrontal cortex. Application of phencyclidine (PCP) is commonly used to identify compounds with antipsychotic potential. In our previous studies, we showed that NRG1 application reduces PCP-induced behavior impairments and GABA level changes in the PFC and hippocampus. In the present study we explored the ability of NRG1 to counterbalance PCP-induced alterations of the GABA-producing enzymes GAD65 and GAD67 and the calcium binding protein parvalbumin (PV) in primary frontal cortex cultures. Dissociated frontal cortex cultures from C57BL6 mice (post natal day 1-2) were

maintained for seven days before being treated with NRG1, PCP or both for 24h. Expression of GAD65, GAD67, PV, DISC1, NMDA-NR1 and synaptophysin mRNA levels were quantified via qPCR. GAD67 and PV levels were significantly increased and GAD65 decreased following PCP application in the frontal cortex cultures. Combined treatment with NRG1 dose-dependently prevented the PCP-induced changes. NRG1 at the lowest concentration reduced GAD65 and 67 levels, with no further effect on other targets or at higher concentrations. Together with our previous behavioral and microdialysis findings, these results demonstrate a critical role of NRG1 in neurotransmission relevant to schizophrenia. With NRG1 application proven safe in phase II trials for heart disease, our results suggest that NRG1 treatment has a promising treatment potential for patients with schizophrenia.

Disclosures: **M. Engel:** None. **X. Huang:** None. **E. Frank:** None. **L. Ooi:** None.

Poster

525. Schizophrenia: Experimental Therapeutics and Preclinical Pharmacology

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 525.07/CC31

Topic: C.15. Schizophrenia and Bi-polar Disorder

Title: Potential implication of the kynurenine pathway in a juvenile two-hit animal model of schizophrenia: Reversal of PPI deficits by the inhibition of indoleamine 2,3-dioxygenase

Authors: *J. DESLAURIERS[1], P. SARRET[1], S. GRIGNON[1,2]
[1]Physiol. and Biophysics, [2]Psychiatry, Univ. de Sherbrooke, Sherbrooke, QC, Canada

Abstract: The levels of kynurenic acid, a metabolite of kynurenine pathway of tryptophan degradation, have shown to be increased in the CSF and in postmortem prefrontal cortex (PFC) of schizophrenia patients. Also, chronic treatment with antipsychotics normalized the same levels in patients, supporting the hypothesis of the implication of kynurenine pathway in schizophrenia. We have previously developed a two-hit animal model, based on prenatal immune challenge (PIC) followed by juvenile restraint stress (RS), showing that PIC combined to RS induced a potentiated disruption of prepulse inhibition of acoustic startle (PPI) in mice. These findings led us to assess whether an inhibitor of indoleamine 2,3-dioxygenase (IDO), the enzyme that catalyzes the degradation of L-tryptophan to N-formylkynurenine, could prevent PPI deficits in this juvenile *in vivo* model of schizophrenia. Gestational mice were injected with polyIC (20 mg/kg; IP) or with saline at gestational day 12. Mice pups were then submitted or not to RS for 2 hours, for three consecutive days, from postnatal days 33 to 35. Three hours before each period

of RS, pups were treated with either 1-methyl-D-tryptophan (1-MT) (50 mg/kg; IP), an inhibitor of IDO, or vehicle. PPI was assessed 24 hours after the last period of RS. According to previous findings, we found that PIC combined with RS resulted in a significant decrease in PPI (-39%; $P < 0.001$, compared to control group). Importantly, the inhibition of IDO, through the administration of 1-MT, prevented the development of PPI deficit (+26%; $P < 0.05$, compared to vehicle) in mice submitted to both insults. Also, the combination of PIC and RS lead to increased cortical and striatal mRNA IDO levels (+61%; $P < 0.01$ and +41%; $P < 0.05$, respectively, as compared to saline without RS). The treatment with 1-MT reduced cortical and striatal mRNA IDO levels (-43%; $P < 0.01$ and -44%; $P < 0.01$, respectively, as compared to vehicle) in mice submitted to both insults. Also, as previously demonstrated, PIC followed by RS reduced GAD67 mRNA levels in the PFC (-29%; $P < 0.05$) and the striatum (-35%; $P < 0.05$) of polyIC-treated dams' offspring submitted to juvenile RS. Importantly, treatment with 1-MT prevented GABAergic abnormalities by increasing cortical and striatal GAD67 mRNA expression (+44%; $P < 0.05$ and +51%; $P < 0.05$, respectively, as compared with vehicle) in mice submitted to both insults. These results support the hypothesis that the kynurenine pathway plays an important role in the development of PPI deficits, a well-known behavior associated with schizophrenia, and in GABAergic abnormalities, recognized as a core feature of schizophrenia and found to be disturbed in our two-hits model.

Disclosures: J. Deslauriers: None. **P. Sarret:** None. **S. Grignon:** None.

Poster

525. Schizophrenia: Experimental Therapeutics and Preclinical Pharmacology

Location: Halls A-C

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Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: NHMRC Program Grant 519461

Australian Postgraduate Award

Title: Muscarinic M_4 receptor regulation of psychosis-like behaviors induced by a dopamine D_1 receptor-selective agonist in mice

Authors: *A.-Y. CHEN, A. CHRISTOPOULOS, M. CANALS, D. T. MALONE
Drug Discovery Biol., Monash Inst. of Pharmaceut. Sci., Parkville, Australia

Abstract: Muscarinic M_4 receptors are found most abundantly in the striatum and are thought to modulate striatal dopaminergic activity. Specifically, in the striatum, M_4 receptors are co-localized with dopamine D_1 receptors on GABAergic output neurons and have been shown to functionally antagonize D_1 receptor-mediated activities in M_4 receptor knockout studies. However, the specific interaction between these two receptors has yet to be explored *in vivo* due to the lack of receptor subtype selectivity of M_4 receptor orthosteric agonists. In order to study the ability of M_4 receptors to regulate D_1 receptor-induced behaviors in C57BL/6J mice, a M_4 receptor positive allosteric modulator (LY2033298), an acetylcholinesterase inhibitor (donepezil), and a D_1 receptor-selective agonist (R(+)-6-Br-APB) were used. Allosteric modulators bind to the site of the receptor that is topographically distinct from that of the endogenous ligand and have the potential to possess increased receptor subtype selectivity. R(+)-6-Br-APB disrupted prepulse inhibition and increased locomotor activity in C57BL/6J mice, which are models of aspects of psychosis-like behavior that involve the striatum. LY2033298, when treated in combination with a sub-effective dose of donepezil, which increases endogenous acetylcholine levels, was able to significantly reverse R(+)-6-Br-APB-induced disruption of prepulse inhibition and increase of locomotor activity. These results demonstrate that selective M_4 receptor activation can indeed regulate D_1 receptor-mediated behaviors, as well as add to the increasing evidence of M_4 receptors as a novel target for the treatment of psychosis.

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Poster

525. Schizophrenia: Experimental Therapeutics and Preclinical Pharmacology

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 525.09/CC33

Topic: C.15. Schizophrenia and Bi-polar Disorder

Title: The role of ERK and AKT kinases in the antipsychotic efficacy of Clozapine

Authors: *M. SCARSELLI[1], S. ARINGHIERI[2], S. KOLACHALAM[2], C. GERACE[2], V. VERDESCA[2], M. CAPANNOLO[3], R. MAGGIO[3]
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Abstract: Clozapine is the most effective antipsychotic to date, thus considered the “gold standard” for the treatment of schizophrenia. Among atypical antipsychotic drugs, clozapine is the most efficacious in treating positive, negative, and cognitive symptoms in schizophrenia, particularly in patients resistant to other antipsychotics. However, its benefits are counterbalanced by relevant side effects such as the risk of severe hematological effects. The mechanism of action of Clozapine is still unclear, multifactorial and probably based on its affinities for different receptors and different targets. Relevant to this, the new concept of “biased agonism” has made drug actions even more complicated, whereas some ligands can behave as agonists or antagonists on the same receptor depending from the pathway examined. Indeed, for example, clozapine can act as an agonist or antagonist on 5HT2a serotonin receptor depending from the target taken in consideration. In our study, we decided to investigate the action of clozapine and other antipsychotics as agonists on kinases activity such as ERKs (Extracellular signal-regulated kinases) and AKT (Protein kinase B). These kinases have a prominent role in synaptogenesis, neurogenesis and neuronal survival, and they might be important for antipsychotic efficacy particular for negative and cognitive symptoms in schizophrenia. In our experiments, we found that clozapine has a unique profile in ERK and AKT activation. This evidence might be relevant to explain why Clozapine is the “gold standard” for the treatment of schizophrenia.

Disclosures: **M. Scarselli:** None. **S. Aringhieri:** None. **S. Kolachalam:** None. **C. Gerace:** None. **V. Verdesca:** None. **M. Capannolo:** None. **R. Maggio:** None.

Poster

525. Schizophrenia: Experimental Therapeutics and Preclinical Pharmacology

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 525.10/CC34

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: JSPS

Title: Analysis of effects by repetitive Transcranial Magnetic Stimulation (rTMS) on neurosychatricand neurodegenerative disorder

Authors: ***T. IKEDA**[1], N. NUKINA[2]
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Abstract: rTMS is a noninvasive technique to induce electric current in the brain and is supposed to be beneficial for the treatment of patients with depression, schizophrenia and neurodegenerative disorders. We reported previously that rTMS modulates monoamine tranporter. However, the mechanisms underlying the effects of rTMS are still unclear. We analyzed the changes in mRNA expression in mouse brain that occurred after rTMS with an Affymetrix GeneChip. Following 20days of rTMS, many genes were differentially expressed in the mouse brain. Down-regulation of Period 2, 3 and hypocretin mRNA expression levels and a subsequent decrease in food and water intake were observed. HSP70 mRNA expression levels were up-regulated after transient and chronic rTMS. In N2A 150Q cells, an up-regulation of HSP70 mRNA and protein levels and subsequent cell-protective effects were observed after chronic rTMS. In addition, dopamine receptor 2 mRNA expression levels were down-regulated, and a subsequent decrease in the binding of [3H]raclopride was observed. A decrease in the Bmax and no change in the KD of [3H]raclopride binding was observed in the binding assay.These results indicated that the modulation of several genes may be involved in the therapeutic mechanisms of acute and chronic rTMS for patients with neuropsychiatric disorders.

Disclosures: **T. Ikeda:** None. **N. Nukina:** None.

Poster

525. Schizophrenia: Experimental Therapeutics and Preclinical Pharmacology

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 525.11/CC35

Topic: F.02. Animal Cognition and Behavior

Support: NIH grant DA030425

NIH grant MH091945

NIH grant MH093650

Title: modified DRL task in ad libitum fed rats: Effects of Sigma 1 receptor blockade

Authors: *P. COTTONE[1], A. BLASIO[1], K. C. RICE[2], M. R. IYER[2], V. SABINO[1]
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Abstract: BACKGROUND: Impulsivity can be defined as a predisposition toward rapid, unplanned reactions to internal or external stimuli, and a diminished regard to the negative

consequences of such reactions. Existing animal models of impulsivity frequently use food restriction/deprivation to increase subjects' motivation. Food restriction/deprivation represents an energy-homeostasis limitation, which may confound the interpretation of results and limit the applicability of these models. The first aim of this study was to validate face and convergent validities of a modified differential reinforcement of low rates (DRL) task, which assesses impulsive action in ad libitum fed rats. The second aim of this study was to evaluate the effects of Sigma receptor (SigR) ligands on the modified DRL task. METHODS: Ad libitum fed male Wistar rats were trained in a DRL-15 task during which a press on the active lever resulted in the delivery of a supersaccharin solution, consisting of 1.5% w/v glucose and 0.4% w/v saccharin. Delivery only occurred if at least 15 s had elapsed since the previous lever press. If rats made a premature lever press, the 15 s time period would reset; thus, rats were only reinforced if they withheld a response for longer than 15 s. Responses on the inactive lever were recorded. To pharmacologically validate the task, we tested the effects of the non-competitive NMDA receptor antagonist MK-801 and the partial dopamine receptor agonist Aripiprazole, which have been demonstrated to respectively increase and decrease impulsive action. We also tested the effects of the SigR ligands BD1063 and DTG on impulsive action using the modified DRL task. RESULTS: Rats quickly learned the DRL task, and impulsivity was a very stable and consistent trait. MK-801 dose-dependently increased premature responding in this modified DRL task. Conversely, Aripiprazole dose-dependently decreased premature responding. The Sig1R agonist BD1063 decreased premature responding only in high-impulsive rats. The SigR agonist DTG on the other hand had no effect on impulsive responding. No effects on inactive lever responding were observed following treatment with any of the drugs tested. CONCLUSIONS: Here we have validated a rodent task of impulsive action, which eliminates typical energy-homeostasis limitations and, therefore, opens new avenues in the study of impulsivity in preclinical feeding and obesity research. In addition, we show that Sig1R represents a novel pharmacological target for the modulation of impulsive action.

Disclosures: **P. Cottone:** None. **A. Blasio:** None. **K.C. Rice:** None. **M.R. Iyer:** None. **V. Sabino:** None.

Poster

525. Schizophrenia: Experimental Therapeutics and Preclinical Pharmacology

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 525.12/CC36

Topic: G.04. Physiological Methods

Support: NSC 101-2321-B-182-012

NSC101-2221-E-182 -002 -MY3

NSC 102-2221-E-182 -020 -MY3

CMRPG590022G

Title: Neuromodulation induced by focused ultrasound-induced blood-brain barrier opening combined with intravenous GABA agonists

Authors: *H.-Y. LAI[1,2], P.-C. CHU[3], Y.-T. CHANG[2], T.-Y. CHEN[1], H.-L. LIU[3], Y.-C. PEI[1,2]
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Abstract: Focused ultrasound (FUS) administered in the presence of microbubbles can temporarily disrupting the blood-brain barrier (BBB). FUS-induced BBB opening has been applied in the drug deliver for the treatment of brain tumor as it could elicit a dramatic increase of drug penetration at the target brain area. Furthermore, our previous studies demonstrated that FUS-induced BBB opening was accompanied with neuromodulation as evidenced by the changes in somatosensory evoked potentials (SSEPs) and blood-oxygen-level dependent (BOLD) signals. In theory, FUS-induced BBB opening combined the drug delivery could elicit facilitation, inhibition, or other effects. In the present study, we proposed that FUS-induced BBB opening combined with the intravenous administration of neuronal suppressant, such as GABA agonists, could induce suppression of local neuronal activities. To induce BBB disruption of SD rats, we presented 0.35 MPa FUS to the left primary somatosensory cortex forelimb region (S1FL,1 mm posterior and 4 mm lateral to the bregma) for 90 s with concurrent injection of intravenous microbubbles (SonoVue® SF6-coated, 2-5 μm mean diameter, 10 μl/kg). We also injected intravenously gabapentin (10 or 120 mg/kg) or muscimol (0.2 or 0.4 mg/kg) that were intended to be flow through the BBB disruption to induced neuromodulation. SSEPs elicited by electrical forepaw stimulation and recorded by epidural electrodes implanted on the scalp were evaluated before and after FUS (every 10 min in the first hour, and 1-day post-FUS). Compare to the control group, both the 0.2 and 0.4 mg/kg muscimol group showed significant suppression of SSEP amplitude (time window 10-20 ms after stimulus onset) in the first hour ($p<0.05$), and SSEPs recovered at 1-day follow-up. The effect was more robust in the 0.4 mg/kg muscimol group. Similar to the results for SSEP amplitude, the FUS prolonged SSEP latency in the 0.2 and 0.4 mg/kg muscimol groups. On the contrary, both gabapentin groups showed no changes in SSEP amplitude or latency after FUS. These results indicate that muscimol of 0.2 mg/kg can induce measurable neuromodulation in the local brain. This present study proposed a novel non-invasive, reversible and local neuromodulation method that is suitable for neurophysiological experiments and clinical applications.

Disclosures: **H. Lai:** None. **P. Chu:** None. **Y. Chang:** None. **T. Chen:** None. **H. Liu:** None. **Y. Pei:** None.

Poster

525. Schizophrenia: Experimental Therapeutics and Preclinical Pharmacology

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 525.13/DD1

Topic: C.15. Schizophrenia and Bi-polar Disorder

Title: Validation of alanine serine cysteine transporter-1 as a novel target for schizophrenia using siRNA and inducible knock-down mice

Authors: ***S. PARMENTIER-BATTEUR**[1], L. WARREN[2], V. KUZMICK GRAUFELDS[7], M. J. MARINO[8], R. GENTZEL[3], K. M. SMITH[4], S. JAYARAMAN[5], J. D. VARDIGAN[3], T. ROSAHL[3], M. TADIN-STRAPPS[3], B. C. MAGLIARO[6], A. J. COOKE[3], J. J. RENGER[3]
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Abstract: One novel approach to improving cognitive function and reducing negative symptoms of schizophrenic patients is enhancing NMDA-R function by acting on the glycine modulatory site (GMS). The inhibition of the alanine serine cysteine transporter-1 (Asc-1) was proposed as a novel therapeutic strategy targeting this mechanism. The rationale is that inhibition of Asc-1 transporter potentiates NMDA-R activity via an increase in synaptic levels of the GMS ligand, D-serine, by blocking its re-uptake. However, although this target is mechanistically attractive, it also presents some target validation challenges including the lack of tool compounds and viable adult knockout mice. We therefore used siRNA and inducible knock-down (iKD) mice to evaluate the effects of Asc-1 knock-down on NMDA-R function both *in vitro* and *in vivo*. In rat neuronal cultures, siRNA-induced Asc-1 knock-down resulted in a decrease in D-serine uptake that was associated with an enhanced NMDA-R activity measured by the phosphorylation levels of NMDA-R subunit 1 (pNR1). Intracerebral injection of Asc-1 siRNA produced an increase in basal synaptic levels of D-serine measured by microdialaysis that was also associated with elevated pNR1 levels. Asc-1 iKD mice showed a reversal of the hyperlocomotion induced by the NMDA partial agonist (L-687,414) and increased levels of pNR1 in both the cortex and hippocampus. Finally, the electrophysiological recording in hippocampal slices showed larger

NMDA-mediated field response in slices from Asc-1 iKD mice compared to wild type mice, suggesting an enhanced NMDA-R synaptic transmission associated with Asc-1 knock-down. Taken together, these results provide evidence for the role of Asc-1 in enhancing NMDA-R function and neurotransmission, and validate Asc-1 inhibition as a potential novel strategy for the treatment of schizophrenia.

Disclosures: **S. Parmentier-Batteur:** A. Employment/Salary (full or part-time):; Merck Research Laboratories. **L. Warren:** A. Employment/Salary (full or part-time):; Merck. **V. Kuzmick Graufelds:** A. Employment/Salary (full or part-time):; Merck. **M.J. Marino:** A. Employment/Salary (full or part-time):; Merck. **R. Gentzel:** A. Employment/Salary (full or part-time):; Merck. **K.M. Smith:** A. Employment/Salary (full or part-time):; Merck. **S. Jayaraman:** None. **J.D. Vardigan:** A. Employment/Salary (full or part-time):; Merck. **T. Rosahl:** A. Employment/Salary (full or part-time):; Merck. **M. Tadin-Strapps:** A. Employment/Salary (full or part-time):; Merck. **B.C. Magliaro:** A. Employment/Salary (full or part-time):; merck. **A.J. Cooke:** A. Employment/Salary (full or part-time):; Merck. **J.J. Renger:** A. Employment/Salary (full or part-time):; Merck.

Poster

525. Schizophrenia: Experimental Therapeutics and Preclinical Pharmacology

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 525.14/DD2

Topic: C.15. Schizophrenia and Bi-polar Disorder

Title: M4 muscarinic acetylcholine receptor-specific modulation of immediate early gene expression in mouse brain

Authors: ***X. WANG**, M. PEARSON, R. GENTZEL, J. USLANER, F. THOMSON
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Abstract: Deficits in the muscarinic cholinergic system have been implicated in the pathophysiology of many neurologic and psychiatric disorders, including Alzheimer's disease (AD) and schizophrenia. Specifically, evidence suggests that activating M4 muscarinic acetylcholine receptor (mAChR) may alleviate psychosis and behavioral disturbances associated with these diseases. To investigate the brain regions activated by selective potentiation of M4 mAChR, we measured immediate early gene (IEG) expression, including arc, c-fos and zif268, in the brain of wildtype and M4 knockout mice. We examined several M4 potentiating compounds, such as M1/M4 preferred mAChR agonist (Xanomeline), an M4 specific agonist

(Sumitomo) and the M4 positive allosteric modulator (PAM) (VU0152100). The expression levels of IEGs were determined by qPCR and *in situ* hybridization in various brain regions, including striatum, cortex and hippocampus. Consistent with the hypothesis that activating M4 receptors in the striatum attenuates dopaminergic and glutamatergic neurotransmission, IEG expression was reduced in wildtype mice administered the M4 activators, but not in M4 knockout mice. The magnitude of IEG reduction was more robust in striatum as compared to cortex and hippocampus likely due to the higher expression levels of M4 receptors in striatal neurons. Thus, these data demonstrate that M4 mAChR plays a pivotal role in regulating neuronal activity in multiple brain regions.

Disclosures: **X. Wang:** A. Employment/Salary (full or part-time):; Merck & Co., Inc. **M. Pearson:** A. Employment/Salary (full or part-time):; Merck & Co., Inc. **R. Gentzel:** A. Employment/Salary (full or part-time):; Merck & Co., Inc. **J. Uslaner:** A. Employment/Salary (full or part-time):; Merck & Co., Inc. **F. Thomson:** A. Employment/Salary (full or part-time):; Merck & Co., Inc.

Poster

525. Schizophrenia: Experimental Therapeutics and Preclinical Pharmacology

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 525.15/DD3

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: NIH Grant MH082441

Title: Effects of a β-arrestin biased ligand on schizophrenia-like behaviors in mice

Authors: *S.-M. PARK[1], C. M. SCHMERBERG[1], R. M. RODRIGUIZ[1], M. G. CARON[2], B. L. ROTH[3], J. JIN[4], W. C. WETSEL[1]
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Abstract: Schizophrenia is a devastating neuropsychiatric disorder characterized by positive, negative, and cognitive symptoms. Currently available FDA-approved antipsychotic drugs are effective in treating positive symptoms, but have limited efficacy on negative or cognitive

symptoms. The current drugs have a common feature in that they all target primarily the dopamine D2 receptor (D2R) for action. Over the past 10 years, it has become evident that D2Rs can also signal through a β-arrestin (βarr*)* mediated-pathway independently from the Gi pathway. There is some evidence that stimulation of D2Rs can result in the recruitment of βarr2 with changes in Akt and GSK-3. Interestingly, alterations in these two signaling molecules have been reported in brains from schizophrenic patients. A recent study has shown that many antipsychotic drugs can perturb both the Gi and βarr pathways. We have developed ligands that are devoid of activity at D2R-mediated Gi protein signaling, but are simultaneously high affinity partial agonists for D2R/βarr2 interactions. The purpose of these studies was to test the effectiveness on one of these ligands, UNC9975, on schizophrenia-like behaviors in mice. UNC9975 was observed to depress the hyperlocomotion of NR1-knockdown (NR1-KD) and dopamine transporter knockout (KO) mice in the open field. The compound rescued prepulse inhibition (PPI) in NR1-KD mice. It also rescued phencyclidine-disrupted PPI in βarr2 wild-type mice at a lower dose than in βarr2-KO mice, supporting the idea that UNC9975 acts primarily through the βarr pathway. In a test for social behavior, UNC9975 increased social affiliation in NR1-KD mice and it reduced immobility of the vesicular monoamine 2 heterozygotes in the tail suspension test. Moreover, it decreased conditioned avoidance responses without impairing associative memory from fear conditioning, indicating its effective antipsychotic property. UNC9975 was found also to produce much less catalepsy than haloperidol in the horizontal bar test. Together these findings suggest that the new βarr-biased compound is efficacious in reducing positive- and negative-like symptoms of schizophrenia without having untoward extrapyramidal side-effects in mice.

Disclosures: S. Park: None. **C.M. Schmerberg:** None. **R.M. Rodriguiz:** None. **M.G. Caron:** None. **B.L. Roth:** None. **J. Jin:** None. **W.C. Wetsel:** None.

Poster

525. Schizophrenia: Experimental Therapeutics and Preclinical Pharmacology

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 525.16/DD4

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: TUMS Grant 16005

Title: Nitric oxide signaling system as an augmentative therapeutic target in schizophrenia: experience from a trial with l-lysine

Authors: *M. FAROKHNIA[1], P. MAZAHERI[1], H. YEKEHTAZ[1], N. BEHBAHANI-NEJAD[2], S. AKHONDZADEH[1]
[1]Tehran Univ. of Med. Schools, Tehran, Iran, Islamic Republic of; [2]Dept. of Mol. and Integrative Physiol., Univ. of Michigan, Ann Arbor, MI

Abstract: Background: Many schizophrenic patients do not respond optimally to the antipsychotics monotherapy and augmentation with novel agents is a promising strategy to address this issue. L-lysine reduces nitric oxide (NO) synthesis in the brain as it competes with l-arginine for the common cationic amino acid transporter. Considering the evidenced contribution of NO signaling system in the pathophysiology of schizophrenia, we aimed to evaluate the efficacy and safety of l-lysine as an add-on to risperidone in patients suffering from chronic schizophrenia. Methods: In a double-blind, placebo-controlled, parallel-group, randomized clinical trial, 72 patients with chronic schizophrenia (DSM-IV criteria, disease duration ≥ 2 years) and a total score of ≥ 60 on the positive and negative syndrome scale (PANSS) were randomized to receive risperidone (up to 6 mg/day) plus either l-lysine capsules (6 mg/day) or placebo for 8 weeks. The efficacy assessment measure of this study was PANSS and its different subscales by which patients were evaluated every other week after the baseline visit (weeks 0, 2, 4, 6, and 8). Probable adverse events were closely monitored as well. Results: By the study endpoint, l-lysine-treated patients experienced greater improvement than the placebo group as shown by significant effect of time × treatment interaction for the PANSS total [$F(1.96, 137.21)=12.16$, $P<0.001$], negative [$F(2.01, 140.73)=14.46$, $P<0.001$], and general psychopathology [$F(1.86, 130.39)=9.20$, $P<0.001$] scores, but not for the positive symptoms [$F(2.56, 179.27)=0.56$, $P=0.61$]. No significant difference was detected between two groups in the frequency of adverse events. Conclusions: Inhibiting the NO overproduction seems to be an effective way to attenuate some of the schizophrenic symptoms. In the present study, l-lysine was an efficacious and tolerable add-on therapy for improving negative and general psychopathology symptoms in chronic schizophrenia. Further well-designed clinical trials with larger sample sizes and longer follow-up durations are warranted.

Disclosures: M. Farokhnia: None. **P. Mazaheri:** None. **H. Yekehtaz:** None. **N. Behbahani-Nejad:** None. **S. Akhondzadeh:** None.

Poster

525. Schizophrenia: Experimental Therapeutics and Preclinical Pharmacology

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 525.17/DD5

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: Australian NHMRC Project Grant APP1008473

Title: Differential effects of antipsychotics on serotonin transporter, 5-HT2A and 5-HT2C receptor binding density in the brain of male and female juvenile rats

Authors: *C. DENG, J. LIAN
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Abstract: Introduction: Antipsychotic drugs (APDs) were developed to treat schizophrenia in adults, however, they have been widely and increasingly prescribed (mostly "off label") for short-term control of various mental disorders in children and adolescents, without understanding the underlying mechanisms. Therefore this study investigated the effects of aripiprazole, olanzapine and risperidone (the three most widely-used APDs in children and adolescents) on the binding sites of serotonergic 5-HT2A, 5-HT2C receptors (5-HT2AR, 5-HT2CR) and 5-HT transporters (5-HTT) in various brain regions of juvenile rats. Methods: Male and female Sprague Dawley rats were treated orally with aripiprazole (1mg/kg, t.i.d.), olanzapine (1mg/kg, t.i.d.), risperidone (0.3mg/kg, t.i.d.) or vehicle (control; n = 6/group) starting from postnatal day (PD) 23 (±1 day) for 20 days (a period corresponding to the childhood-adolescent period in humans). Quantitative autoradiography was applied to detect the density of [^{3}H]ketanserin, [^{3}H]mesulergine and [^{3}H]paroxetine binding sites to 5-HT2AR, 5-HT2CR and 5-HTT. Results: Olanzapine significantly decreased 5-HT2AR binding in the nucleus accumbens (NAc), and 5-HT2AR and 5-HT2CR binding in the prefrontal cortex (PFC) and cingulate cortex (Cg) of both male and female rats. There were also some gender differences; male rats have higher 5-HT2CR binding density in the NAc than female rats. Olanzapine decreased 5-HT2CR binding in the NAc of only male rats. Risperidone decreased 5-HT2AR/5-HT2CR binding in the PFC of female rats. However, aripiprazole had no effects on 5-HT2AR and 5-HT2CR binding compared with controls. None of the three APDs had any effect on 5-HTT binding. Conclusion: These results suggested that early treatment with these antipsychotics had different effects on the 5-HT2AR and 5-HT2CR binding, and some of these changes were gender-dependent. It illustrates the importance of investigating how individual antipsychotic drugs act in the developing brain of both genders.

Disclosures: C. Deng: None. **J. Lian:** None.

Poster

525. Schizophrenia: Experimental Therapeutics and Preclinical Pharmacology

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 525.18/DD6

Topic: C.15. Schizophrenia and Bi-polar Disorder

Title: MT-3014, a novel PDE10A inhibitor I. pharmacological characterization of MT-3014

Authors: M. TAKAKUWA, Y. WATANABE, M. MURATA, J. ANABUKI, M. KATSU, K. TAKASHINA, *H. YASUMATSU
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Abstract: Phosphodiesterase 10A (PDE10A), a dual hydrolase of cAMP and cGMP, is highly expressed in striatal medium spiny neurons. Inhibition of PDE10A is expected to be a novel therapeutic approach for schizophrenia. In this study, we evaluated the pharmacological profile of a novel PDE10A inhibitor MT-3014. First, *in vitro* profile of MT-3014 was evaluated. MT-3014 inhibited PDE10A with an IC50 value of 0.37 nmol/L. Its selectivity against other PDE families or other 52 targets (enzymes, ion channels, receptors and transporters) was more than 300-fold. Second, MT-3014 was evaluated in rat models to assess antipsychotic potential. MT-3014 inhibited the MK-801-induced hyperactivity and suppressed the conditioned avoidance responses. The ED50 value in each test was less than 1 mg/kg, p.o., which was equivalent to that of risperidone. Third, MT-3014 was evaluated in rat models of cognitive impairment. MT-3014 attenuated the MK-801-induced prepulse inhibition (PPI) deficits and reversed MK-801-induced novel object recognition (NOR) deficits at the same dose ranges as those which demonstrated the antipsychotic potential above. On the other hand, risperidone required much higher dose to attenuate MK-801-induced PPI deficits and failed to reverse MK-801-induced NOR deficits. Finally, side effects-liability of MT-3014 was evaluated. Although MT-3014 induced catalepsy, its magnitude was less than that of risperidone. MT-3014 did not increase plasma prolactin concentration, though risperidone increased it. Also, repeated administration of MT-3014 (once daily for 21days) did not increase the body weight compared to the vehicle-treated group. The above results indicate MT-3014 possesses not only antipsychotic activity but also procognitive potential. Additionally, MT-3014 has less potential to exhibit side-effects seen with present antipsychotics. Therefore, MT-3014 could be a new generation of therapeutic agent for schizophrenia.

Disclosures: M. Takakuwa: None. **Y. Watanabe:** None. **M. Murata:** None. **J. Anabuki:** None. **M. Katsu:** None. **K. Takashina:** None. **H. Yasumatsu:** None.

Poster

525. Schizophrenia: Experimental Therapeutics and Preclinical Pharmacology

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Program#/Poster#: 525.19/DD7

Topic: C.15. Schizophrenia and Bi-polar Disorder

Title: MT-3014, a novel PDE10A inhibitor II.The relationship between the inhibitory effect of MT-3014 on conditioned avoidance response and the brain PDE10A occupancy in rats

Authors: Y. WATANABE[1], T. SAIJO[2], K. KOJIMA[2], S. SATO[3], M. TAKAKUWA[1], K. TAKASHINA[1], *K. TESHIMA[4,1], H. YASUMATSU[1]
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Abstract: MT-3014, a potent and selective PDE10A inhibitor, suppresses the rat conditioned avoidance response (CAR) which is used as a test with high predictive validity for antipsychotic activity. In this study, we evaluated the relationship between the inhibitory effect of MT-3014 on CAR and the brain PDE10A occupancy in rats. The inhibitory effect on CAR peaked at 1 h after oral administration (ED50=0.52 mg/kg) and was decreased in a time-dependent manner. The plasma concentration correlated with its inhibitory effect on CAR and was 50.07 ± 11.56 ng/mL (mean±S.E.M, n=3) at the dose of 0.5 mg/kg. To evaluate the brain PDE10A occupancy of MT-3014, the rat PET imaging study was conducted using a PDE10A PET tracer, ^{11}C-IMA107. Specific binding of ^{11}C-IMA107 was localized to the striatum and it was dose-dependently decreased by pre-treatment of MT-3014. To determine the relationship between the PDE10A occupancy of MT-3014 and its plasma concentration, blood samples were collected during the PET scan. The plasma concentration required to achieve a 50% PDE10A occupancy (EC50) was estimated to be 58.00 ± 5.80 ng/mL (mean±S.E.M., n=16 from 8 rats) using Emax model. Above results indicate that the ED50 in CAR and the EC50 in the PET imaging are achieved almost same plasma concentration.

Disclosures: Y. Watanabe: None. **T. Saijo:** None. **S. Sato:** None. **M. Takakuwa:** None. **K. Takashina:** None. **K. Teshima:** None. **H. Yasumatsu:** None. **K. Kojima:** None.

Poster

525. Schizophrenia: Experimental Therapeutics and Preclinical Pharmacology

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Program#/Poster#: 525.20/DD8

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: NIH Grant R01 MH091816

Title: Modulation of dopamine release in the nucleus accumbens core by lithium treatment

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Abstract: Lithium (Li+) is a prototypical mood stabilizer with efficacy in the treatment of bipolar disorder and reduction of suicidal behavior. Chronic Li+ treatment lowers extracellular dopamine (DA) concentrations in NAc, as assessed by microdialysis. However, it is unclear whether this finding reflects alterations of basal extracellular DA levels or changes in the magnitude or duration of phasic DA transients. To address this issue, we used fast-scanning cyclic voltammetry (FSCV) to analyze changes in extracellular DA concentrations in the NAc core evoked by electrical stimulation of the ventral tegmental area (VTA) with sub-second temporal resolution. We tested the effects of chronic and acute Li+ treatments on DA release in mice. In the chronic Li+ treatment experiment, mice were fed LiCl containing chow or control chow for at least three weeks prior to FSCV. For acute treatment experiment, 300 mg/kg LiCl or vehicle was injected i.p. five hours prior to FSCV recordings. A bipolar stimulating electrode was placed in the VTA and a carbon fiber electrode was placed in the NAc core. DA transients were evoked by trains of increasing amplitude, 60 rectangular, biphasic pulses that were applied to the VTA. In depletion experiments, control or chronically lithium treated mice were given repeated stimulus trains of either 20Hz-20 pulses or 60 Hz-60 pulses. Mice treated chronically with Li+ manifested significantly decreased peak amplitudes of stimulation-evoked DA transients in the NAc core. Chronic Li+ treatment did not significantly alter the rise time or decay time of the DA transients. Repeated stimulation experiments revealed that while DA release in the lithium group was attenuated early on, but this difference disappeared in further stimulations and both groups had similar depletion curves in the 60Hz-60 pulses run. Acute Li+ treatment did not significantly alter the peak amplitude or decay constant of stimulus-evoked DA transients. These results demonstrate that chronic but not acute Li+ treatment attenuates phasic DA release in the NAc core evoked by electrical stimulation of the VTA without altering the kinetics of DA release or reuptake. We also showed that this difference was not due to the availability of DA. DAergic neurotransmission in the NAc has been associated with both mania-like and depression-like behaviors in animal models. The amplitude and timing of DA transients in the NAc are critical in reward prediction, motivational and cognitive control of behavior, and impulsivity. Because impulsivity is strongly associated with suicide, our findings suggest that reduced DA release in the NAc may be part of the mechanism of lithium's anti-suicidal effects.

Disclosures: **A. Can:** None. **R. Cachope:** None. **D.O. Frost:** None. **J.F. Cheer:** None. **T.D. Gould:** None.

Poster

525. Schizophrenia: Experimental Therapeutics and Preclinical Pharmacology

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 525.21/DD9

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: KAKENHI 24300196

Title: Ketamine acts on 5-HT1B receptors in the nucleus accumbens and ventral pallidum: a possible role for its antidepressant action

Authors: H. YAMANAKA[1], C. YOKOYAMA[1], H. MIZUMA[1], H. DOI[1], S. J. FINNEMA[3], C. HALLDIN[3], *H. ONOE[2]
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Abstract: Ketamine is a unique anesthetic reagent known to produce various psychotic symptoms, such as hallucinations and dissociative state. Interestingly, ketamine has been reported to elicit fast-acting and long-lasting antidepressant effect in patients with major depression. Since existing antidepressants take several weeks to achieve their full therapeutic effect, ketamine seems to be an attractive antidepressant with some unique actions. Although recent studies provide insight into the molecular mechanisms of the effects of ketamine, its antidepressant mechanism has not been fully elucidated. To understand the involvement of the brain serotonergic system in the actions of ketamine, we performed a positron emission tomography (PET) study on non-human primates. Four rhesus monkeys underwent PET studies with two serotonin (5-HT)-related PET radioligands, [^{11}C]AZ10419369 and [^{11}C]DASB, which are highly selective for the 5-HT1B receptor and serotonin transporter (SERT), respectively. Preclinical evidence suggests that the 5-HT1B receptor plays an important role in the pathophysiology of depression, and SERT is a target protein for some existing antidepressants, such as 5-HT selective reuptake inhibitors and tricyclic antidepressants. Voxel-based analysis with the standardized brain MRI revealed that ketamine administration significantly increased 5-HT1B receptor binding in two brain regions: the nucleus accumbens and ventral pallidum, both of which are key neural substrates in motivation and reward systems, while it significantly reduced SERT binding in these brain regions. Pretreatment with NBQX, a potent antagonist of

the glutamate AMPA receptor, blocked the action of ketamine on 5-HT1B receptor but not SERT binding. This indicates the involvement of AMPA receptor activation in ketamine-induced alterations of 5-HT1B receptor binding. Because NBQX is known to block the antidepressant effect of ketamine in rodents, alterations in the serotonergic neurotransmission, particularly upregulation of postsynaptic 5-HT1B receptors in the nucleus accumbens and ventral pallidum may be critically involved in the antidepressant action of ketamine. A recent clinical PET study has also shown that 5-HT1B receptor binding within the identical brain regions is significantly low in patients with major depressive disorder compared with that in healthy subjects. Therefore, PET imaging studies for 5-HT1B receptor binding might be useful for a diagnosis of major depression as well as for the development of novel antidepressants.

Disclosures: H. Yamanaka: None. **H. Onoe:** None. **C. Yokoyama:** None. **H. Mizuma:** None. **H. Doi:** None. **S.J. Finnema:** None. **C. Halldin:** None.

Poster

525. Schizophrenia: Experimental Therapeutics and Preclinical Pharmacology

Location: Halls A-C

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Program#/Poster#: 525.22/DD10

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: R01MH094358

Title: Deconstructing auditory verbal hallucinations: a phenomenological approach

Authors: *C. ROSEN[1], K. A. CHASE[2], H. GIN[2], R. P. SHARMA[2]
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Abstract: Background: Auditory Verbal Hallucinations (AVHs) are an important transdiagnostic phenomenon occurring in schizophrenia, bipolar disorder, major depression and existing along a continuum within the general population (van Os, 2000; Badcock and Hugdahl, 2014). AVHs are sensory experiences that occur in the absence of external stimuli with a sufficient similarity to the real percept that they are attributed to an external source, outside of the individuals control. AVHs are frightening experiences, leading to high levels of distress, social isolation, and functional disability (Hayward, 2013). The heterogeneity of AVHs is substantial, and may be experienced as either ego-syntonic or ego-dystonic, and can involve one or multiple voices with distinct personalities that give commands, insult or affirm the patient (McCarthy-Jones, 2013). Lastly, AVHs have similar characteristics and attributes of real individuals, thus are experienced

as an integrated individual coherent relationship (Paulik, 2012). Methods: The Maastricht Interview was administered to participants who endorsed current auditory hallucinations (Romme & Escher, 2000). The interview consists of a comprehensive evaluation of AVH regarding the following aspects of the experience, characteristics, history, triggers, content, impact, relationship with AVH, and coping strategies. The phenomenological interview was recorded using reflection, clarification, requests for examples and descriptions, listening techniques to establish the context and construction of the experience, and reflection on meaning. Open coding of the qualitative interview was followed by selective identification of emergent themes. Results: The phenomenology of AVHs was examined in subjects who have experienced AVHs for more than 20 years. We identified three primary categories and themes in our analysis specific to participants': 1.) Conceptual understanding of AVHs, 2.) Characteristics of AVHs, and 3.) Ability to influence AVHs. Discussion: AVHs are a complex and fascinating phenomenon that patients may attribute to a Higher Power, spirits, ghosts, guardian angels, and, more recently, messages generated by radio or computers. AVHs are extremely heterogeneous in terms of frequency, duration, location, content, personification, acoustic quality, linguistic complexity, and ongoing commentary or voices conversing. The phenomenological approach in deconstructing AVH elucidates common themes, thereby decreasing the overall heterogeneity for ultimate uses in better treatment outcomes and constructs of possible neurobiological mechanisms of action.

Disclosures: C. Rosen: None. **K.A. Chase:** None. **H. Gin:** None. **R.P. Sharma:** None.

Poster

526. Opiate Reinforcement

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 526.01/DD11

Topic: C.17. Drugs of Abuse and Addiction

Support: RO1 DA025674

RO3 DA034886

Title: Transgenerational effects of female adolescent morphine exposure on offspring morphine self-administration, reinstatement, and gene expression

Authors: *F. M. VASSOLER, E. M. BYRNES
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Abstract: The prescription of opiates has escalated dramatically over the past decade, resulting in increased access and misuse of these highly addictive medications. The use in adolescent populations, and in particular adolescent female populations, is particularly concerning because opioids play a significant role in neuroendocrine function and development. Thus, exposure to opioids during this critical developmental period could have significant consequences for the female, as well as her future offspring. Here we utilize a rat model established in the laboratory to examine the influence of adolescent morphine exposure, followed by prolonged abstinence on the behavior of the progeny. Female Sprague dawley rats were administered daily subcutaneous (s.c.) injections of morphine sulphate for 10 days using an increasing dosing regimen and beginning at postnatal day 30 (P30). The dose started at 5 mg/kg and increased every other day by 5 mg/kg. Therefore, on P30 and P31 the animals received 5 mg/kg, on P32 and P33 10 mg/kg and so forth until P40 when they received 25 mg/kg. Age-matched control animals received saline injections. A minimum of 20 days later (on or after P60) the females were mated with naïve males. Morphine self-administration, extinction and reinstatement in the offspring were examined during adulthood (P60). The acquisition of morphine self-administration was examined at two doses: 0.25 or 0.75 mg/kg/infusion. At the low dose, a main effect of maternal treatment was observed demonstrating that MorF1 animals self-administered significantly more morphine than the SalF1 animals. This effect appeared to be driven by the MorF1 males, who took significantly more morphine than the SalF1 males ($p<0.05$). In contrast, at the higher dose, there was no difference in the acquisition between the male animals but the MorF1 females took significantly less morphine than the SalF1 females ($p<0.05$). Following extinction, reinstatement was induced with a 1 mg/kg intraperitoneal (i.p.) priming injection. We found significant attenuation of reinstatement in both male and female MorF1 animals (following high dose self-administration). This suggests that female adolescent exposure to opioids, even following prolonged abstinence, affects the offspring's response to morphine. Experiments examining the transcriptome of the nucleus accumbens at baseline and in response to a morphine injection using RNA deep sequencing are ongoing.

Disclosures: F.M. Vassoler: None. **E.M. Byrnes:** None.

Poster

526. Opiate Reinforcement

Location: Halls A-C

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Program#/Poster#: 526.02/DD12

Topic: C.17. Drugs of Abuse and Addiction

Support: NSERC

FQRS

Title: Augmentation of heroin seeking following chronic food restriction in the rat: A role for mesolimbic and nigrostriatal dopamine?

Authors: *T. M. D'CUNHA, E. DAOUD, A. BISHOP, L. HAMEL, F. SEDKI, U. SHALEV
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Abstract: In human drug addicts chronic food restriction or dieting augments drug taking and increases the risk for relapse during abstinence. We recently reported that chronic food restriction during a period of prolonged withdrawal augments heroin seeking in rats with a history of heroin self-administration. Previous research on chronically food restricted (FDR) rats has found alterations in the mesolimbic dopamine (DA) system, a critical component of the reward system. Moreover, it has been suggested that the nigrostriatal DA system is also a critical component in reward and addiction. Here we assessed levels of extracellular DA in various terminal regions of the mesolimbic and nigrostriatal DA pathways, including the nucleus accumbens (NAc) shell and core, the basolateral amygdala (BLA), and the dorsolateral striatum (DLS). Rats were trained to self-administer heroin for 10 days. Next, rats were moved to the animal colony for 14 days of withdrawal. During the withdrawal period rats were given unrestricted access to food or subjected to a mild chronic food restriction that decreased their body weight to approximately 90% of their original body weight. On the 14th day of food restriction rats were returned to the operant chambers for a drug seeking test conducted under extinction conditions. Extracellular DA was assessed using *in vivo* microdialysis and HPLC during baseline conditions in the animal facility and then during the 3 hour drug seeking test. Food restriction significantly augmented heroin seeking compared to sated control rats. In the NAc shell there was a significant increase in DA only in the FDR group upon re-exposure to the drug context and throughout the drug seeking test. Conversely, DA in the NAc core increased during re-exposure to the drug context but returned to baseline during the drug seeking test regardless of food condition. Similarly, in the DLS DA increased at initiation of the drug seeking test in both groups. No changes in DA were found in the BLA during the drug seeking test, or between FDR and sated rats. Taken together, these results suggest that food restriction results in changes in DA release in only the NAc shell during the augmentation of heroin seeking. Future studies will investigate if DA antagonists can block the augmentation of heroin seeking induced by chronic food restriction.

Disclosures: T.M. D'Cunha: None. **E. Daoud:** None. **A. Bishop:** None. **L. Hamel:** None. **F. Sedki:** None. **U. Shalev:** None.

Poster

526. Opiate Reinforcement

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Topic: C.17. Drugs of Abuse and Addiction

Support: P01 DA031656

F31 DA030799

Title: A classically conditioned opioid cue acquires greater control over motivated behavior and induces greater Fos protein expression in rats prone to attribute incentive salience to a food cue

Authors: *L. M. YAGER[1], K. K. PITCHERS[1], S. B. FLAGEL[2,3], T. E. ROBINSON[1]
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Abstract: Cues associated with natural or drug rewards can acquire such powerful motivational control over behavior that individuals sometimes have difficulty resisting them. There is, however, considerable individual variation in the ability of reward cues to gain such control over behavior, which is due, at least in part, to intrinsic individual variation in the extent to which reward cues are attributed with incentive salience. For example, if a spatially discrete stimulus (the conditioned stimulus, CS) is repeatedly paired with delivery of a food reward (the unconditioned stimulus, US), in some rats (sign-trackers, STs), the food cue itself becomes attractive, eliciting approach and engagement with it, and desired, in that rats will work to obtain it. In other rats (goal-trackers, GTs) the food cue itself is less attractive, presentation of the CS instead elicits approach to the location where food will be delivered, and GTs do not work as avidly to gain access to the cue. Importantly, the propensity to attribute incentive salience to a food cue predicts the extent to which a discrete cocaine cue acquires motivational properties. Although we have shown that a cocaine cue acquires greater control over motivated behavior in STs relative to GTs, we do not know if this generalizes to other drug classes. Thus, the first aim of the experiments reported here was to determine whether the propensity of an individual to attribute incentive salience to a food cue predicts the extent to which a cue associated with administration of an opioid drug (remifentanil) acquires incentive motivational properties. The second aim was to begin to explore whether the neurobiological correlates underlying individual variation in the attribution of incentive salience to food and opioid cues are similar. To do this we measured Fos protein expression elicited by presentation of either a food or opioid cue in STs and GTs. Consistent with previous studies using cocaine, we found that relative to GTs, STs

were more attracted to a classically conditioned opioid cue (they approached it) and they found the opioid cue more desired (they worked for it). We also found that in order for either a food or an opioid cue to engage cortico-striatal-thalamic circuitry it must be imbued with incentive salience, as indicated by the fact that such cues induced Fos protein expression preferentially in STs.

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Poster

526. Opiate Reinforcement

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Program#/Poster#: 526.04/DD14

Topic: C.17. Drugs of Abuse and Addiction

Title: Sex, drugs, and food: The role of sex hormones in chronic food restricted-induced augmentation of heroin seeking in female rats under withdrawal

Authors: *F. SEDKI, J. GREGORY GARDNER, A. LUMINARE, I. AKHTAR, T. D'CUNHA, J. DUCHESNEAU
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Abstract: Food restriction enhances drug seeking following prolonged withdrawal from drug taking. Recently, we reported augmented heroin seeking in chronically food restricted male rats under withdrawal, an effect that has yet to be examined in female rats. Women and female rats possess an increased vulnerability to drugs of abuse, which may be mediated by fluctuations in ovarian hormones. As such, it is hypothesized that progesterone and estradiol play opposing roles in drug seeking. Estradiol may enhance drug seeking, while progesterone may attenuate drug seeking. Previous studies have predominantly investigated the effects sex hormones on cocaine-trained rats, and there are relatively few reports on the effects in heroin-trained rats. Thus, we investigated the role of estradiol and progesterone in the augmentation of heroin seeking in chronically food restricted female rats, under withdrawal. Methods: Female Long-Evans rats were trained to self-administer heroin for 10-12 days in operant conditioning chambers. Next, rats were moved to the animal colony and maintained on free access to food (sated group) or subjected to 14 days of mild chronic food restriction (FDR group), which sustained their body weight at 90% of their baseline body weight. On day 14, rats underwent a 3 h heroin seeking test under extinction conditions, in the operant conditioning chambers. Exp. 1:

Rats underwent bilateral ovariectomy (OVX) or sham surgery, as well as estradiol (low maintenance dose of approximately 35 pg/ml E2 in plasma) or cholesterol (CH) silastic capsule implantation (s.c.) prior to the food restriction phase. Exp. 2: Rats underwent bilateral OVX and were repeatedly administered with a high dose of E2 (0.5 mg/kg; s.c.) for 8 days prior to testing. Exp. 3: Progesterone injections (0.0, 2.0 mg/kg) were administered 24 h and 2 h prior to testing. Results: In all experiments, food restriction resulted in augmented heroin seeking, compared to the sated controls. Specifically, in Exp. 1 food restriction resulted in increased heroin seeking in the OVX-CH and sham-CH groups, compared to their respective sated controls. In contrast, E2 replacement attenuated the food restriction effect. Preliminary data from Exp. 2 suggests a similar pattern as in Exp. 1. Injections of progesterone in Exp. 3 had no effect on heroin seeking in both the sated and FDR groups. Conclusions: Overall, the effect of food restriction on heroin seeking in female rats under withdrawal is as robust as in males. Interestingly, low-dose E2 replacement attenuated the food restriction effect in the OVX rats, possibly due to its anorexic properties. However, progesterone does not modulate heroin seeking in female rats.

Disclosures: **F. Sedki:** None. **J. Gregory Gardner:** None. **A. Luminare:** None. **I. Akhtar:** None. **T. D'Cunha:** None. **J. Duchesneau:** None.

Poster

526. Opiate Reinforcement

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Program#/Poster#: 526.05/DD15

Topic: C.17. Drugs of Abuse and Addiction

Support: NIDA/IRP

Title: Role of projections from ventral subiculum to nucleus accumbens shell in context-induced reinstatement of heroin seeking

Authors: *J. M. BOSSERT[1], R. M. ST. LAURENT[1], N. J. MARCHANT[1,3], H. WANG[2], M. MORALES[2], Y. SHAHAM[1]
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Abstract: Background: In humans, exposure to contexts previously associated with heroin use can provoke relapse. In rats, exposure to heroin-paired contexts after extinction of drug-

reinforced responding in different contexts reinstates heroin seeking. We previously demonstrated a causal role for projections from ventral medial prefrontal cortex to accumbens shell in this reinstatement. Here, we examine the contribution of glutamate projections from ventral subiculum to accumbens shell. We combined Fos with the retrograde tracer Fluoro-Gold to assess activation of this pathway during context-induced reinstatement. We then employed an anatomical disconnection procedure to examine the interaction between glutamatergic projections from ventral subiculum to accumbens shell and local dopamine D1 postsynaptic receptors in this reinstatement. Methods: We trained rats to self-administer heroin for 12 days; drug infusions were paired with a discrete tone-light cue. Lever pressing was subsequently extinguished in a non-drug-associated context in the presence of the discrete cue. We then tested the rats in the heroin- or extinction-associated contexts under extinction conditions. Results: Exposure to the heroin but not extinction context reinstated lever pressing. Context-induced reinstatement was associated with increased Fos expression in ventral subiculum neurons, including those projecting to accumbens shell. We also found that inactivation of ventral subiculum in one hemisphere combined with dopamine D1 receptor blockade into contralateral or ipsilateral accumbens shell decreased this reinstatement. Conclusions: Results suggest that the glutamatergic projections from ventral subiculum to accumbens shell are part of a circuit in which activation provokes context-induced relapse to heroin seeking.

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Poster

526. Opiate Reinforcement

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Topic: C.17. Drugs of Abuse and Addiction

Support: Center for the Study of Traumatic Stress

Title: Effects of chronic intravenous morphine self-administration on *in vivo* brain glucose utilization (18FDG-PET) and transcriptome expression (RNA Sequencing) in rats

Authors: *K. CHOI[1,4], T. LE[1,4], G. SUKUMAR[2], C. M. WILSON[3,5], R. G. SELWYN[3,5], C. L. DALGARD[2,4], R. J. URSANO[1,4]
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Abstract: Addiction to opiates is a major public health problem and non-medical use of prescription opiates has risen substantially. Although chronic use of opiates can induce physical and psychological dependence, molecular mechanisms that contribute to the opioid dependence are not well understood. Using chronic intravenous morphine self-administration (MSA), brain imaging (18FDG-PET and CT) and RNA sequencing, we investigated the effects of withdrawal from chronic MSA on *in vivo* regional glucose utilization and transcriptome expression levels in rats. Male Sprague Dawley rats self-administered morphine (0.5 mg/kg/infusion) or saline for 3 weeks (4 hours/day, 5 days per week), and glucose uptake and transcriptome expression levels were measured during spontaneous withdrawal. The animals reliably self-administered intravenous morphine with average daily intake of 7 mg/kg for three weeks. Glucose uptake was decreased in the brainstem in 2-day withdrawal from MSA. Morphine challenge dramatically increased glucose uptake in multiple brain regions including the nucleus accumbens, hypothalamus, amygdala and brainstem. The animals also exhibited behavioral sensitization to morphine challenge. RNA sequencing analysis with the nucleus accumbens tissue (1-week withdrawal) identified a substantial number of genes (FDR-adjusted q-value < 0.05) that are associated with biological functions such as synapse, neuron development, ion channels and neurotransmitter receptors. Spontaneous withdrawal from chronic MSA induced robust changes in *in vivo* glucose utilization and gene expression in the brain. By combining a clinically relevant animal model, a non-invasive brain imaging and a high-throughput transcriptome sequencing, we demonstrated the utility of investigating the biological basis of opioid addiction.

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Poster

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Support: NIH Grant DA12964

NIH Grant DA16176

Title: Environmental enrichment protects against self-administration of the short-acting opioid remifentanil

Authors: *R. S. HOFFORD, M. T. BARDO
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Abstract: Rearing environment affects many behavioral outcomes, including self-administration of drugs of abuse. In particular, several studies have shown that rats raised in an isolated condition (IC) self-administer more amphetamine, methylphenidate, and cocaine than rats raised in an enriched condition (EC) at low unit doses. The data on the effects of rearing environment on opioid self-administration are less clear. This study examined the acquisition of self-administration of the short-acting opioid remifentanil (1 μg/kg) in IC, EC, and pair-housed controls (social condition, SC). Additionally, after acquisition, these rats were allowed to administer several different doses of remifentanil (0, 0.1, 0.3, 1, 3, and 10 μg/kg) in semi-random order to generate a dose-response curve for each group. Given IC rats' propensity to self-administer more stimulants at low unit doses, it was hypothesized that IC rats would acquire self-administration more rapidly and would take more remifentanil at low doses compared to EC rats. As hypothesized, IC rats acquired self-administration more quickly than EC and SC rats. In addition, at stable responding, IC rats self-administered more remifentanil than EC rats; the increased responding was evident at both low doses and higher doses of remifentanil. IC and SC rats did not differ in their intake of remifentanil during the maintenance and dose-response phases of the experiment. These findings support previous literature suggesting that environmental enrichment can protect against self-administration of drugs of abuse. However, the exact mechanism underlying this protection may be different for opioids and stimulants.

Disclosures: R.S. Hofford: None. **M.T. Bardo:** None.

Poster

526. Opiate Reinforcement

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Program#/Poster#: 526.08/DD18

Topic: C.17. Drugs of Abuse and Addiction

Title: Sex differences in conditioned morphine reward

Authors: D. G. WEIDEMANN, A. K. DENOBREGA, H. HANIF, S. A. M. BOBZEAN, *L. I. PERROTTI
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Abstract: Compared to males, women and female rodents are more responsive to cues associated with drug reward. Recent studies suggest that these sex differences are due, in part, to activational effects of rising and falling levels of ovarian hormones throughout the reproductive cycle of females. However, to date, most of the research examining sex differences in drug reward has been conducted using psychostimulants. Thus, the aim of the present study was to investigate sex differences in morphine conditioned place preference. To this end, adult female and male Long Evans rats were conditioned to associate one of two visually and tactically distinct environments with one of four doses of morphine sulfate (0, 1.25, 2.5, or 5mg/kg) or saline over three 60 minute alternating (morning and afternoon) conditioning sessions. Conditioned place preference (CPP) was defined as a significant increase in the amount of time spent in the morphine-paired environment during the 30 minute post-conditioning test compared to the amount of time spent in this same compartment during the 30 minute pre-conditioning test. Preliminary data analyses determined that female rats expressed CPP to the higher conditioning dose of morphine (5mg/kg; $p<0.01$), while male rats expressed CPP only at the lower conditioning doses of the drug (1.25, 2.5mg/kg; $p<0.05$). These findings demonstrate that sex significantly influences the expression of morphine CPP. Experiments are currently underway to examine a wider range of morphine doses as well as the influence of the estrous cycle on morphine CPP.

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Poster

526. Opiate Reinforcement

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grant MH085607

State of Florida, Executive Office of Governor's Dept. of Economic Opportunity

Title: Exposure to HIV-1 Tat protein potentiates the rewarding effects of morphine, and reinstates extinguished conditioned place preference

Authors: *J. P. MCLAUGHLIN[1], M. L. GANNO[2], Y. ZHANG[3], M. J. KREEK[3], J. J. PARIS[2]
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Abstract: Aims: While exposure to the HIV-1-accessory protein Tat is known to increase striatal dopamine levels, the functional consequences of Tat protein on the behavioral response to abused drugs are little known. Accordingly, we hypothesized that HIV-1 Tat expression in brain would modulate the rewarding effects of morphine. Methods: Using the GT-tg bigenic mouse model, where brain-selective Tat expression is controlled by activation of a doxycycline (Dox) promotor, we tested the effects of Tat protein on morphine-conditioned place preference (CPP) and consumption in a two bottle choice (TBC) assay. Microdialysis was performed with additional mice expressing Tat protein and repeatedly administered opioid treatment. Results: Western blot analysis confirmed the expression of Tat protein in GT-tg bigenic mice correlated with dose and duration of Dox treatment. In initial behavioral testing, although GT-tg bigenic mice expressing Tat demonstrated saline-conditioned place preferences similar to uninduced littermates and saline- or Dox-treated C57BL/6J mice, Tat expression for 7 days significantly doubled morphine-CPP. Subsequent characterization found the potentiation of CPP for morphine to be dependent on the magnitude of exposure to Tat protein. Consistent with this observation, exposure to Tat protein increased the levels of dopamine released in response to heroin in GT-tg mice. Of interest, among GT-tg bigenic mice demonstrating extinction of morphine-CPP, subsequent expression of Tat protein for 7 days resulted in the reinstatement of the extinguished place preference response in previously uninduced mice. Conclusions: Overall, these data suggest that expression of HIV-1 Tat protein in mouse brain potentiated heroin-induced dopamine release and the rewarding effects of morphine in a dose- and duration-dependent matter. These results suggest that exposure to Tat protein is sufficient to increase the rewarding effects of abused substances. Moreover, the Tat-induced reinstatement of an extinguished place preference for morphine suggests a biological means by which HIV infection may increase the vulnerability to substance abuse and relapse in abstinent subjects.

Disclosures: J.P. McLaughlin: None. **M.L. Ganno:** None. **Y. Zhang:** None. **M.J. Kreek:** None. **J.J. Paris:** None.

Poster

526. Opiate Reinforcement

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 526.10/DD20

Topic: C.17. Drugs of Abuse and Addiction

Support: SAP 4100055576

Title: Tracking sleep in a rodent model of heroin addiction

Authors: *A. A. COFFEY, Z. GUAN, P. S. GRIGSON, J. FANG
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Abstract: Addiction is a chronic disease characterized by multiple relapses. In the United States, heroin abuse is increasing dramatically. Drug abuse and sleep disturbances interact to form a vicious cycle where drug abuse disrupts sleep and sleep deficits, in turn, spur on drug abuse. With opioid abuse, the most commonly cited sleep deficit is a decrease in rapid eye movement (REM) sleep during withdrawal. Here, we sought to characterize the effects of drug on sleep patterns throughout the entire cycle of heroin addiction, including acquisition, abstinence, and a drug-induced reinstatement. To our knowledge, ours is the first study to examine the impact of acquisition of drug-taking, abstinence, and extinction/reinstatement on patterns of sleep. This comprehensive analysis allows us to relate sleep deficits to differences in drug taking behavior, including the amount of drug taken, the motivation to take drug, and the propensity to relapse. We used electroencephalography (EEG) and electromyography (EMG) to measure sleep patterns in male Sprague-Dawley rats over 20 days of acquisition, 14 days of abstinence, and a single day of extinction and reinstatement, a rodent model of relapse. Compared to saline controls, acquisition of heroin self-administration was associated with a poor quality of sleep as indicated by more rapid transitions between sleep and wake states. Ultimately, characterization of the drug-induced dysregulation of sleep may reveal new avenues for treatment.

Disclosures: **A.A. Coffey:** None. **P.S. Grigson:** None. **Z. Guan:** None. **J. Fang:** None.

Poster

526. Opiate Reinforcement

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Dr. Miriam and Sheldon G. Adelson Medical Research Foundation (MJK)

Title: Mouse models of the OPRM1 (A118G) polymorphism: Differential heroin self-administration behavior compared to wild type mice

Authors: *Y. ZHANG[1], R. PICETTI[2], E. R. BUTELMAN[3], A. HO[3], J. BLENDY[4], M. KREEK[3]
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Abstract: Rationale: Mu opioid receptors (MOPRs) are the target of opioids, which are currently responsible for massive addiction morbidity in the U.S. The gene coding for the human MOPR (OPRM1) has an important functional single nucleotide polymorphism (SNP), A118G, in which an adenine-to-guanine substitution exchanges an asparagine for an aspartic acid at a putative N-glycosylation site. The OPRM1 A118G genotype results in substantially increased risk of heroin addiction; however the neurobiological mechanism for this increased risk is not fully understood. Objectives: The current study examined heroin self-administration (SA) behavior in A112G (G/G) mice in extended SA sessions. Methods: Adult male G/G and wild type litter mate (A/A) mice were allowed to SA heroin (0.25 mg/kg/unit dose, FR1 with a nose poke response) for 4 hours/day for 10 consecutive days. Half of the mice then continued in a heroin dose-response study, while extinction from heroin SA was studied in the other half. Results: Male G/G mice responded for heroin significantly more often than A/A mice in the initial 10 days of heroin SA and in the subsequent dose-response study. The number of responses at the active hole escalated over the 10 consecutive daily SA sessions regardless of genotypes. There were no significant differences in extinction of SA between the A/A and G/G mice. Conclusion: male G/G mice self-administered more heroin than did A/A mice over a 10-day period. These are the first studies to examine the acquisition of heroin SA in this mouse model of the clinically relevant A118G OPRM1 SNP and may lead to a better understanding of the neurobiological and behavioral mechanisms that underlie greater risk of heroin addiction in carriers of the A118G SNP.

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Poster

526. Opiate Reinforcement

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Topic: C.17. Drugs of Abuse and Addiction

Title: Endomorphin analogs fully substitute for morphine without suppressing operant responses for food in a discrimination model: Potential role in opioid addiction pharmacotherapy

Authors: *M. R. NILGES[1], J. E. ZADINA[2,3]
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Abstract: Endomorphins (EM) have the highest selectivity of any endogenous peptide for the pain modulating mu-opioid receptor (MOR). We have synthesized several metabolically stable, blood-brain barrier permeable EM analogs that have equal or greater analgesic potency than morphine, the current gold standard MOR agonist. Morphine and other exogenously derived opioid analgesics produce detrimental side effects such as respiratory depression, tolerance, glial cell activation, and have a large potential for abuse. We have shown that EM analogs do not induce respiratory depression, produce substantially less tolerance than morphine, and do not induce markers of microglia or astrocyte activation. In abuse liability assays, intravenous (i.v.) morphine produced a robust and dose-dependent conditioned place preference (CPP) effect, but the analogs did not produce CPP or aversive effects. In self-administration models, rats with 12-hour per day access to an i.v. EM analog infusion lever pressed no more than controls with access to i.v. vehicle infusions, suggesting the analogs lack reinforcing qualities. Furthermore, EM analogs appear to penetrate the blood-brain barrier since i.c.v. naloxone-methiodide attenuated the antinociceptive responses of the analogs. In this study we trained rats to discriminate i.v. infusions of morphine from vehicle in a 2-lever food choice drug-discrimination (DD) task to assess the substitution potential of the analogs for morphine. Methadone suppresses motor/appetitive responses for food in the DD model at doses that substitute for morphine. A compound which lacks CPP and self-administration, but substitutes for morphine in the DD model without motor/appetitive impairment, has potential as a pharmacotherapy for opioid addiction. We found that analogs 2 and 4 fully substituted for morphine, but did not impair motor/appetitive responding. The analogs fully substituted for morphine after randomized bolus injections (0.3 - 5.6 mg/kg i.v.) and after cumulative injections made in escalating ¼ log doses. Data provided here suggest the analogs may provide better therapy for opioid addiction than methadone, since motor impairment is a major limitation of methadone maintenance therapy. These data indicate EM analogs lack reinforcement qualities, provide potent CNS mediated analgesia, substitute for morphine in the DD model, and are novel candidates for opioid addiction pharmacotherapy.

Disclosures: M.R. Nilges: None. **J.E. Zadina:** None.

Poster

527. Monoamines and Behavior: Serotonin and Histamine

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Topic: C.18. Behavioral Pharmacology

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Title: Differential effect of selective 5-HT7 receptor antagonist on reversal learning and attentional set shifting in rats

Authors: *A. HRNJADOVIC[1], P. J. ALLEN[2], B. KOCSIS[2]
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Abstract: Previous work has demonstrated that serotonin 7 receptor (5-HT7) antagonism ameliorates impairments in selective cognitive domains in animal models of schizophrenia induced by NMDA receptor blockade, particularly in novel object recognition and attentional set shifting tests (ASST), but not in prepulse inhibition. Rodent ASST (Birrell and Brown, 2000) allows for the identification of several types of cognitive rigidity, such as failure on reversals (when the +/- cues are switched), and intradimensional (ID; when a new pair of cues of the same dimension is introduced) and extradimensional set-shifting (ED; when the rat has to switch between cue dimensions). Importantly, failure on the reversals versus on the ID/ED shift were shown to develop after lesioning, respectively, the medial (mPFC) and orbital (OFC) regions of the prefrontal cortex. Since 5-HT7 receptors show stronger OFC expression compared with mPFC, we hypothesized that it might differentially affect these types of cognitive rigidity. Six male Sprague-Dawley rats were tested in the ASST, several times each, after subcutaneous administration of saline, MK-801 (NMDAR antagonist, 0.2 mg/kg), SB-269970 (5-HT7R antagonist, 1 mg/kg), and co-administration of the two compounds. Due to behavioral impairments induced by MK-801, the rats did not take food for the first ~2 hrs after injection, even if food was scattered in the recording arena. Therefore, ASST started only when the rats were able to find the food, hold it, and eat. Accordingly, SB-269970 was administered following two protocols: 1) shortly before MK-801 or 2) shortly before MK-801 and ~2 hrs later (i.e. immediately before starting the ASST). We found that MK-801 impaired performance on all discrimination phases of ASST whereas administration of SB-269970 alone had no effect. When co-administered with MK-801 however, SB-269970 reversed the effect of NMDA receptor blockade selectively on the reversal phases of ASST. When an additional dose of the 5-HT7 antagonist was given ~2 hrs after MK-801 injection (i.e. at the start of ASST) it also restored performance on the ED shift. We conclude that 5-HT7 receptor mechanisms may provide a

specific contribution to the complex of cognitive deficits in schizophrenia and possibly other psychiatric diseases, e.g. anorexia nervosa, which also express different forms of cognitive inflexibility.

Disclosures: A. Hrnjadovic: None. **P.J. Allen:** None. **B. Kocsis:** None.

Poster

527. Monoamines and Behavior: Serotonin and Histamine

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NCRG Early Stage Investigator Award to KMN

Title: Distinct circuits underlie the effects of 5-HT1B receptors on aggression and impulsivity

Authors: *K. M. NAUTIYAL[1,2], K. TANAKA[5], M. M. BARR[6], Y. LE DANTEC[7], L. TRITSCHLER[7], D. J. DAVID[7], A. M. GARDIER[7], C. BLANCO[1,3], S. AHMARI[8], R. HEN[1,4]
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Abstract: Impulsive and aggressive behaviors are both mediated by the serotonin 1B receptor (5-HT1BR), an inhibitory GPCR expressed on both serotonergic and non-serotonergic neurons. However the circuit(s) underlying these effects have not been delineated, nor has the developmental contribution been assessed. In order to address these questions, we developed a novel mouse model to control spatial and temporal expression of the 5-HT1BR. A transgenic line was created by replacing the endogenous htr1b coding region with a cassette containing a floxed tetracycline operator and htr1b cDNA (tetO1B). Crossing tetO1B mice to mouse lines expressing the tetracycline-dependent transcriptional silencer (tTS) or Cre transgenes under the control of various promoters, allowed for tissue specific knock-down of 5-HT1BR, the former of which

could be rescued by treatment with doxycyline. Mice were tested for increased aggression using a male-male territorial aggression assay. Impulsivity was measured in operant behavior paradigms which tested response inhibition including differential reinforcement of low-rate responding and Go/No-go tasks. Whole-life, whole-brain knockout (β-Actin -tTS/tetO1B mice) resulted in high levels of aggression and impulsivity. Interestingly, rescue of 5-HT1BR expression in adulthood reversed the impulsive, but not the aggressive phenotype. The aggressive phenotype was ameliorated following post-natal rescue of 5-HT1BR expression, with a sensitive period around P21-28. To localize the receptors involved in mediating these behaviors, we first knocked out 5-HT1B autoreceptors, which are expressed on serotonin neurons (Pet-1-Cre/tetO1B mice). These mice were neither aggressive nor impulsive, suggesting a lack of involvement of autoreceptors. Next, a subset of heteroreceptors located in the cortex, striatum and hippocampus were knocked out (CaMKII-tTS/tetO1B). These mice displayed increased aggressive, but not impulsive behavior. This suggests that receptors in cortex, striatum, and/or hippocampus modulate aggressive behavior, and a different subset of heteroreceptors mediate impulsive behavior. To begin to dissect the mechanisms underlying the 5-HT1BR effect on impulsivity, we measured *in vivo* extracellular levels of dopamine (DA) in the nucleus accumbens (NAc), a known modulator of response inhibition. Levels of DA were correlated with impulsive behavior - increased following a lack of 5-HT1BRs throughout life and reversed with adult rescue of the receptor. Overall, our data suggest that 5-HT1BRs modulate aggression and impulsivity through distinct mechanisms and that 5-HT1BR effects on DA may modulate impulsivity.

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Poster

527. Monoamines and Behavior: Serotonin and Histamine

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Topic: C.18. Behavioral Pharmacology

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Brain & Behavior Research Foundation

Title: Development of a discrete trials task to assess serotonergic modulation of interval timing in mice

Authors: *A. L. HALBERSTADT[1], K. SCHEFFERS[2], A. D. FLYNN[3], J. W. YOUNG[1], M. A. GEYER[1]
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Abstract: The perception of time is essential for survival, is required for the precise organization of sequences of activity, and the anticipation of behavioral outcomes and future events. One form of temporal perception is interval timing which refers to the discrimination of durations typically in the seconds to minutes range. Schizophrenia is associated with timing deficits. It has been proposed that impaired temporal processing is a core deficit of schizophrenia, contributing to cognitive dysfunction, hallucinations, and inappropriate behavior. There is also evidence that the serotonergic system, which is believed to play a role in the neuropathology of schizophrenia, regulates temporal perception. Unfortunately, little is known about the neural substrates that are involved in serotonergic modulation of timing. We have developed a discrete trials interval timing task in mice that can be used to elucidate the neural and receptor mechanisms underlying the modulation of interval timing by both endogenous serotonin and hallucinogenic drugs. In the discrete trials task, a lamp is illuminated for a variable duration, and then two levers are presented. Responding on lever A is reinforced if the stimulus duration is < 6.5 s while responding on lever B is reinforced if the stimulus duration is > 6.5 s. C57BL/6J mice were trained to discriminate between short (2.5 and 5 s) and long (8 and 10.5 s) stimulus durations, and then challenged with a wider range of test stimuli. We examined whether task performance was affected by 5HT2A receptor ligands. We found that mice can learn to reliably discriminate between the short and long duration training stimuli (>80% accuracy for the two extreme stimulus durations). Challenge studies revealed that the proportion lever B responding increased with the stimulus duration. The 5HT2A/2C agonist and serotonergic hallucinogen 2,5-dimethoxy-4-iodoamphetamine (DOI; 0.25-1 mg/kg, IP) altered interval timing ($F(18,216)=2.42$, $p<0.002$) and reduced T50 (time corresponding to %B = 50). A reduction of T50 indicates a change in the period of the internal pacemaker. The selective 5HT2A antagonist M100907 (0.1 mg/kg IP) also altered interval timing and reduced T50. These findings suggest that 5HT2A receptors modulate temporal discrimination. Our goal is to use this behavioral paradigm to investigate the regulation of interval timing by the serotonergic system and determine the neural sites involved in this effect. It is possible that the disruption of temporal perception induced by hallucinogens could represent an animal model for schizophrenia research, potentially facilitating the development of novel agents with antipsychotic activity.

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Poster

527. Monoamines and Behavior: Serotonin and Histamine

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Svenska Läkaresällskapet (SLS-384001)

Title: Prenatal exposure to antenatal depression and antidepressants alters the ROCK2 expression

Authors: *J. D. OLIVIER, H. ÅKERUD, H. KAIHOLA, Å. EDVINSSON, A. SKALKIDOU, I. SUNDSTRÖM-POROMAA
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Abstract: Depressive symptoms during pregnancy affect about 25% of the pregnant women. Several studies have shown that antenatal depression has an impact on both the mother and the fetus. The use of antidepressants during pregnancy to relief the mother's symptoms has significantly increased during the last decade. Although no gross teratogenic effects on the offspring have been reported the effects of antidepressant treatment during pregnancy remain largely unknown. To unravel the molecular mechanisms underlying antenatal depression and antidepressant treatment a microarray was performed on human fetal placentae. Results show that, among others, ROCK2 was down-regulated in both depressed and antidepressant cases. In contrast to the RNA expression, a small increase in ROCK2 protein levels was found in total tissue lysate in both depressed and antidepressant cases. Moreover, immunohistochemistry of the placenta revealed that ROCK2 expression was slightly increased in trophoblasts, stromal, and endothelial cells of depressed and antidepressant cases. As ROCK2 is part of the Rho-ROCK signaling pathway, which plays a role in cardiovascular diseases, a rat model for depression (heterozygous SERT ko-rat) was used to further unravel the role of ROCK2 in prenatal depression and antidepressant treatment. Pregnant rats were treated from gestational day (G)1 till G19 with either fluoxetine or placebo. On G20 rats were sacrificed and placenta, fetal heart and fetal brain were collected. Rat experiments to validate and extent the human data are now ongoing. Together, the results of this study will give us more insights into the role of ROCK2 in prenatal depression and antidepressant treatment and the effects on the offspring.

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Poster

527. Monoamines and Behavior: Serotonin and Histamine

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Title: Individual wheel running activity of laboratory rats using a radio frequency identification technology

Authors: *N. KUBOTA[1], S. YANAGITA[1], Y. TAKANO[2], K. TAKEDA[1]
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Abstract: Spontaneous wheel running is a common physical exercise model to enhance physiological and psychological health in rodents. The effects of exercise on physiological and psychological health are known to be often regulated by amount of physical exercise. Thus, previous experimental data for wheel running have been collected in individual housing in order to measure the amount of individual physical activity. Recently, numerous studies have suggested that individual housing influences hypothalamic pituitary adrenal axis activity to stress while social housing can buffer the influence of stress responsiveness. Therefore, we cannot rule out a possibility of negative influences of living alone on beneficial effects of exercise. However, it is difficult to indentify individual animals in social housing. In this study, we tried to monitor individual wheel running activity in individual or social housing using radio frequency identification (RFID) technology in laboratory rats. We also assessed brain monoamine levels, which are crucial for health benefits on brain, using high-performance liquid chromatography. Male Wistar rats were implanted with subcutaneous microchips, i.e., electronic identification devices, providing each animal with a unique identification number. Animals were randomly assigned to either individual or social living conditions, and were housed in plastic cages with an attached running wheel for 4 weeks. Each cage was equipped to monitor an individual animal's access to running wheel using microchip-scale system. Daily wheel revolutions were recorded digitally, and running distance was calculated by multiplying wheel circumference by the number of revolutions. Four weeks of spontaneous wheel running made differences in both daily running distance and brain monoamine levels between individual and social living conditions. These results suggest a possibility that this RFID monitoring system facilitates the collection of individual wheel running data of rats in social housing.

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Poster

527. Monoamines and Behavior: Serotonin and Histamine

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Title: A highly efficient approach for specific targeting of postnatal brain serotonin synthesis

Authors: *M. S. WHITNEY, E. DENERIS
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Abstract: Deficient serotonin (5-HT) signaling in the brain has been implicated in the pathogenesis of many devastating and highly prevalent psychiatric disorders. However, the importance of postnatal 5-HT for the maintenance of 5-HT neuron function and normal behavior is still poorly understood. This is partly due to the lack of specificity and efficiency of traditional techniques used to alter the 5-HT system in animal models. For example, many studies have employed genetic approaches to target transcription factors required for fetal 5-HT neuron development and function, which result in substantial decreases in the expression of *tryptophan hydroxylase 2* (*Tph2*), the rate-limiting enzyme in brain 5-HT synthesis, and deficiencies in 5-HT levels. However, those approaches also affect many other serotonergic genes not directly related to 5-HT synthesis, bringing into question the specific cause of the behavioral deficits seen in those models. Importantly, mouse models with germline targeting of *Tph2,* in which brain 5-HT synthesis is never initiated, have confirmed that 5-HT deficiencies beginning in early fetal life are sufficient to cause behavioral abnormalities in adulthood. Still, it remains to be determined if maintenance of postnatal 5-HT levels is critical for the functional integrity of 5-HT neurons and normal behavior. To address these fundamental questions, we have developed an efficient approach to decrease brain 5-HT synthesis, specifically, at different postnatal time points. *Tph2* expression is targeted by stereotaxic injection of a viral vector expressing Cre recombinase, AAV-Cre, into the dorsal raphe nucleus (DRN) of adult *Tph2fl/*Δ mice. This technique results in highly reproducible and severe losses of postnatal Tph2 and 5-HT. Representative targeting in the adult DRN resulted in a 91.8% (±0.9) decrease in *Tph2* mRNA, a 99.5% (±0.3) reduction in Tph2+ neurons, and a 93.9% (±1.9) decrease in forebrain 5-HT levels. Expression of aromatic L-amino acid decarboxylase, the other enzyme involved in 5-HT synthesis, was unchanged in AAV-Cre-targeted 5-HT neurons marked with Rosa-YFP, demonstrating that 5-HT neurons remain in normal numbers and loss of Tph2 and 5-HT are not due to 5-HT neuronal death. Preliminary data suggests the development of gene expression changes following chronic loss of 5-HT. Current studies are focused on investigating the impact of postnatal 5-HT deficiency on gene expression, anxiety-like behaviors, fear conditioning, behavioral inhibition, and the response to chronic stress.

Disclosures: M.S. Whitney: None. **E. Deneris:** None.

Poster

527. Monoamines and Behavior: Serotonin and Histamine

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Topic: C.18. Behavioral Pharmacology

Title: Stimulation of serotonin 5-ht3 receptors cause vomiting via the activation of ca/camkii-dependent erk1/2 signaling in the least shrew (cryptotis parva)

Authors: W. ZHONG, T. HUTCHINSON, S. CHEBOLU, *N. A. DARMANI
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Abstract: Activation of Ca^{2+}-permeable 5-HT_3 receptors (5-HT_3Rs) by the selective 5-HT_3 receptor agonist 2-methyl serotonin (2-Me-5-HT) can induce vomiting. We have previously demonstrated that L-type Ca^{2+} channel blockers, nifedipine and amlodipine or the selective 5-HT_3R antagonist palonosetron, suppress the induced vomiting in the least shrew. To clarify the contribution of Ca^{2+}-linked intracellular cascades in brainstem, we have examined the involvement of interaction of 5-HT_3R with calmodulin, Ca^{2+}/calmodulin-dependent protein kinase IIα (CaMKIIα) and extracellular signal-regulated kinase 1/2 (ERK1/2) signals in 2-Me-5-HT-induced emesis. Administration of 2-Me-5-HT (5 mg/kg, i.p.) significantly: i) enhanced the 5-HT_3R-calmodulin interaction in the brainstem as revealed by both immunoprecipitation, and colocalization in the area postrema by immunohistochemistry; and ii) activated CaMKIIα in brainstem as shown by Western blot and immunohistochemistry. These effects were suppressed by palonosetron pretreatment. Amlodipine also suppressed the 5-HT_3R-calmodulin interaction and CaMKIIα phosphorylation in the brainstem caused by 2-Me-5-HT. Furthermore, 2-Me-5-HT-induced emesis and CaMKIIα activation in the brainstem were blocked by dantrolene, an inhibitor of Ca^{2+}-release through ryanodine receptor channels in ER, but not by 2-APB, an inhibitor of ER inositol-1, 4, 5-triphosphate receptor. Inhibitors of CaMKII (KN93) and ERK1/2 (PD98059) dose-dependently suppressed emesis caused by 2-Me-5-HT. Moreover, 2-Me-5-HT activated ERK1/2 in the brainstem, which was abrogated by palonosetron, KN93, PD98059, amlodipine, and dantrolene. This study demonstrates that Ca^{2+} mobilization via extracellular Ca^{2+} influx through 5-HT_3Rs/L-type Ca^{2+} channels and intracellular Ca^{2+} release via RyRs on ER, initiate Ca^{2+}-dependent sequential activation of CaMKIIα and ERK1/2, which contribute to the 5-HT_3R-mediated, 2-Me-5-HT-evoked emesis.

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Poster

527. Monoamines and Behavior: Serotonin and Histamine

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Topic: C.18. Behavioral Pharmacology

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Bertil Hållsten's foundation (NA)

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Swedish brain foundation (NA)

Title: Anxiogenic-like effect of acute administration of two selective serotonin reuptake inhibitors - Repeated measurements of freezing behavior during presentation of brief acoustic stimuli

Authors: *S. M. HAGSÄTER, J. THORÉN, R. PETTERSSON, E. ERIKSSON
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Abstract: Objectives: Acute administration of selective serotonin reuptake inhibitors (SSRIs) may exacerbate anxiety. In a previous study we found an increase of "anxiety" after SSRI administration in a paradigm exploiting presentation of brief acoustic stimuli as an augmenter of freezing behavior. Hence, this paradigm appears to be feasible for investigations of agents with anxiogenic effects. In the current study we wanted to explore the repeatability of the animal response in this paradigm, and to what extent the baseline "anxiety" of the animals is of importance to the drug response. Animal response to two different SSRIs, escitalopram and paroxetine, was assessed. **Methods**: A total of 72 male Sprague Dawley rats were tested in two separate experiments. In both experiments repeated testing sessions consisting of 20 acoustic stimuli (0.2 s, 90 dB white noise bursts) with an interstimulus interval of 30 seconds were used. Experiment I: Rats (n=24) were tested on two separate days (day 1 and 18) in two identical sessions (no injections were given prior to testing). Experiment II: Rats (n=48) were first tested in one session (day 1) without injections similar to in experiment I. On day 5 and 18 half of the rats received a single injection of escitalopram (escitalopram oxalate, 10 mg/kg) one hour before testing. On day 12 (the same) half of the rats received a single injection of paroxetine (paroxetine hydrochloride, 10 mg/kg) one hour before testing. The remaining rats received 1 ml of 0.9% saline on day 5, 12 and 18. Effects of drug injections were analyzed separately for rats trichotomized in groups based on freezing response on day 1. **Results**: Experiment I: A strong

correlation was found for repeated testing in this paradigm ($r_{d1\text{-}d18} = .87$, $p_{d1\text{-}d18} < .0001$). Experiment II: For low anxiety rats (n=16) drug treated animals displayed increased freezing on all days versus control ($p_{d5} < .001$, $p_{d12} < .001$,$p_{d18} < .01$). Medium anxiety rats (n=16) displayed increased freezing on day 12 and day 18 ($p_5 = .42$, $p_{12} < .05$, $p_{18} < .01$). No significant effect of drugs was found for rats displaying high anxiety at baseline ($p_{d5} = .94$, $p_{d12} = .84$, $p_{d18} = .69$). **Conclusion**: As demonstrated by correlation analysis, freezing behavior measured during presentation of brief acoustic stimuli displays very high inter-test stability and repeatability for individual subjects. Acute administration of SSRI resulted in a pronounced increase in freezing for rats displaying low anxiety at baseline. Notably, there was no significant effect on freezing for animals displaying high anxiety at baseline.

Disclosures: S.M. Hagsäter: None. **J. Thorén:** None. **R. Pettersson:** None. **E. Eriksson:** None.

Poster

527. Monoamines and Behavior: Serotonin and Histamine

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 527.09/DD31

Topic: C.18. Behavioral Pharmacology

Support: BBSRC grant BB/J016756/1

Title: Increased impulsivity in the stop-signal reaction time task in a mouse model of Prader-Willi syndrome: Role of 5-HT2C receptors

Authors: J. R. DAVIES[1], T. HUMBY[3], L. S. WILKINSON[3], *A. R. ISLES[2]
[1]MRC Ctr. for Neuropsychiatric Genet. and Genomics, [2]Psychological Med., Neurosci. and Mental Hlth. Res. Inst., Cardiff, United Kingdom; [3]Sch. of Psychology, Cardiff Univ., Cardiff, United Kingdom

Abstract: Prader-Willi syndrome (PWS), is a neurodevelopmental disorder caused by deletion or inactivation of paternally expressed imprinted genes on human chromosome 15q11-q13, leads to an array of disabilities including compulsive hyperphagia and deficits in social behaviour, such as social withdrawal, temper tantrums, perseverative speech and behaviour, mental rigidity, stereotyped behaviour and impulsiveness. The PWS genetic interval contains several brain-expressed small nucleolar (sno)RNA species that are subject to genomic imprinting. One of these, *snord115*, negatively regulates editing and alternative splicing of the 5-HT_{2C} receptor

(*5htr2c*) pre-RNA which is thought to play an important role in regulation of behavioural inhibition and impulsivity[1,2]. In this study we have used the imprinting centre (IC) deletion mouse model for PWS (PWS-IC(+/-)), which results in a loss of all paternal gene expression for the PWS interval[3], including *snord115*, which leads to an increase in abundance of the less functional isoforms of *5htr2c*. Our previous results showed no effect of IC deletion on premature responding in the five-choice serial reaction time task (5-CSRTT)[4], however, an enhanced increase in impulsivity was seen relative to controls when the behaviour was probed with 5-HT_{2C} selective drugs. More recent studies have identified the contribution of 5-HT_{2C} receptors to inhibitory control, in tasks assessing impulsive action, such as the stop-signal reaction time task (SSRTT)[2]. In the SSRTT, control of responding was manipulated by altering the onset of an auditory 'stop-signal' during the go response. All mice showed the anticipated increases in impulsive responding as the stop-signal was moved closer to the end of the go response, but PWS-IC(+/-) mice showed increased impulsivity relative to wild-type controls at all stop-signal positions. Studies are on-going to investigate the effects of manipulating this behaviour with selective agonists and antagonists of the 5-HT_{2C} receptor. These data, in comparison with our previous work[3], suggest specific facets of inhibitory behaviour may be impaired in PWS-IC(+/-) mice and that *snord115* via its actions on the 5-HT_{2C} receptor gene may mediate this dissociation. **References** 1. Winstanley et al 2004 *Psychopharmacology* 176, 376-85 2. Humby et al. 2013 *Neuropsychopharmacology*.38, 2150-9. 3. Doe et al 2010 *Human Molecular Genetics*18, 2140-8

Disclosures: J.R. Davies: None. **T. Humby:** None. **L.S. Wilkinson:** None. **A.R. Isles:** None.

Poster

527. Monoamines and Behavior: Serotonin and Histamine

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Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 527.10/DD32

Topic: C.18. Behavioral Pharmacology

Support: NIMH Grant R01MH097718

Title: The roles of 5-Hydroxytryptamine 2A and 2C receptors in maternal behaviors in rats

Authors: *J. GAO, M. LI
Dept. of Psychology, Univ. of Nebraska-Lincoln, Lincoln, NE

Abstract: Maternal behavior in rats is a highly motivated and well-organized social behavior. Recently, we found a disruptive effect of 5-HT2C receptor agonism (but not 5-HT2A receptor antagonism) on maternal behavior in rats. In the present study, we explored how activation of 5-HT2A alone or both 5-HT2A and 5-HT2C receptors may influence the expression of maternal behavior. In addition, we examined the neuroanatomical basis of the central action of 5-HT2C receptor blockade on maternal behaviors. In Experiment 1, using a within-subject design, we examined the roles of 5-HT2A agonist and 5-HT2C antagonist on maternal behaviors (n=8 for each drug). On postpartum Days (PND) 4, 6, 8, and 10, Sprague-Dawley mother rats were injected with the 5-HT2A agonist TCB-2 (0, 1.0, 2.5 or 5.0 mg/kg, sc), or the 5-HT2C antagonist SB242084 (0, 0.2, 0.6 or 1.0 mg/kg, sc). Maternal behavior was tested for 10 min at 30 min before and 30 min, 120 min, 24 h after injection. TCB-2 (not SB242084) dose-dependently disrupted pup retrieval, pup licking, pup nursing, and nest building. In Experiment 2, using a between-subject design, we examined the roles of 5-HT2A agonism and the combined agonism on 5-HT2A and 5-HT2C. On PND 4, 6, 8, and 10, separate groups of mother rats were injected with saline, TCB-2 (2.5 mg/kg, sc), or the combined TCB-2 (2.5 mg/kg) and MK212 (5-HT2C agonist, 1.0 mg/kg), sc. Maternal behavior was tested at 30 min before and 30, 90, and 150 min after injection. Injection of TCB-2 or TCB-2+MK212 significantly disrupted various maternal behaviors. There appeared to be an additive effect in the TCB-2+MK212 condition. There was no apparent change in the magnitude of the disruptive effect of TCB-2 and TCB-2+MK212 across the test days (no sensitization or tolerance). In Experiment 3, 6 female rats were tested for their maternal behaviors at 10 min after central infusion of saline (on PND 3), or MK212 at 25, 75, or 250 ng/side (on PND 5, 7, and 9 respectively) into the nucleus accumbens shell (NAs). In Experiment 4, 12 rats were tested at 10 min and 60 min after infusion of saline, MK212 25, 250 ng/side, or 1.0, 2.0, 5.0 ug/side into medial prefrontal cortex (mPFC) on PND 4, 6, 8, and 10. Results show that intra-NAs or intra-mPFC MK212 infusion had no effect on various maternal behaviors. These findings suggest that activation of either 5-HT2A or 5-HT2C receptor has a detrimental effect on rat maternal behavior. The central action of 5-HT2C activation does not appear to occur in the NAs and mPFC. Future work will further investigate the neuroanatomical basis of 5-HT 2A/2C agonists and identify the antagonistic action between these two receptor systems.

Disclosures: **J. Gao:** None. **M. Li:** None.

Poster

527. Monoamines and Behavior: Serotonin and Histamine

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 527.11/EE1

Topic: C.18. Behavioral Pharmacology

Title: Monoamine precursor injections influence individual difference of spontaneous physical activity in rats

Authors: *S. YANAGITA[1], N. KUBOTA[1], Y. TAKANO[1], T. MATSUZAWA[1], T. ISHIWATA[2], K. TAKEDA[1]
[1]liberal arts, Tokyo Univ. of Sci., Chiba, Japan; [2]Rikkyo Univ., Saitama, Japan

Abstract: Daily amount of physical activity is an important factor to confer the physiological and psychological health benefits. Spontaneous wheel running is a common physical exercise model in order to enhance physiological and psychological health in rodents. It is well known that daily spontaneous wheel running distance gradually increases over several weeks from the starting day of running. We have investigated a novel methodological target to increase physical activity in these rat models. In the previous study, we showed that spontaneous activity in running wheel was implicated in the levels of brain serotonin (5-HT) and dopamine, and suggesting that the change of the balance of brain 5-HT and dopamien contents could regulate daily amount of physical activity. In this study, we examined the effects of 5-HT and dopamine precursor injections on daily spontaneous activity in physically active rats. Male Wistar rats were housed individually in cages with or without an attached running wheel and were randomly assigned to either physically active or sedentary conditions. Physically active rats were allowed voluntary access to their wheels for 4 weeks. After 3 weeks from the start of running, half the number of the rats in each group was injected 5-HT precursor, 5-Hydroxytryptophan (5-HTP), or dopamine precursor, levodopa (L-Dopa), every other day for a week (i.p. 60mg/g). We also assessed the levels of brain serotonin, dopamine, and its metabolite using HPLC. The results indicated that there is no significant difference between 5-HTP injected rats and vehicle control on the daily spontaneous wheel running distance in physically active conditions. The brain levels of 5-HT did not be influenced, although the levels of 5-HT metabolites and turn over tended to be increased by 5-HTP injections. Continuous L-Dopa injections influenced daily individual difference of running distance in physically active rats. Present results indicate that physically active state did not be regulated by only 5-HT contents, and lead to a hypothesis that the daily amount of physical activity could be regulated by the change of balance of brain 5-HT and dopamine contents.

Disclosures: S. Yanagita: None. **N. Kubota:** None. **Y. Takano:** None. **T. Matsuzawa:** None. **T. Ishiwata:** None. **K. Takeda:** None.

Poster

527. Monoamines and Behavior: Serotonin and Histamine

Location: Halls A-C

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Program#/Poster#: 527.12/EE2

Topic: C.18. Behavioral Pharmacology

Support: P51OD011132

5T32DA015040-10

Title: Mechanisms of 3,4-methylenedioxymethamphetamine(MDMA)-induced enhancement of prosocial behavior and sensitization in mice

Authors: *D. W. CURRY[1,4], A. BELKOFF[2,4], L. L. HOWELL[3,4]
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[4]Neuropharm. and Neurologic Dis., Yerkes Natl. Primate Res. Ctr., Atlanta, GA

Abstract: S,R(+/-)-3,4-methylenedioxymethamphetamine (MDMA) is an amphetamine derivative that became popular as a recreational drug (ecstasy) and therapeutic tool during the 1970's and early 1980's. Escalating use led to its prohibition but scientific interest in the drug has persisted due to its unique prosocial effects. Under clinical observation, volunteers report that MDMA increases feelings of closeness towards others, empathy, and gregariousness. In addition to these acute effects, there is evidence of enduring therapeutic effects such as improved interpersonal functioning and significant symptom reduction in PTSD patients. Ongoing studies are now investigating its potential for treating adults with autism. However, serious limitations remain to wider clinical use of MDMA, particularly its suspected neurotoxicity and history as an abused substance. Thus, there is significant impetus to determine the mechanisms that underlie MDMA's prosocial and therapeutic effects so that new medications can be developed that isolate and more safely harness these effects. We found that racemic MDMA (7.8 mg/kg) robustly increases social interaction in male mice and that subsequent treatments lead to a sensitization of this effect, indicating that neural adaptations are taking place. These adaptations may explain MDMA's long term therapeutic effects, but the mechanism remains unknown. Recent studies have suggested that 5-HT2A receptor activation may be necessary for sensitization to occur. We found that mice treated with the selective 5-HT2A antagonist M100907 (0.3 mg/kg) prior to MDMA during 3 conditioning sessions showed normal sensitization when test 48 hours later with a novel conspecific in a social interaction test. Only a high (1 mg/kg) dose disrupted sensitization to MDMA's prosocial effects, indicating that there may be important off-target effects that are responsible for blocking sensitization. When given as a pretreatment on test day,

neither dose decreased the acute prosocial effects of MDMA. *In vivo* microdialysis was used to determine the neurochemical sensitization to MDMA and the effect of M100907 pretreatment on dopamine and serotonin release. These results indicate that activation of 5-HT2A receptors is not necessary for the acute prosocial effects of MDMA and that their role in sensitization is unclear. Because MDMA is both a widely used drug of abuse and a potential therapeutic, there is great impetus to better understand its acute and long-term behavioral and neurobiological effects. Ongoing studies are investigating the importance of other receptors including 5-HT1A, 5-HT1B, and oxytocin receptors.

Disclosures: D.W. Curry: None. **A. Belkoff:** None. **L.L. Howell:** None.

Poster

527. Monoamines and Behavior: Serotonin and Histamine

Location: Halls A-C

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Program#/Poster#: 527.13/EE3

Topic: C.18. Behavioral Pharmacology

Support: PROLAB LARC/IBRO/CNPq- Brasil/Colômbia

Title: 5-HT2A receptors expression in prelimbic cortex of rats selectively breed for high and low anxiety traits

Authors: *L. A. LEÓN[1], F. P. CÁRDENAS[2], S. ZARATE[2], V. C. GOMES[3], J. LANDEIRA-FERNANDEZ[4]
[1]Physics/Chemistry, Univ. of Sao Paulo, Ribeirao Preto, Brazil; [2]Univ. de los Andes, Bogotá, Colombia; [3]Univ. Federal de São João Del-Rei, São João Del-Rei, Brazil; [4]Pontificia Univ. Catolica de Rio de Janeiro, Rio de Janeiro, Brazil

Abstract: Introduction: Genetic vulnerability to stress is considered to be a high risk factor in the development of psychopathologies, such anxiety disorders and depression. Several data have consistently showed the involvement of serotoninergic (5HT2A) neurotransmission of limbic structures in the genesis of such disorders. However, there are few studies exploring changes in the expression of 5HT2A receptors in the prelimbic cortex in the eclosion of anxiolytic-like or ansiogenic-like behavior. Importantly, the activity of 5HT2A receptors in this region is considered to be associated with structures responsible for the activation of defensive responses, like the amygdaloid complex. Methods: In the present study, we employed as subjects male rats selectively breed for High (n=12) and Low (n=12) conditioned freezing in response to contextual

cues previously associated with footshocks, animals that are known as the Carioca high-and low-conditioned freezing (CHF and CLF) rats. Recent reports have consistently showed that these lines present marked differences in both learned and innate fear responses and in several biological markers of anxiety. Also they had showed differences in the anxiolytic-like response when tested with the antagonist of 5HT2A receptors. In this sense, we evaluated the amount of 5HT2A receptors in the prelimbic cortex of both lines, after submitted them to the contextual fear conditioning protocol. Results: Our preliminary results indicate significant differences between the two lines, CHF and CLF rats, in the 5HT2A receptors expresion in the prelimbic cortex than CLF animals. This results support previous findings where the antagonist of 5HT2A receptor, ketanserin, showed differential effects for both lines of rats. These findings will help us in the understanding of neurobiological mechanisms that underlies vulnerability to stress.

Disclosures: L.A. León: None. **F.P. Cárdenas:** None. **V.C. Gomes:** None. **J. Landeira-Fernandez:** None. **S. Zarate:** None.

Poster

527. Monoamines and Behavior: Serotonin and Histamine

Location: Halls A-C

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Program#/Poster#: 527.14/EE4

Topic: C.18. Behavioral Pharmacology

Support: U.S. Public Health Service Grant DA012514

Office of Research Infrastructure Programs grant OD P51OD011132

Title: Mdma increases prosocial behavior and vocalizations in squirrel monkeys

Authors: *E. G. PITTS, M. T. LOGUN, L. L. HOWELL
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Abstract: Several mental illnesses include disruption of social interactions, including autism, post-traumatic stress disorder (PTSD), and drug dependence. 3,4-methylenedioxy-N-methylamphetamine (MDMA) has been show to increase feelings of openness and trust, positive emotions, and sociability. Recently, MDMA has been examined as a therapeutic adjunct in the treatment of PTSD, with promising results. Yet, the mechanisms underlying the prosocial effects of MDMA are still not known. We are using a group housed non-human primate model to examine the prosocial effects of MDMA. Squirrel monkeys metabolize MDMA in a manner

similar to humans and provide a strongly translational model in which to examine the mechanisms underlying the prosocial effects of MDMA. We administered MDMA (racemic and its enantiomers) or saline intramuscularly to four group housed male squirrel monkeys. We then used a behavioral ethogram and vocalizations to examine the effects on behavior. We have found that racemic MDMA causes dose-dependent increases in affiliative behavior and vocalizations. The enantiomers of MDMA are known to have different neurotransmitter release and receptor binding profiles. We have also found that, although the enantiomers have different behavior profiles, they both increase prosocial behaviors. Using this established non-human primate model, we will be able to pharmacologically examine the mechanisms mediating the prosocial effects of MDMA.

Disclosures: E.G. Pitts: None. **M.T. Logun:** None. **L.L. Howell:** None.

Poster

527. Monoamines and Behavior: Serotonin and Histamine

Location: Halls A-C

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Program#/Poster#: 527.15/EE5

Topic: C.18. Behavioral Pharmacology

Support: FAPESP-Process Number 2013/03065-6

Title: Role of 5-HT1A receptors located in the dorsal sub-region and lateral wings of the dorsal raphe nucleus in the modulation of inhibitory avoidance and escape behaviors

Authors: *R. L. POBBE, A. SPIACCI JR, H. ZANGROSSI JR
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Abstract: A wealth of evidence indicates that the dorsal raphe nucleus (DR) is not a homogenous structure, but an aggregate of different subpopulations of serotonergic and non-serotonergic neurons that are morphologically and functionally distinct. This study was performed to assess the effects of the pharmacologic manipulation of 5-HT1A receptors placed within the dorsal sub-region (DRd) and the lateral wings (lwDR) of the DR on the expression of defensive responses generated by the elevated T-maze. This model allows the measurement, in the same rat, of two subtypes of anxiety-related responses, inhibitory avoidance and escape. Male Wistar rats were tested in the elevated T-maze ten minutes after intra-DRd or intra-lwDR administration of different doses of the 5-HT1A receptor antagonist WAY-100635, and of the 5-HT1A receptor agonist 8-OH-DPAT. The results showed that intra-DRd infusion of WAY-

100635 facilitated inhibitory avoidance acquisition, and impaired escape expression; interestingly, intra-lwDR administration of this same antagonist impaired escape performance and exerted no effect on inhibitory avoidance acquisition. In addition, intra-DRd injection of 8-OH-DPAT impaired inhibitory avoidance acquisition, and facilitated escape expression, while the infusion of this agonist into the lwDR facilitated escape response and did not alter the avoidance task. Overall, our results indicate that whereas 5-HT1A receptors in the DRd exert opposed control on inhibitory avoidance and escape behaviors, the pharmacologic manipulation of 5-HT1A receptors located in the lwDR preferentially alters the latter response.

Disclosures: R.L. Pobbe: None. **A. Spiacci Jr:** None. **H. Zangrossi Jr:** None.

Poster

527. Monoamines and Behavior: Serotonin and Histamine

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 527.16/EE6

Topic: C.18. Behavioral Pharmacology

Title: Wfs1-deficient mice display altered glycemic control and immobility behaviour in response to antidepressants treatment

Authors: *R. REIMETS, M. LOOMETS, M. PLAAS, S. RAUD, T. VISNAPUU, E. VASAR
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Abstract: Mutations in the WFS1 gene predispose to type 2 diabetes and they also make humans more susceptible to mood disorders. On the other hand, there is a strong association between antidepressant treatment and the risk for diabetes. The aim of this study was to investigate the behavioral and metabolic effects of three classes of antidepressants in Wfs1-deficient mice. Namely, the tricyclic antidepressants (TCA) desipramine and amitriptyline, the selective serotonin reuptake inhibitor (SSRI) escitalopram and the atypical antidepressant bupropion were studied in terms of immobility behaviour in the tail suspension test (TST) and blood glucose levels in the glucose tolerance test (GTT). Results showed that the basal time spent immobile in the TST by Wfs1-deficient mice did not differ from that of their heterozygous or wild-type littermates. Moreover, the doses of desipramine, amitriptyline and escitalopram necessary to significantly reduce immobility time compared to drug-naive animals of the same genotype, were lower in homozygous and heterozygous mice than in their wild-type littermates. In the GTT, homozygous Wfs1-deficient mice exhibited impaired glucose tolerance. The hyperglycemic effect was aggrevated by both of the tested TCA-s, causing more severe and

long-lasting hyperglycemia and therefore indicating changes in monoamine signaling caused by Wfs1-deficiency which is not specific to the central nervous system. By contrast, the glucose tolerance of Wfs1-deficient mice was not affected by escitalopram. Together, results from this study indicate that, compared to other classes of antidepressants, SSRI-s might be more suitable for alleviation of depressive symptoms in patients with WFS1 mutations because they did not worsen deficits in glycemic control.

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Poster

527. Monoamines and Behavior: Serotonin and Histamine

Location: Halls A-C

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Program#/Poster#: 527.17/EE7

Topic: C.18. Behavioral Pharmacology

Support: American Pain Society Award in Chronic Pain

Duquesne University Faculty Development Fund

Title: Characterization of serotonin receptor subtype 2C (5-HT2C) in pain and depression using novel compounds derived from marine cyanobacteria

Authors: *N. C. LAX, C. M. IGNATZ, K. AHMED, K. J. TIDGEWELL, B. J. KOLBER
Duquesne Univ., Pittsburgh, PA

Abstract: Chronic pain and major depressive disorder are widespread conditions in the United States. Interestingly, these conditions often occur comorbidly, with each individual disease amplifying the symptoms of the other. Many medications available on the market today for treating pain or depression target G-protein coupled receptors (GPCRs), implying that this class of receptors may be involved in the development of the comorbidity of these conditions. Our lab has sought to characterize a poorly understood GPCR, the serotonin 2C (5-HT2C) receptor, and the role that it plays in comorbid pain and depression. Our approach for targeting this receptor uses compounds isolated from filamentous marine cyanobacteria collected off of the coast of Panama in the Pacific Ocean. Compounds from this cyanobacterial collection show strong affinity for the 5-HT2C receptor. These compounds were screened for *in vivo* activity using a series of pain and depression behavioral assays. Compounds were delivered in male C57Bl/6J

mice via intra-cerebroventricular (ICV) cannulas. Compounds were tested in naïve mice or in mice subjected to a model of comorbid pain and depression, the Spared Nerve Injury (SNI) surgery. SNI surgery involves ligating two of the three branches of the sciatic nerve, the tibial and common peroneal branches, while leaving the third branch, the sural branch, intact. SNI surgery induces mechanical hypersensitivity in the ipsilateral paw (modeling pain) and also induces depression-like behavior. We have found that ICV injections of the compound isolated from the marine cyanobacterium induce effects in several standard behavioral assays. Our results suggest that the 5-HT2C receptor may be a key target in the future development of compounds used to treat comorbid pain and depression.

Disclosures: **N.C. Lax:** None. **C.M. Ignatz:** None. **K. Ahmed:** None. **K.J. Tidgewell:** None. **B.J. Kolber:** None.

Poster

528. Drug Discovery and Development: Neurodegenerative Diseases II

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 528.01/EE8

Topic: C.19. Drug Discovery and Development

Support: HHSN271200800033C

Title: The national institute for neurological disorders and stroke (ninds) repository biomarker discovery collection is a public resource of biomaterials for neurodegenerative disorders

Authors: *G. BALABURSKI[1], S. HEIL[1], A. GREEN[1], C. KOPIL[2], M. FRASER[2], M. SUTHERLAND[3], K. GWINN[3], R. CORRIVEAU[3], C. TARN[1]
[1]Coriell Inst. For Med. Res., Camden, NJ; [2]Michael J Fox Fndn., New York, NY; [3]Natl. Inst. for Neurolog. Disorders and Stroke, Bethesda, MD

Abstract: Neurological diseases are devastating to patients and their families and present significant research and diagnostic challenge. Biomarkers for diagnosis, disease onset, disease progression and therapeutic response are urgently needed to improve diagnostic and clinical outcomes of patients with neurodegenerative diseases such as Parkinson's and Huntington's disease. The mission of the NINDS Repository is to provide genetic support for scientists investigating the pathogenesis of the central and peripheral nervous systems, and accelerate discovery of causes and risks of neurological disease by sharing biomaterials and de-identified clinical data. The NINDS Repository is an integral component in the effort to identify and

validate biomarkers of neurological disorders by the virtue of being a centralized facility for storage, processing, and distribution of biofluids (cerebrospinal fluid, plasma, serum, whole-blood, urine) and nucleic acids (DNA and RNA). To harmonize sample collection across different clinical sites and minimize pre-analytical variables, the NINDS Repository: (i) ensures that samples collected for biomarker discovery are of premier quality by collaboratively establishing unified standards for sample collection; (ii) provides rapid feedback to clinical sites regarding sample appearance and quality; (iii) maintains secure, high quality sample storage conditions with real-time monitoring and recording systems and(iv) performs standardized laboratory processing and quality assurance using validated operating procedures. To further enhance the investigation and discovery of novel biomarkers the NINDS Repository is establishing large, long term longitudinal collections of biological samples obtained from affected and neurologically healthy individuals. Currently the NINDS Repository collects samples under multiple NINDS sponsored biomarker initiatives such as the Parkinson's Disease Biomarkers Program (PDBP), the Frontotemporal Degeneration (FTD) MAPT Carrier (FTD-MAPT) study, the Neurobiological Predictors of Huntington's Disease study (PREDICT-HD), as well as a jointly sponsored study (BioFIND) on Parkinson's Disease in collaboration with the Michael J. Fox Foundation. Thus, the NINDS Repository is a vital resource for research designed to discover and validate biomarkers of neurological disorders. Biomarker discovery samples are available upon request either directly from the NINDS Repository web catalog (http://ccr.coriell.org/NINDS), or via NIH-sponsored resources with links to the online catalog

Disclosures: **G. Balaburski:** None. **S. Heil:** None. **A. Green:** None. **C. Kopil:** None. **M. Fraser:** None. **M. Sutherland:** None. **K. Gwinn:** None. **R. Corriveau:** None. **C. Tarn:** None.

Poster

528. Drug Discovery and Development: Neurodegenerative Diseases II

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Program#/Poster#: 528.02/EE9

Topic: C.19. Drug Discovery and Development

Support: NIH R01NS087611 to ANM

Seahorse Bioscience to ANM

NIH P30DK063491 to CMM

Title: Oxidation of alternative energy substrates in response to reduced mitochondrial pyruvate transport is experimentally neuroprotective

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Abstract: The persistent lack of effective therapies for chronic forms of neurodegenerative disease reinforces the need to explore novel approaches. To that end, a growing body of data provides a strong rationale for improvement of brain energy metabolism as a general approach. We find that neuronal energy metabolism can be improved through metabolic conditioning, and this conditioning can be achieved through mild inhibition of certain catabolic pathways. Specifically, pharmacologic inhibition of mitochondrial pyruvate uptake can acutely increase plasma membrane glucose uptake and enhance the oxidation of ketone bodies and branched chain amino acids. This alternative substrate oxidation can potentiate metabolic flexibility and resistance to excitotoxic injury of cortical neurons. Addition of beta-hydroxybutyrate +/- leucine to the culture medium provided roughly 25% protection from excitotoxic neuronal death, and inhibition of the pyruvate carrier further increased neuroprotection to about 50%. In experiments with cultured cells using metabolic flux analysis, chronic genetic and pharmacological inhibition of mitochondrial pyruvate uptake potentiates the rewiring of cellular metabolism, increasing glutamine and fatty acid oxidation to meet cellular energetic demands and increasing NAD(P)H production via malic enzyme. The data suggest that mild inhibition of substrate transport or oxidation is experimentally neuroprotective and may be a therapeutic target for the treatment neurodegenerative disease.

Disclosures: **A.S. Divakaruni:** F. Consulting Fees (e.g., advisory boards); Seahorse Bioscience. **A.Y. Andreyev:** None. **T.P. Ciaraldi:** None. **C.R. Green:** None. **M. Wallace:** None. **C.M. Metallo:** None. **A.N. Murphy:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Seahorse Bioscience. F. Consulting Fees (e.g., advisory boards); Seahorse Bioscience.

Poster

528. Drug Discovery and Development: Neurodegenerative Diseases II

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 528.03/EE10

Topic: C.19. Drug Discovery and Development

Support: NINDS 1R43NS076060 (CBB)

Title: Validation of a high content screening system to develop therapeutics for enhancing OPC differentiation

Authors: ***J. S. LUNN**[1], M. TORRES-CASTILLO[1], S. MEDICETTY[1], B. TRAPP[2], B. BAI[1], B. BAI[1]
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Abstract: Multiple Sclerosis (MS) is a chronic inflammatory demyelinating disease of the central nervous system (CNS). The majority of current therapies focus solely on modulating the immune system but do not address remyelination. The endogenous oligodendrocyte progenitor cells (OPC) within the brain are capable of spontaneous remyelination, but despite abundant numbers, eventually this regenerative mechanism also fails. The presence of the endogenous OPC population provides us an opportunity for therapeutic intervention in MS. Stimulation of OPC proliferation and differentiation could enhance long term recovery from MS injury. We have developed a high content screening method to test the capacity of small molecules to induce OPC differentiation using primary OPC cultures from transgenic animals expressing EGFP downstream of the PLP promoter. OPCs treated with T3 showed 2.8 fold increase in EGFP positive differentiated oligodendrocytes compared to DMSO. Densitometric analysis of western blots demonstrated a similar (2.3 fold) increase in PLP protein levels in OPCs treated with T3 when compared to DMSO controls. Furthermore, OPCs treated with T3 demonstrated increased phenotypic differentiation when immunolabled for PLP and MBP demonstrating robust oligodendrocyte differentiation. Together our data demonstrates that the expression of the PLP:EGFP transgene is a valid method to accurately represent the extent of differentiation in our OPC culture system to identify and evaluate new therapeutic molecules for MS.

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Poster

528. Drug Discovery and Development: Neurodegenerative Diseases II

Location: Halls A-C

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Program#/Poster#: 528.04/EE11

Topic: C.19. Drug Discovery and Development

Title: Targeting extracellular cyclophilin A-mediated neurovascular dysfunction

Authors: *R. D. BELL[1], G. SUIDAN[2], A. CAMERON[2], M. ILARDI[2], N. KABLAOUI[2], K. FONSECA[2]
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Abstract: The neurovascular unit (NVU) is composed of endothelium, pericytes, vascular smooth muscle cells, perivascular macrophages and glial cells. Cellular communication within the NVU controls blood-brain barrier (BBB) integrity, cerebral blood flow and the chemical composition of the CNS required for proper functioning of neuronal circuits. Importantly, NVU dysfunction has been implicated in many CNS disorders, including Alzheimer's disease, multiple sclerosis, stroke and schizophrenia. Cyclophilin A (CypA), a ubiquitously expressed and abundant cytosolic protein that plays an essential role in the immunosuppressant effect of cyclosporine A has been linked to vascular dysfunction and neurodegeneration. Preclinical data in APOE4 transgenic mice hows that elevated CypA causes significant NVU dysfunction and BBB damage, primarily due to NFκB-MMP9 mediated degradation of basement membrane and tight junction complexes as wellas direct neurovascular cell death. Loss of function of CypA via a conventional genetic knockout strategy or acute treatment with cyclosporine A (CsA) provides a significant degree of protection against APOE4-based neurovascular pathology. Recently, it has been demonstrated that increased extracellular CypA (eCypA) is found in the circulation of patients with vascular diseases, such as hypertension and diabetes, as well as in the cerebrospinal fluid of APOE4 carriers. Moreover, eCypA is actively secreted from vascular and white blood cells during various pathologic conditions and mediates pro-inflammatory signaling cascades. However, the respective contribution of extracellular versus intracellular CypA mediating neurovascular damage remains elusive. Here, we show eCypA is significantly elevated in the plasma and cerebral spinal fluid (CSF) of CD-1 mice following a lipopolysaccharide (LPS)-induced model of systemic inflammation and BBB damage. We then demonstrate that pharmacological inhibition of eCypA using a published cell impermeable CsA analog following LPS administration improves BBB integrity. More specifically, the CSF to plasma albumin quotient as well as fibrinogen extravasation into brain parenchyma are both significantly reduced following eCypA inhibition in LPS-treated mice. In summary, accumulating evidence suggests that inhibition of eCypA may be a novel strategy to improve the neurovascular dysfunction associated with CNS disorders.

Disclosures: **R.D. Bell:** A. Employment/Salary (full or part-time):; Pfizer Inc. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Pfizer Inc. **G. Suidan:** None. **A. Cameron:** None. **M. Ilardi:** None. **N. Kablaoui:** None. **K. Fonseca:** None.

Poster

528. Drug Discovery and Development: Neurodegenerative Diseases II

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Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 528.05/EE12

Topic: C.19. Drug Discovery and Development

Title: Transgenic mice that over-express NAAA in microglia: A tool to understand the role of NAAA in neuroinflammation

Authors: *A. RIBEIRO, S. PONTIS, A. GUIJARRO, D. PIOMELLI
Fondazione Inst. Italiano Di Tecnologia, Genova, Italy

Abstract: N-acylethanolamine acid amidase (NAAA) is a lipid amidase that preferentially hydrolyzes palmitoylethanolamide (PEA), an endogenous PPAR-alpha agonist with marked analgesic, anti-inflammatory and neuro-protective properties. Although the anti-inflammatory effects of PEA have been investigated, the role of NAAA in inflammatory diseases remains unclear. To fill this knowledge gap, we generated a knock-in (ki) mouse with conditional NAAA overexpression in macrophages and microglia. The coding sequence of NAAA isoform 1 was inserted within the Rosa26 locus, under the control of the ubiquitous CAGG promoter. A floxed (loxP flanked) transcriptional STOP cassette was incorporated between the transgene and the CAGG promoter. The resulting model is a NAAA conditional ki heterozygous mouse in which the expression of the transgene is dependent upon Cre recombinase. To achieve NAAA overexpression in microglia and macrophages, ki mice were crossed with CD11b-Cre transgenic mice [B6.Cg-Tg(ITGAM-cre)2781Gkl/Flmg; obtained from the Alexander Fleming Institute (Athens, GC) through the EMMA-European Mouse Mutants Archive]. Mice were genotyped using a downstream of the short homology arm and upstream of the NAAA-pA transgene sequence. For characterization of mice phenotype (n=5) we analyzed NAAA mRNA levels by qRT-PCR in the spinal cord, lungs, spleen, and liver of knock-in mice compared to wild-type (wt) littermates. Additionally, we analyzed NAAA protein expression in the spinal cord of ki by immunohistochemistry. The levels of NAAA mRNA were higher in ki mice than in wt littermates: 1.7-fold increase in the spinal cord, 1.9-fold increase in the lungs, 1.7-fold increase in the spleen, and 3.1-fold increase in the liver. Immunofluorescence analysis showed an increase in NAAA expression in Iba1-positive cells (microglia) in spinal cord of ki mice compared to wt littermates. Moreover, in ki mice it is possible to individualize two morphologically distinct types of Iba1-positive cells: (1) activated-like microglia expressing high levels of NAAA, which show large and ameboid shape; and (2) non-activated microglia expressing lower levels of NAAA with characteristic ramified form. Only the latter cell type is observed in wt littermates.

The generation of a mouse strain over-expressing NAAA in microglia opens new avenues to understand the role of this enzyme in inflammatory diseases of the central nervous system.

Disclosures: **A. Ribeiro:** None. **S. Pontis:** None. **A. Guijarro:** None. **D. Piomelli:** None.

Poster

528. Drug Discovery and Development: Neurodegenerative Diseases II

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Program#/Poster#: 528.06/EE13

Topic: C.19. Drug Discovery and Development

Support: Boehringer Ingelheim Ulm University BioCenter (BIU)

Title: Comparative evaluation of target engagement for monoacylglycerol lipase (MAGL) inhibitors and cyclooxygenase (COX) inhibitors: Impact of tandem mass spectrometry (LC-MS/MS) and automated behavioral analysis in mice

Authors: ***C. PORAZIK**[1,2], A. WITTING[2], B. FERGER[1]
[1]CNS Dis. Res., Boehringer Ingelheim Pharma GmbH & Co. KG, Biberach, Germany; [2]Dept. of Neurol., Ulm Univ., Ulm, Germany

Abstract: Inhibition of the endocannabinoid metabolism of 2-arachidonoylglycerol (2-AG) by monoacylglycerol lipase (MAGL) has been studied in various animal models to elucidate the role of endocannobinoids (eCB) in disorders such as cancer, pain and neurodegenerative diseases. Newer findings suggest a prominent role for MAGL also in regulation of brain prostaglandin levels. Here, we investigate the effects of the commercially available MAGL inhibitors JZL184, KML29 and MJN110 on target engagement and behavior in comparison to the cyclooxygenase (COX) 1 selective inhibitor SC560, the COX2 selective inhibitors refecoxib and celecoxib and the non-selective COX1/2 inhibitor ibuprofen. We set up and validated a sensitive liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) method to determine levels of 2-arachidonoylglycerol (2-AG) and anandamide (AEA) as well as arachidonic acid (AA) and prostaglandins (PGs) PGE_2, PGD_2, $PGF_{2\alpha}$ and TXB_2 out of different matrices. Male adult C57BL/6JRj mice were treated with MAGL and COX inhibitors (30 mg/kg) or vehicle. Brains and peripheral organs were removed 60 min after treatment and tissues were processed for LC-MS/MS analysis. Additionally, MAGL and COX inhibitors were analyzed in a separate study to investigate their effects on potential motor behavior impairment using an automated system to quantify rearing behavior and distance travelled in an open field by

interruptions of light beam arrays. The MAGL inhibitors significantly increased 2-AG levels and decreased AA and PGs in the brain and peripheral tissues. COX inhibition reduced PGs but had no effect on 2-AG and AA levels. Notably, SC560 was most effective in reducing PGs levels in the brain. Several compounds of this study showed a time-dependent hypomotility in the motor activity analysis. Interestingly, MJN110 which was very potent in increasing brain 2-AG did not show motor impairment in the behavioral observation test. In conclusion, we set up a reliable LC-MS/MS biomarker method to quantify changes in brain and peripheral tissue in the endocannabinoid and eicosanoid pathway induced by MAGL and COX inhibitors. These changes are indicative for target engagement and will help to elucidate the role of endocannabinoids in neurodegenerative diseases.

Disclosures: C. Porazik: None. **A. Witting:** None. **B. Ferger:** A. Employment/Salary (full or part-time):; B.F. is a full time employee of Boehringer Ingelheim Pharma GmbH & Co. KG..

Poster

528. Drug Discovery and Development: Neurodegenerative Diseases II

Location: Halls A-C

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Program#/Poster#: 528.07/EE14

Topic: C.19. Drug Discovery and Development

Title: CNS102 (trans-geranylgeranyl acetone) protects against kainic acid- induced neurotoxicity in rat through multiple signaling pathways

Authors: *Y. PAN[1], F. ERMINI[1], J. BRIDGEWATER[1], H. LISTER[1], W. G. HAAG[1,2], A. ARGADE[1], H. SERIZAWA[1]
[1]Coyote Pharmaceuticals, Menlo Park, CA; [2]Optim Sci. Corp., San Ramon, CA

Abstract: Mixture of trans- and cis- isomers of geranylgeranyl acetone (GGA), as an established drug product (teprenone) for treatment of ulcer, has been reported as neuroprotective in multiple disease models. Based on the prediction that the trans-isomer is a better conformational fit to the structure of GGA putative target geranylgeranyl transferase I (GGTase I), we purified the trans-GGA (named as CNS102). We then tested CNS102 efficacy in a kainic acid (KA) induced excitotoxicity model in rat. Sub-chronic oral administration of CNS102, at doses dramatically lower than reported for teprenone, significantly reduced CA3 neuronal death at 72 hours after KA challenge in CNS102- vs. vehicle-treated rats, indicating that CNS102 can protect against KA-induced hippocampal injury. We further measured induction of mRNA and protein for multiple heat shock proteins (HSPs) by CNS102 vs. vehicle in hippocampi following KA injury.

Consistent with reports that GGA is a known HSP inducer, we observed differential regulation of HSP family members with CNS102. Protection by CNS102 in our rat models may thus be mediated at least partially through HSP dependent neuroprotective mechanism. Consistently, our *in vitro* mechanistic studies in neuroblastoma 2A (N2A) cells revealed that CNS102 induces the expression of a luciferase reporter driven by i-HSP70 5'-UTR, and increases expression of selective HSPs. Interestingly, we also observed that CNS102 increases Rap1A prenylation and the level of active RhoA; both of these small GTPases have been implicated in modulating neuronal morphogenesis and function. This idea is further supported by our findings that CNS102 can promote the neurite outgrowth of N2A cells. Taken together, our results indicate that CNS102 targets pathways that underlie the etiology of multiple neurodegenerative diseases including Amyotrophic Lateral Sclerosis, and therefore may present itself as an effective drug candidate for therapeutic development.

Disclosures: **Y. Pan:** A. Employment/Salary (full or part-time):; Coyote Pharmaceuticals. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Coyote Pharmaceuticals. **F. Ermini:** A. Employment/Salary (full or part-time):; Coyote Pharmaceuticals. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Coyote Pharmaceuticals. **J. Bridgewater:** A. Employment/Salary (full or part-time):; Coyote Pharmaceuticals. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Coyote Pharmaceuticals. **H. Lister:** A. Employment/Salary (full or part-time):; Coyote Pharmaceuticals. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Coyote Pharmaceuticals. **W.G. Haag:** 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.; Coyote Pharmaceutical. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Coyote Pharmaceuticals. **A. Argade:** A. Employment/Salary (full or part-time):; Coyote Pharmaceuticals. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Coyote Pharmaceuticals. **H. Serizawa:** A. Employment/Salary (full or part-time):; Coyote Pharmaceuticals. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Coyote Pharmaceuticals.

Poster

528. Drug Discovery and Development: Neurodegenerative Diseases II

Location: Halls A-C

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Program#/Poster#: 528.08/EE15

Topic: C.19. Drug Discovery and Development

Title: CNS102, a new drug in development for amyotrophic lateral sclerosis, improves survival and behavior in SOD1 mice

Authors: *F. ERMINI[1], Y. PAN[1], W. G. HAAG[1,2], A. ARGADE[1], H. SERIZAWA[1]
[1]Coyote Pharmaceuticals, Menlo Park, CA; [2]Optim Sci., San Ramon, CA

Abstract: The SOD1(G93A) transgenic mouse is a well-characterized model for familial amyotrophic lateral sclerosis (ALS). Here we evaluate the effects of CNS102, the purified all-trans isomer of geranylgeranyl acetone (GGA), on disease onset, motor behavior and survival in SOD1 mice. Mice were orally administered CNS102 (12 mg/kg), riluzole (8 mg/kg) or vehicle daily starting at P39 and assessed for motoric deficits, body weight and survival. Using a cat walk system for analysis of motor function, CNS102-treated SOD1 mice performed better, demonstrating improved gait, stride length and running speed. In addition, CNS102 significantly improved grip strength and hind leg extension by tail hang assay. Neuroscore analyses showed that the rate of increase was 33% greater in vehicle- vs. CNS102-treated SOD1mice, indicating that progression is delayed with CNS102. Physiologically, CNS102 delayed the loss of body weight vs. vehicle-treated animals, and the difference corresponds to a rate reduction of over 40%. Importantly, survival was prolonged in the CNS102-treated SOD1 mice as compared to those treated with riluzole or vehicle. It is important to note that CNS102 is efficacious at a much lower dose (12 mg/kg) compared to those reported for the mixed isomer GGA (teprenone) in other models of neurodegeneration (i.e. over 600 mg/kg), and chronic dosing (approximately 3 months) with CNS102 revealed no adverse effects in the SOD1 mice and wild type controls. These pre-clinical studies using a low dose CNS102 indicate its potential as a human therapeutic for ALS.

Disclosures: **F. Ermini:** A. Employment/Salary (full or part-time):; Coyote Pharmaceuticals. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Coyote Pharmaceuticals. **Y. Pan:** A. Employment/Salary (full or part-time):; Coyote Pharmaceuticals. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Coyote Pharmaceuticals. **W.G. Haag:** 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.; Coyote Pharmaceutical. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds);

Coyote Pharmaceuticals. **A. Argade:** A. Employment/Salary (full or part-time):; Coyote Pharmaceuticals. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); ankush_argade@yahoo.com. **H. Serizawa:** A. Employment/Salary (full or part-time):; Coyote Pharmaceuticals. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Coyote Pharmaceuticals.

Poster

528. Drug Discovery and Development: Neurodegenerative Diseases II

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 528.09/EE16

Topic: C.19. Drug Discovery and Development

Support: NHMRC 632876

NHMRC 1037746

Title: The dimerization state of the human translocator protein (TSPO) is differentially affected by its ligands

Authors: *S. CHUA[1], A. MASEDUNSKA[2], S. BANISTER[3], Y. D. KE[1], P. GUNNING[2], M. KASSIOU[3], L. M. ITTNER*[1]
[1]Dementia Res. Unit (DRU), [2]Dept. of Anat., UNSW, Sydney, Australia; [3]Dept. of Chem., Univ. of Sydney, Sydney, Australia

Abstract: The translocator protein (TSPO) is a 5 transmembrane protein that resides in the outer mitochondrial membrane where it is involved in the regulation of cholesterol transport and neurosteroid synthesis. It has been found to be upregulated in activated microglia and astrocytes in both acute and chronic neuroinflammatory conditions. As such, recent work had focused intensely on developing TSPO as an *in vivo* brain imaging and drug treatment target. The functional form of the TSPO receptor is thought to be a dimer, however, the effects of pharmacological ligands on dimerization remains largely unknown. In this study, ligand effects on complex formation of TSPO and its missense variant, A147T, were studied using co-immunoprecipitation and confocal microscopy in 293T and HeLa cells respectively. Co-immunoprecipitation of co-transfected TSPO and A147T revealed the formation of spontaneous and stable dimers. However, various TSPO ligands had distinctly different effects on the formation of these dimers. It was shown that the diagnostic ligand PK11195, and significantly

more so DPA-714, but not Ro5-4864 disrupted TSPO dimer formation. Whilst the A147T polymorphism had no effect on dimer formation, it greatly enhanced the disruptive effect of PK11195. In addition to the effect of ligands, it was demonstrated that deletion of the cholesterol-recognition amino acid consensus sequence at the extreme carboxyl-terminus of TSPO affects homodimerisation. Hence, it can be concluded that the spontaneous and stable formation of TSPO homodimers is a ligand sensitive process and modulated by specific domains within the protein.

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Poster

528. Drug Discovery and Development: Neurodegenerative Diseases II

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Topic: C.19. Drug Discovery and Development

Support: MS Society Fast Forward Grant

Title: NDC-1308, a gain of function estradiol analog for inducing remyelination in multiple sclerosis patients

Authors: *S. H. NYE, J. G. YARGER
Discovery, ENDECE Neural, LLC, Mequon, WI

Abstract: Several estrogens have advanced to clinical trials, yet they impact only the neuroprotective aspects of multiple sclerosis (MS), rather than the elusive remyelination activity needed to repair the damaged myelin sheath. We previously reported that NDC-1308, an estrogen receptor (ER) agonist, activates known intracellular pathways for oligodendrocyte progenitor cell (OPC) differentiation. Unlike E2, NDC-1308 induces mouse OPCs to differentiate into mature oligodendrocytes *in vitro*. Here, we determined the efficacy of NDC-1308 to repair the damaged myelin sheath in a validated animal model of demyelination. Intended outcomes also include: i) an initial benefit-risk assessment for NDC-1308, ii) determining the dosing parameters for non-clinical IND-enabling studies, and iii) design of the Phase 1 clinical study. NDC-1308 was formulated for injection using an SBE-beta-cyclodextrin and assessed for its ability to induce remyelination in the cuprizone mouse model of demyelination. Male mice were treated for 12-weeks with cuprizone and rapamycin to cause

demyelination of white and gray matter regions of the brain. The demyelinated mice were administered NDC-1308 by intraperitoneal (60 mg/Kg, i.p., q.d) or subcutaneous (15-60 mg/Kg, s.c., b.i.d.) injections for 3 or 6 weeks. Blood was collected at termination for clinical chemistry analysis, along with reproductive tracts for pathology, and brain regions for assessing the level of NDC-1308 remyelination. A 3-week NDC-1308 treatment in cuprizone demyelinated mice resulted in greater than 20% ($P<0.005$) and 16% ($P<0.05$) remyelination of cortical and hippocampal regions, respectively. At 6 weeks of NDC-1308 treatment, remyelination in hippocampal regions increased to 30% ($P<0.0001$). However, E2 did not induce remyelination. Thus, NDC-1308 has apparently retained several qualities of E2, while gaining the important ability to induce remyelination of axons *in vivo*. All animals tolerated the chronic NDC-1308 treatments well. Observed animal behavior and clinical chemistries were normal, and there was no evidence of aberrant mammary tissue. These results suggest there could be a significant benefit for treating MS patients with NDC-1308. Non-clinical IND-enabling studies and a first-in-human Phase 1 study are planned for 2015.

Disclosures: **S.H. Nye:** A. Employment/Salary (full or part-time):; ENDECE Neural. 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.; MS Society. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); ENDECE Neural. **J.G. Yarger:** A. Employment/Salary (full or part-time):; ENDECE Neural. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); ENDECE Neural.

Poster

528. Drug Discovery and Development: Neurodegenerative Diseases II

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 528.11/EE18

Topic: C.19. Drug Discovery and Development

Title: The Nrf2 transcriptional target, OSGIN1, contributes to the cytoprotective properties of monomethyl fumarate

Authors: ***M. S. BRENNAN**[1], M. F. MATOS[2], C. SUN[2], S. SZAK[2], R. H. SCANNEVIN[2]
[1]Neurobio., Biogen Idec and Boston Univ., Cambridge, MA; [2]Neurobio., Biogen Idec, Cambridge, MA

Abstract: **Background**. Delayed-release dimethyl fumarate (DMF) is an oral therapeutic marketed for the treatment of multiple sclerosis (MS); however the detailed mechanisms that underlie the immunomodulatory and cytoprotective properties of DMF are not well understood. Previous data supports activation of the nuclear factor (erythroid-derived 2)-like 2 (Nrf2) pathway as a primary pharmacodynamic response to DMF or monomethyl fumarate (MMF, the primary metabolite of DMF) treatment and these responses may be differentially regulated in distinct cell types. In the central nervous system (CNS), we have identified the Nrf2 transcriptional target gene OSGIN1 to be significantly upregulated following DMF treatment *in vivo*; however, the contribution of this gene to the pharmacodynamic properties of DMF or MMF has not been previously described. **Objective**. To characterize the *in vitro* effects of MMF on OSGIN1 expression, and evaluate the necessity of OSGIN1 in mediating cytoprotective effects against toxic oxidative stress. **Methods.** Primary human astrocyte cultures were transfected with siRNA targeted against OSGIN1 or Nrf2 and treated with MMF, followed by toxic oxidative challenge with hydrogen peroxide. Cell viability was measured after treatment/insult and RT-PCR was conducted to determine transcriptional levels of OSGIN1, as well as the OSGIN1 associated genes PADI4 and p53. The potential involvement of PADI4 and p53 in MMF-mediated cytoprotection was also analyzed via siRNA knockdown. Furthermore, we analyzed the importance of OSGIN1 and its associated genes on cell proliferation since OSGIN1 has been previously identified as a regulator of the cell cycle. **Results.** siRNA knockdown of OSGIN1 significantly reduced the ability of MMF to protect human astrocytes against a toxic oxidative stress, and abrogated the MMF-induced increase in PADI4 expression levels. Additionally, the loss of PADI4 and p53 via siRNA knockdown also reduced the cytoprotective effects of MMF in human astrocytes against oxidative stress. **Conclusions**. These data support a role for the Nrf2 transcriptional target, OSGIN1, as an important mediator of cytoprotection in the CNS following DMF/MMF administration. Furthermore, these data identify a potential mechanism for DMF/MMF-mediated cytoprotection in human astrocytes that functions in an Nrf2-dependent manner.

Disclosures: **M.S. Brennan:** A. Employment/Salary (full or part-time):; Biogen Idec. **M.F. Matos:** A. Employment/Salary (full or part-time):; Biogen Idec. **C. Sun:** A. Employment/Salary (full or part-time):; Biogen Idec. **S. Szak:** A. Employment/Salary (full or part-time):; Biogen Idec. **R.H. Scannevin:** A. Employment/Salary (full or part-time):; Biogen Idec.

Poster

528. Drug Discovery and Development: Neurodegenerative Diseases II

Location: Halls A-C

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Topic: C.19. Drug Discovery and Development

Support: Adelson Medical Research Foundation

Title: Preventing neurodegeneration by inhibiting the correct HDACs: Molecular and pharmacological strategies

Authors: *M. BOURASSA, R. R. RATAN
Burke-Cornell Med. Res. Inst., White Plains, NY

Abstract: Histone deacetylase inhibitors have demonstrated significant potential as therapeutics for a wide variety of neurological conditions, including stroke and Alzheimer's disease. By inhibiting HDACs, the deacetylation of histones is blocked, allowing acetylated regions of the genome to remain transcriptionally active. HDAC inhibitors have the significant advantage over other potential drugs in their ability to promote the transcription of a large cassette of neuroprotective genes. By studying them in detail, we can optimize their protective and restorative properties and reduce or eliminate their side effects. As HDAC inhibitors neuroprotective in an *in vitro* model of oxidative stress affect Class I HDACs (HDAC1, 2, 3 and 8), we have endeavored to identify which Class I HDAC(s) accounts for this neuroprotection. We have found that HDAC2 knock down is neuroprotective in the glutathione depletion model of oxidative stress. Thus we are testing the effects of HDAC2 knock down in neuroprotection in a mouse model ischemic stroke. By using the HDAC inhibitor Scriptaid, which inhibits several HDACs, including HDAC2 we also are investigating a genetic biomarker of neuroprotection to improve *in vivo* testing of HDAC inhibitors. As HDAC inhibitors alter the expression of a large number of genes, there are many potential biomarkers that could be used to identify the most neuroprotective dose. With the results from these experiments we hope to significantly expand our knowledge of how HDAC inhibitors protect neurons and how to optimize the most protective doses using biomarkers. In the future, this information will aid in the design of better HDAC inhibitors that could be used as even more effective drug treatments for a host of neurological conditions.

Disclosures: M. Bourassa: None. **R.R. Ratan:** None.

Poster

528. Drug Discovery and Development: Neurodegenerative Diseases II

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 528.13/EE20

Topic: C.19. Drug Discovery and Development

Support: grant from the Walloon Region (Belgium) – (Conventions N°6368 & 6827).

Title: UCB0255, a new and selective synaptic vesicle protein 2A (SV2A) ligand with promising efficacy for cognitive deficits

Authors: *E. R. DETRAIT, E. JNOFF, F. BROUTA, K. LECLERCQ, E. JIGOREL, M. WOOD, M. GILLARD, H. KLITGAARD, A. MATAGNE, Y. LAMBERTY, B. KENDA, L. PROVINS
UCB Biopharma, Braine-L' Alleud, Belgium

Abstract: SV2A is a family member of synaptic vesicle proteins, involved in the control of neurotransmitters exocytosis end endocytosis. SV2A knock down triggers spontaneous seizures and the antiepileptic SV2A ligand, levetiracetam, reduces neurotransmitters release, thereby acting as a positive SV2A modulator. We report here a new class of negative SV2A modulators that appear to constitute a promising new medication to improve cognitive deficits. Among these, UCB0255 was identified as a preclinical candidate for further drug development. UCB0255 displays high selectivity and affinity (p*K*i=7.9) for SV2A but is devoid of antiepileptic properties. In contrast, UCB0255 opposes seizure protection afforded by levetiracetam in the audiogenic mouse seizure model, differentiating it from anticonvulsant SV2A ligands. UCB0255 displayed pro-cognitive activity in a variety of tests and challenges that may be relevant to cognitive disorders observed in neuropsychiatric conditions. UCB0255 showed efficacy in mice against beta-amyloid$_{(25-35)}$-induced deficit in working memory, long-term contextual memory and spatial reference memory. Using the object recognition paradigm in rats, UCB0255 was able to alleviate 24h delay-dependent forgetting of a familiar object, following administration before or after the acquisition trial, and counteracted scopolamine and subchronic phencyclidine-induced recognition memory deficits. The activity brought performance back to control level in most tests and the active doses ranged from 0.03 to 3 mg/kg across all tests/models, which corresponds to SV2A occupancy ranging from 5 to 90%. UCB0255 displays drug-like properties with a promising PK profile including good oral bioavailability in rat, and a wide safety margin of multiples of pharmacologically active doses in 4-week rat and 2-week dog toxicity studies as well as a favorable preliminary developability assessment. UCB0255 is a selective and high affinity SV2A negative modulator. Its new mechanism associated with pro-cognitive properties holds promise for the treatment of cognitive deficits associated with neurological and neuropsychiatric disorders.

Disclosures: **E.R. Detrait:** A. Employment/Salary (full or part-time):; UCB Biopharma. **E. Jnoff:** A. Employment/Salary (full or part-time):; UCB Biopharma. **F. Brouta:** A. Employment/Salary (full or part-time):; UCB Biopharma. **K. Leclercq:** A. Employment/Salary (full or part-time):; UCB Biopharma. **E. Jigorel:** A. Employment/Salary (full or part-time):;

UCB Biopharma. **M. Wood:** A. Employment/Salary (full or part-time):; UCB Biopharma. **M. Gillard:** A. Employment/Salary (full or part-time):; UCB Biopharma. **H. Klitgaard:** A. Employment/Salary (full or part-time):; UCB Biopharma. **A. Matagne:** A. Employment/Salary (full or part-time):; UCB Biopharma. **Y. Lamberty:** A. Employment/Salary (full or part-time):; UCB Biopharma. **B. Kenda:** A. Employment/Salary (full or part-time):; UCB Biopharma. **L. Provins:** A. Employment/Salary (full or part-time):; UCB Biopharma.

Poster

528. Drug Discovery and Development: Neurodegenerative Diseases II

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 528.14/EE21

Topic: C.19. Drug Discovery and Development

Support: NIH Common Fund

Title: Ameliorating the CNS Niemann-Pick type C phenotype by modulating Wnt signaling

Authors: *A. G. EFTHYMIOU[1], J. STEINER[2], M. S. RAO[2], N. MALIK[2]
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Abstract: Niemann-Pick type C (NPC) is a familial disorder that has devastating consequences on early development with multisystem effects. The disease involves neurodegeneration of the central nervous system that cannot be cured or modified through therapeutic treatment. Several drugs to treat this disorder have been identified in assays using rodent models and human fibroblasts from NPC patients, which are functionally different from the neural cells that express the neurodegenerative effects of NPC. In an effort to generate a more relevant cellular model for Niemann-Pick type C we generated an iPSC line from an NPC patient fibroblast sample and subsequently differentiated these cells to neural stem cells, neurons, and astrocytes. Few differences were seen at the neural stem cell stage by whole transcriptome array analysis, with no visible cholesterol accumulation or lysosomal abnormalities. Gene expression analysis indicated that Niemann-Pick type C iPSC-derived neurons displayed abnormal calcium signaling, suggesting that alterations in this pathway may underlie the phenotype. Candidate drugs including dantrolene and curcumin could rescue consistent specific loss of cortical neurons differentiated in culture from NPC neural stem cells. Further examination of the gene expression profiles of the cells revealed that Wnt signaling and ryanodine receptor expression were significantly altered. Our data suggest that altered Wnt signaling may be an important early event

in the etiology of the disease and that modulating Wnt or calcium signaling may be important even at early stages of Niemann-Pick type C.

Disclosures: **A.G. Efthymiou:** None. **J. Steiner:** None. **M.S. Rao:** None. **N. Malik:** None.

Poster

528. Drug Discovery and Development: Neurodegenerative Diseases II

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 528.15/EE22

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH/NINDS R01NS080844

NSC 102-2320-B-030-011

Title: Systemic neonatal lipopolysaccharide-induced brain injury can be ameliorated by melatonin

Authors: ***L.-T. TIEN**[1], Y.-J. LEE[1], L.-W. FAN[2]
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Abstract: Our previous study showed that lipopolysaccharide (LPS)-induced brain injury in the neonatal rat is associated with nitrosative and oxidative stress. The present study was conducted to examine whether melatonin, an endogenous molecule with antioxidant properties, reduces systemic LPS-induced nitrosative and oxidative damage in the neonatal rat brain. Intraperitoneal (i.p.) injection of LPS (2 mg/kg) was administered to Sprague-Dawley rat pups on postnatal day 5 (P5), and i.p. administration of melatonin (20 mg/kg) or vehicle was performed 5 minutes after LPS injection. Sensorimotor behavioral tests were performed 24 h after LPS exposure, and brain injury was examined after these tests. The results show that systemic LPS exposure resulted in impaired sensorimotor behavioral performance, and acute brain injury, as indicated by the loss of oligodendrocyte immunoreactivity and a decrease in mitochondrial activity in the neonatal rat brain. Melatonin treatment significantly reduced LPS-induced neurobehavioral disturbances and brain damage in neonatal rats. The neuroprotective effect of melatonin was associated with attenuation of LPS-induced nitrosative and oxidative stress, as indicated by the decreased nitrotyrosine- and 4-hydroxynonenal-positive staining in the brain following melatonin and LPS exposure in neonatal rats. Further, melatonin significantly attenuated LPS-induced increases in

the number of activated microglia in the neonatal rat brain. The protection provided by melatonin was also associated with a reduced number of inducible nitric oxide synthase (iNOS)+ cells, which were double-labeled with ED1 (microglia). Our results show that melatonin prevents the brain injury and neurobehavioral disturbances induced by systemic LPS exposure in neonatal rats, and its neuroprotective effects are associated with its impact on nitrosative and oxidative stress.

Disclosures: L. Tien: None. **Y. Lee:** None. **L. Fan:** None.

Poster

528. Drug Discovery and Development: Neurodegenerative Diseases II

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 528.16/EE23

Topic: C.19. Drug Discovery and Development

Support: Pfizer Global Research and Development

Title: Investigations of the dose-dependent effects of the PDE10A inhibitor PF-2545920 on primate regional cerebral glucose metabolism in the resting state

Authors: *G. V. WILLIAMS[1,2], D. W. CAMPBELL[1,2], C. J. SCHMIDT[3], M. M. ZALESKA[3], C. M. SANDIEGO[1], R. E. CARSON[4], S. A. CASTNER[1,2]
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Abstract: Phosphodiesterase (PDE) inhibitors alter neuronal function in particular brain regions by modulating cyclic nucleotide signaling. PDE10 is found almost exclusively in striatum and is linked to both cAMP and cGMP. While little is known of the role of PDE10 in cognition, there is interest in the use of PDE10 inhibitors as antipsychotics and cognitive enhancers for neurological disorders. Recently, we demonstrated that the dose-dependent effects of the AMPA potentiator PF-04958242 on [18F]fluorodeoxyglucose (FDG) uptake tested under normal conditions predicts its dose-dependent ability to prevent the disruption of cognition by ketamine. Here, we have investigated whether the dose-dependent effects of the PDE10A inhibitor PF-2545920 on glucose metabolism in the resting state might also reveal its potential to enhance frontostriatal function. A group of 8 animals were administered PF-2545920 (0.067 and 0.2 mg/kg, SC) or vehicle 2 hrs prior to FDG injection (~5 mCi, IV). FDG was administered when the animals were

sitting in the resting state where they remained for 20 - 25 min. They were then anesthetized with propofol and positioned in a microPET Focus 220 scanner where a 9 min transmission scan was acquired first, followed by a 20 min emission scan, commencing 60 - 75 min post-FDG injection. A nonlinear group template constructed from MRIs was used to spatially normalize the PET images prior to statistical parametric mapping (SPM). Univariate analysis revealed significant dose-dependent effects on metabolism, some of which survived multiple comparisons. In comparison to vehicle, PF-2545920 at 0.067 mg/kg induced elevations in metabolism in L. anterior cingulate (ACC) and lateral orbital cortices, as well as L. middle and superior temporal gyri. Conversely, this dose reduced metabolism in L. occipital gyrus as well as R. hippocampus/presubiculum, medial GPi, thalamus, motor cortex, and cerebellum. PF-2545920 at 0.2 mg/kg altered this pattern of influence, elevating activity in L. dorsal arcuate sulcus/dorsolateral prefrontal (dlPFC), infralimbic, and lateral orbital cortices, lateral amygdala n., ACC, hippocampus, cuneus and body of caudate n. as well as R. dlPFC and posterior cingulate cortex. This higher dose also suppressed activity in L. occipital gyrus, anterior ventromedial caudate n. and putamen, as well as R. thalamus, parietal cortex and cerebellum. These results were consistent with those derived from covariance with plasma exposure. These findings indicate that this PDE10A inhibitor not only influences corticostriatal and extrapyramidal circuitry but also modulates activity in a wider circuitry involved in cognition.

Disclosures: **G.V. Williams:** 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.; Pfizer Global Research and Development. **D.W. Campbell:** 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.; Pfizer Global Research and Development. **S.A. Castner:** 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.; Pfizer Global Research and Development. **C.J. Schmidt:** A. Employment/Salary (full or part-time):; Pfizer Global Research and Development. **M.M. Zaleska:** A. Employment/Salary (full or part-time):; Pfizer Global Research and Development. **R.E. Carson:** 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.; Pfizer Global Research and Development. **C.M. Sandiego:** 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.; Pfizer Global Research and Development.

Poster

528. Drug Discovery and Development: Neurodegenerative Diseases II

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 528.17/EE24

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIA R01 AG038739

Vanderbilt Brain Institute

Title: The protective role of vitamin C against Alzheimer's disease

Authors: *S. DIXIT[1], F. E. HARRISON[2]
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Abstract: Neurodegenerative diseases, such as Alzheimer's disease, involve high levels of oxidative stress. Vitamin C (VC) is a vital antioxidant molecule hypothesized to protect against the oxidative damage associated with Alzheimer's disease pathology. In fact, the highest concentrations of VC in the body are found in the brain, suggesting an important role of VC in neuronal health. VC is transported into the brain via the high-affinity Sodium-dependent Vitamin C Transporter-2 (SVCT2), which causes accumulation of VC in neurons against the concentration gradient. We hypothesize that an increased concentration of VC in neurons, via over expression of SVCT2, will provide greater protection against Alzheimer's disease. Primary cortical neuron cultures were established from wild-type mouse embryos and embryos transgenically modified to overexpress SVCT2 (SVCT2-Tg). Cell viability was measured using MTT assays following challenge with increasing concentrations of hydrogen peroxide to mimic increasing oxidative stress in these cultures. SVCT2-Tg cultures showed significantly greater viability across all doses when compared to wild-type cultures. Similarly, a trend toward greater viability of SVCT2-Tg cultures compared to wild-type cultures was detected when challenged with amyloid beta (1-42), the protein responsible for plaque formation characteristic of Alzheimer's disease. Taken together, these data suggest that VC has a protective function against oxidative stress associated with Alzheimer's disease.

Disclosures: S. Dixit: None. **F.E. Harrison:** None.

Poster

528. Drug Discovery and Development: Neurodegenerative Diseases II

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 528.18/EE25

Topic: C.19. Drug Discovery and Development

Support: DFG grant NI 683/ 4-2

Title: Effect of the nicotinic α4β2-receptor partial agonist varenicline on non-invasive brain stimulation-induced neuroplasticity in the human motor cortex

Authors: *M. A. NITSCHE[1], M.-F. KUO[1], W. PAULUS[2], J. GRUNDEY[2], G. BATSIKADZE[2]
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Abstract: Nicotine alters cognitive functions in animals and humans most likely by modification of brain plasticity. In the human brain, it alters plasticity induced by transcranial direct current stimulation (tDCS) and paired associative stimulation (PAS), probably by interference with calcium-dependent modulation of the glutamatergic system. We aimed to test this hypothesis further by exploring the impact of the α4β2-nicotinic receptor partial agonist varenicline on focal and non-focal plasticity, induced by PAS and tDCS, respectively. We administered low (0.1mg), medium (0.3mg) and high (1.0mg) single doses of varenicline or placebo medication before PAS or tDCS on the left motor cortex of 25 healthy non-smokers. Corticospinal excitability was monitored by single-pulse transcranial magnetic stimulation (TMS)-induced motor evoked potential (MEP) amplitudes up to 36 hours after plasticity induction. Whereas low-dose varenicline had no impact on stimulation-induced neuroplasticity, medium-dose abolished tDCS-induced facilitatory after-effects, favoring focal excitatory plasticity. High-dose application preserved cathodal tDCS-induced excitability diminution and focal excitatory PAS-induced facilitatory plasticity, but abolished anodal tDCS- and inhibitory PAS-induced excitability alterations. These results are comparable to the impact of nicotine receptor activation and might help to further explain the involvement of specific receptor subtypes in the nicotinic impact on neuroplasticity and cognitive functions in healthy subjects and patients with neuropsychiatric diseases.

Disclosures: **M.A. Nitsche:** F. Consulting Fees (e.g., advisory boards); Advisory Board Neuroelectrics. **M. Kuo:** None. **W. Paulus:** None. **J. Grundey:** None. **G. Batsikadze:** None.

Poster

528. Drug Discovery and Development: Neurodegenerative Diseases II

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 528.19/EE26

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: Childhood Cancer Fondation

Swedish Research Council

Title: Lithium reduced hippocampal progenitor cell death, inflammation, central hypothyroidism and cognitive dysfunction after irradiation to the young rat brain

Authors: *C. ZHU[1], K. ZHOU[2], C. XIE[2], Y. ZHANG[3], T. LI[3], Y. SUN[2], Y. XU[4], K. BLOMGREN[5]
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Abstract: Background: There is growing evidence that lithium (Li) is protective in a variety of brain injury paradigms. Cranial radiotherapy in children usually results in cognitive as well as pituitary dysfunction. Objective: Our aim was to evaluate the short- and long-term effects of Li in the immature brain after irradiation (IR). Design/Methods: Male Wistar rats were injected with 2 mmol/kg LiCl i.p. on postnatal day 7 (P7) and additional lithium injections, 1 mmol/kg, were administered at 24 h intervals for up to 14 days (until P20). On P11 the whole brain received a single IR dose of 6 Gy. Blood samples were collected from the tail vein 1, 3, and 5 weeks after IR. Results: Body weight gain was identical in all groups from P7 until P25. After P25 growth rate increased, but irradiated rats now followed a lower growth trajectory. Li did not affect growth rate, neither in controls, nor in irradiated rats. Serum creatinine was not different in Li-treated rats at any age. IR-induced progenitor cell death in the subgranular zone of the hippocampus was reduced by Li treatment. IR-induced inflammation, as judged by the levels of CCL2 (MCP-1), IL-1α, IL-1β, and GRO/KC in the hippocampus 6 h after IR, was reduced by Li. Neurogenesis was reduced by IR, but increased by Li, both in control rats and after IR. Astrogenesis was also reduced by IR, but rescued by Li treatment. Thyroid-Stimulating Hormone (TSH) tripled when the growth rate increased. The normal, growth-related TSH increase was abolished in irradiated rats, but Li treatment restored TSH levels and thereby prevented the central hypothyroidism that otherwise would have developed. Novel object recognition was used to test memory and learning. Recognition index at P60 was reduced 50% by IR, but normalized by Li treatment. In an open field test at P60, the distance moved was decreased and time spent in the open zone was increased after IR, but again normalized by Li treatment. Conclusion: Together, these results demonstrate that Li can be safely administered to young rats during the

second and third weeks of life and prevent both short- and long-term damage to the brain caused by ionizing radiation.

Disclosures: **C. Zhu:** None. **K. Zhou:** None. **C. Xie:** None. **Y. Sun:** None. **Y. Xu:** None. **Y. Zhang:** None. **T. Li:** None. **K. Blomgren:** None.

Poster

528. Drug Discovery and Development: Neurodegenerative Diseases II

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 528.20/EE27

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Title: Treatment with Nymphaea lotus lowers blood pressure, anxiety and improves erectile function in L-Name rats

Authors: ***M. P. KAMENI**, D. D. P. DZEUFIET, P. KAMTCHOUING, T. DIMO
Animal Biol. and Physiol., Univ. of Yaounde 1, Yaounde, Cameroon

Abstract: Background: Erectile dysfunction (ED) is a common, multifactorial disorder that is associated with aging and a range of organic and psychogenic conditions, including hypertension, hypercholesterolemia, diabetes mellitus, cardiovascular disease, and depression and/or anxiety. Aim: To clarify the relation between erectile dysfunction hypertension and anxiety; we evaluated the effects of a chronic oral treatment with L-NAME separately or concomitantly with Losartan or aqueous extract of Nymphaea lotus flowers, on blood pressure and cardiac frequency levels, on anxiety with an elevated alley (Suok test) and on standard patterning of sexual behaviour of male rats. Methods and results: L-NAME (10 mg/kg) only and Losartan (10 mg/kg) were used as negative and positive control respectively. Thirty days lasting treatment with L-NAME increased anxiety and distressed sexual behaviour parameters of sexually experimented adult male rats. Moreover the ejaculatory rate was hit harder by L-NAME than motivation and copulatory patterning. After 30 days withdrawal of L-NAME treatment, the sexual response and anxiety-elevated factors, were recovered. The extract of N. lotus at the doses of 75 and 200 mg/kg were able to reverse partially or totally the inhibitory effects of L-NAME, suggesting that the chronic oral treatment with L-NAME induces erectile and ejaculatory dysfunctions by peripheral mechanisms. Conclusion: Its earlier investigated anti-stress properties is considered to supplement the stimulation of sexual activity as stimulants/anti-depressants have associated effects such as improving libido, erection, ejaculation time and orgasm. The results obtained also suggest that chronic oral treatment with NO synthase inhibitor is a relevant and

powerful model to evaluate the effects of drugs on male sexual disorders in male rats. Keywords: Nymphaea lotus, sexual disorders, anxiety, blood pressure, L-NAME.

Disclosures: **M.P. Kameni:** None. **D.D.P. Dzeufiet:** None. **P. Kamtchouing:** None. **T. Dimo:** None.

Poster

528. Drug Discovery and Development: Neurodegenerative Diseases II

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 528.21/EE28

Topic: A.08. Transplantation and Regeneration

Title: Liraglutide exerts neuroprotective effects for cerebral infarct model of rats

Authors: *J. MORIMOTO[1], K. SATO[1,2], M. KAMEDA[1], T. YASUHARA[1], T. AGARI[1], T. BABA[2], F. WANG[3], A. SHINKO[1], T. WAKAMORI[1], A. TOYOSHIMA[1], H. TAKEUCHI[1], T. SASAKI[1], S. SASADA[1], A. KONDO[1], C. V. BORLONGAN[4], M. MATSUMAE[2], I. DATE[1]
[1]Okayama Univ. Grad Sch. of Med., Okayama, Japan; [2]Neurosurg., Tokai Univ., Tokyo, Japan; [3]Ctr. for Innovative and Translational Medicine, Kochi Univ. Med. Sch., Kochi, Japan; [4]Neurosurg., Univ. of South Florida Col. Med., Tampa, FL

Abstract: Abstract: Background The number of patients suffering from diabetes mellitus (DM) is increasing and cerebral infarct is often associated with DM. Recent studies revealed that glucagon-like peptide-1 (GLP-1) exerted neuroprotective effects. In this study, we investigated whether liraglutide, a GLP-1 analogue, exerts therapeutic effects on cerebral infarct models of rats. Methods Transient middle cerebral artery occlusion was performed for Wistar rats. At 1 hour after reperfusion, liraglutide or saline was intraperitoneally administered. Behavioral test was performed at 1 and 24 hours. Rats were subsequently euthanized for histological evaluation. Peripheral blood was taken for measurement of blood glucose level and evaluation of oxidative stress. Brain tissues were collected to measure the level of vascular endothelial growth factor (VEGF). Results Liraglutide-treated rats significantly preserved motor functions, compared with control rats. Infarct volumes of liraglutide-treated rats decreased, compared with those of control rats. The level of derivatives of reactive oxygen metabolite reduced in liraglutide-treated rats. VEGF level of liraglutide-treated rats in the cerebral cortex was significantly upregulated, compared to that of control rats. Conclusions Liraglutide exerted neuroprotective effects on cerebral infarct model of rats through anti-oxidative effects and VEGF upregulation. Keywords: cerebral infarct; diabetes mellitus; liraglutide; oxidative stress; VEGF

Disclosures: **J. Morimoto:** None. **K. Sato:** None. **M. Kameda:** None. **T. Yasuhara:** None. **T. Agari:** None. **T. Baba:** None. **F. Wang:** None. **A. Shinko:** None. **T. Wakamori:** None. **A. Toyoshima:** None. **H. Takeuchi:** None. **T. Sasaki:** None. **S. Sasada:** None. **A. Kondo:** None. **C.V. Borlongan:** None. **M. Matsumae:** None. **I. Date:** None.

Poster

528. Drug Discovery and Development: Neurodegenerative Diseases II

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 528.22/FF1

Topic: C.19. Drug Discovery and Development

Support: NIH R-21 NS078369

Title: Gentamicin improves survival and outcome after experimental subarachnoid hemorrhage

Authors: R. LISEK[1], V. FRIEDRICH[2], M. G. BAXTER[2], J. B. BEDERSON[1], *F. A. SEHBA[1]
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Abstract: Aneurysmal subarachnoid hemorrhage (SAH) is a medical emergency that requires prompt diagnosis and therapeutic intervention. Almost 12% of patients die before receiving medical attention, 33% within the first 48 hours and up to 50% within the first 30 days. The mechanisms leading to early death after SAH are not understood; as a result no specific therapy or strategy to reduce early mortality currently exists. During our ongoing experimental studies on acute phase injury in rat after SAH, we found that gentamicin treatment improves survival after SAH. Since those studies examined a single dose we then studied gentamicin efficacy at varied doses, using 30 day survival and behavioral status. Animals received a single dose of gentamicin (8, 16 or 32 mg/Kg) or saline 3 hours after the surgery (n=11-14 per dose group). Dose assignment was randomized and the investigator was blinded to dose. Animal survival studied at 7 and 30 day after SAH by Kaplan-Meier curve and animal outcome was measured as changes in general behavior, cognition and neurological status compared to saline injected animal. Survival at 7 days was 36% in saline and 58%, 71% and 66 % in 8, 16 and 32 mg/kg gentamicin groups, respectively. Survival at 30 days post-SAH was 9% in saline, and 58, 26 and 48% in 8, 16 and 32 mg/Kg gentamicin group, respectively. Behavioral status in gentamicin treated groups was not significantly improved over the saline controls. These results show that gentamicin extends animal survival post SAH and this effect is not dose dependent over the range tested. The data suggest that, while gentamicin treatment may not improve behavioral status, but it does not worsen it. Gentamicin is currently not used to improve acute phase survival and neurological

outcome after SAH, nor is any other agent administered clinically for this purpose. This preclinical translational study explores the possibility of developing gentamicin treatment protocols to improve survival in the first days after SAH.

Disclosures: R. Lisek: None. **V. Friedrich:** None. **M.G. Baxter:** None. **J.B. Bederson:** None. **F.A. Sehba:** None.

Poster

529. Auditory Processing: Temporal, Frequency, and Spectral Processing-Perception

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 529.01/FF2

Topic: D.02. Auditory

Support: NSF DDIG 1311194

Title: Neural detection of signals in noise

Authors: *K. M. SCHRODE[1], M. A. BEE[2]
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Abstract: Acoustic communication in social groups in difficult because intense background noise levels dramatically reduce the signal to noise ratio. Often listeners perform better when the amplitude of the background noise fluctuates over time. It is typically thought that this improvement is due to catching “acoustic glimpses” during dips in the noise level. This exploitation of increased signal to noise ratio is known as dip listening. Recently, it was discovered that females of Cope’s gray treefrog (Hyla chrysoscelis), but not the green treefrog (H. cinerea), showed better detection of a conspecific communication signal (or call) in noise when there were temporal fluctuations imposed on the noise. While these results suggested that gray treefrogs may have been using a dip listening mechanism, we cannot rule out stochastic resonance from the temporal fluctuations in the noise as an alternative possible mechanism. To test which mechanism frogs were using, we performed neural recordings from the auditory midbrain while presenting conspecific calls in the presence of sinusoidally amplitude-modulated noise. We manipulated the timing of calls such that they were either centered in a dip or at a peak of the background noise and used signal detection theory to calculate thresholds for detection. Detection thresholds were compared under these manipulations and between species to identify

potential species-differences in the mechanisms underlying exploitation of temporal fluctuations in noise.

Disclosures: K.M. Schrode: None. **M.A. Bee:** None.

Poster

529. Auditory Processing: Temporal, Frequency, and Spectral Processing-Perception

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 529.02/FF3

Topic: D.02. Auditory

Support: NIH/NIDCD R01DC009237

Title: Critical period hearing loss disrupts subsequent amplitude modulation detection

Authors: *M. L. CARAS, D. H. SANES
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Abstract: Transient bouts of pediatric hearing loss increase the risk for disrupted speech and language processing. Though these deficits can persist for years after normal auditory input is restored, their neural and perceptual bases are poorly understood. We addressed this issue in an established model of human auditory processing, the Mongolian gerbil, by inducing moderate, reversible hearing loss in developing pups via bilateral earplug insertion. After restoration of normal auditory input via earplug removal, gerbils were trained and tested on an amplitude modulation (AM) detection task. AM cues are necessary and sufficient for speech comprehension, and AM detection capabilities display a prolonged maturational time course in both gerbils and humans. Here, we report that auditory deprivation from the age of ear canal opening (postnatal day (P)11) to P23 elevates AM detection thresholds compared to control littermates, whereas earplugging at a later time point (P23-35) produces no behavioral impairment. Chronic multi-unit recordings from auditory cortex of freely moving animals engaged in the AM detection task will reveal whether AM detection deficits can be explained by impaired sensory coding. Together, our findings suggest that there is a critical period of development during which the maturation of AM perception is vulnerable to hearing loss and may help to explain speech and language delays associated with temporary hearing loss in children.

Disclosures: M.L. Caras: None. **D.H. Sanes:** None.

Poster

529. Auditory Processing: Temporal, Frequency, and Spectral Processing-Perception

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 529.03/FF4

Topic: D.02. Auditory

Support: Burroughs Wellcome Career at the Scientific Interface Award

Klingenstein Fellowship in Neuroscience

Pennsylvania Lions Club Hearing Research Fellowship

NIH NIDCD R03 DC 013660

Title: Effects of noise-induced tinnitus on frequency discrimination acuity in mice

Authors: L. MWILAMBWE-TSHILOBO, A. J. O. DAVIS, *M. N. GEFFEN
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Abstract: The goal of the present study is to characterize the effects of noise-induced tinnitus on auditory processing. Tinnitus is a subjective phenomenon in which affected individuals perceive a phantom sound (e.g. ringing, buzzing) in the absence of any external acoustic stimulation. A variety of animal models have been developed over the years in order to elucidate the neural mechanisms which underlie this complex auditory disorder. While it has been shown that induction of tinnitus leads to a selective decrease in gap detection in the frequency bands of the ringing sound, whether auditory discrimination is affected in those bands is less understood. Here, we induced tinnitus in mice by prolonged exposure to loud band-pass limited noise, and assayed whether mice exhibited a change in frequency discrimination acuity. We used the acoustic-startle reflex model to assess frequency discrimination (Clause et al. 2011). Tinnitus was induced in mice during a 1 hour exposure to a 10 kHz 120 db SPL tone, and assessed using a gap detection paradigm (Turner et al., 2006). We measured the frequency discrimination threshold for a set of high and low frequency tones embedded within a 22 kHz and 12 kHz background tone respectively prior to noise exposure and at 1, 4, and 8 weeks post-exposure. Tone-exposed mice that demonstrated robust gap detection deficits exhibited an increase in high-tone frequency discrimination threshold. This increase was sustained at 8 week post-exposure. Interestingly, although the low frequency bands were less severely affected during gap detection, frequency discrimination threshold for low tones also increased. These findings reveal that the

induction of tinnitus in the higher frequency bands adversely effects overall frequency discrimination, and suggests that tinnitus adversely affects auditory processing.

Disclosures: L. Mwilambwe-Tshilobo: None. **A.J.O. Davis:** None. **M.N. Geffen:** None.

Poster

529. Auditory Processing: Temporal, Frequency, and Spectral Processing-Perception

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Program#/Poster#: 529.04/FF5

Topic: D.02. Auditory

Support: NIH-NIA P01 AG009524

Title: Aldosterone augments prepulse inhibition but not startle amplitude in middle-aged cba/caj mice

Authors: *J. D. HALONEN[1,2,3], A. HINTON[1,2], X. ZHU[4], A. LOWE[4], J. WALTON[2]
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Abstract: The mineralocorticoid aldosterone (ALD) is released from the adrenal cortex and regulates Na^+ and K^+ flow across cell membranes. Previous research has demonstrated in aged humans that ALD serum levels correlate significantly with pure-tone thresholds, with higher serum ALD levels associated with better hearing. Furthermore, ALD treatment has been shown to significantly improve cochlear function, as measured by the auditory brainstem response, in autoimmune deficient mice. This experiment was designed to investigate if ALD treatment improves behavioral measures of hearing using prepulse inhibition (PPI) of the acoustic startle response (ASR). Middle-aged (15-17 mon.) CBA/CaJ mice were randomly assigned to 2 groups, either placebo or treatment, 1.67 µg per day of D-aldosterone, administered via extended release pellets implanted subcutaneously. The ASR was measured using standard methods and stimuli were presented through speakers located 30 cm directly above the transducer platform. ASR input-output functions were collected by presenting the wideband startle elicitor at intensities ranging from 55-115 dB, in 10 dB increments. Approximately 30 minutes after startle testing, noise PPI testing occurred. Pre-pulse stimuli were presented at 20, 40, 55, and 75 dB SPL, which terminate 30 ms prior to the wideband startle elicitor. Baseline tests were followed by tests at 4, 6, 10, and 16 weeks post hormone pellet treatment. No significant differences were found in the

startle input-output functions at any testing point. However, significant differences at 4, 6, 10 and 16 weeks post treatment were found. ALD treated animals were found to have greater PPI as compared to the control group at 40 dB at 4 weeks and 20, 40, and 55 dB at every test after. The increase in PPI indicates increased salience for low level noise signals and supports the hypothesis that ALD improves hearing sensitivity. These results are the first we are aware of to demonstrate a behavioral effect on prepulse inhibition in CBA/CaJ mice treated with aldosterone. The importance of this behavioral assay ties together our previous *in vitro* work showing the upregulation of a key Na^+-K^+ pump, NKCC1, in the lateral wall of the cochlea following ALD treatment as a possible mechanism to prevent age-related hearing loss . (Work supported by NIH-NIA P01 AG009524)

Disclosures: J.D. Halonen: None. **A. Hinton:** None. **X. Zhu:** None. **A. Lowe:** None. **J. Walton:** None.

Poster

529. Auditory Processing: Temporal, Frequency, and Spectral Processing-Perception

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Topic: D.02. Auditory

Support: NIH-NIA P01 AG009524

Title: Aldosterone modulates both simple and complex auditory processing in the auditory midbrain of aging CBA mice

Authors: E. J. BRECHT[1,2], X. ZHU[1,2], R. D. FRISINA[1,2], *J. P. WALTON[1,2,3]
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Abstract: Aldosterone (ALD) is a mineralocorticoid hormone secreted by the adrenal cortex which plays a primary role in controlling sodium (Na+) and potassium (K+) serum levels within the peripheral and central auditory system. It has been shown that serum ALD levels decline with age in humans and other mammals. Deficiencies or imbalances between Na+/K+-ATPase, K+ channels and Na-K-Cl cotransporter in the cochlea have also been observed to cause morphological and functional changes on cells downstream in the K+ recycling pathway, leading subsequently to hearing loss. A causative role for ALD in age-related hearing loss has yet to be demonstrated; however our group discovered a link between low serum ALD and severity of

presbycusis in elderly humans. While extensive work has been conducted exploring the effects of ALD within the peripheral auditory system, the effects of ALD treatment on the central auditory system have yet to be investigated. The goal of this study was to assess the long-term therapeutic effect of ALD treatment on neural processing of inferior colliculus (IC) auditory midbrain neurons in aged mice. We investigated the effects of ALD treatment (1.67 μg per day via a 120 day slow release, subcutaneous pellet) on the receptive fields (RFs) and the temporal processing abilities of IC neurons in age-paired control and treated mice. Multi-unit activity was assessed via analysis of the changes in RFs based on the minimum response threshold (MT) and width of tuning (Q10 & Q40), with the tonotopic axis divided into low, mid and high frequency regions. Temporal processing was measured using a gap-in-noise signal from which minimum gap thresholds were derived. Following ALD treatment, a significant positive effect for both RFs and temporal processing was observed, as well as a significant improvement in mean MTs of 11, 14 and 17 dB for the three frequency regions, respectively. Tuning was also affected by ALD treatment, evident by a broadening of RFs for Q10 for all regions and Q40 values for high BFs. There was no effect of treatment on the proportion of response types observed. These results suggest that ALD alters RF properties, with improvements in absolute sensitivity, and temporal processing abilities of IC neurons in aging CBA mice. Changes in MCR expression and function may be a contributing factor to central auditory processing deficits found in aged listeners. Furthermore, these results are the first to indicate a role of MCR in neural correlates of temporal processing. The results point to a potential target for future therapeutic interventions to prevent or slow down the progression of age-related hearing loss.

Disclosures: E.J. Brecht: None. **X. Zhu:** None. **R.D. Frisina:** None. **J.P. Walton:** None.

Poster

529. Auditory Processing: Temporal, Frequency, and Spectral Processing-Perception

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Program#/Poster#: 529.06/FF7

Topic: D.02. Auditory

Support: NIH NIA Grant P01 AG009524

Title: The aftermath of hormone replacement therapy in CBA/CaJ middle age female mice

Authors: *T. WILLIAMSON[1], X. ZHU[2], J. P. WALTON[3], R. D. FRISINA[2]
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Abstract: Hormone replacement treatment (HRT- with estradiol (E) and progestin (P)) has been used extensively to reduce menopausal symptoms in aging women sometimes with serious side effects. Previous findings have suggested that estrogen plays a vital role in preserving the cochlea, including hair cell function (Al-Mana et al., Neurosci. 153: 881-900, 2008). Meanwhile, progesterone has been thought to be very detrimental to the ear and the central auditory system (Price et al., Hear. Res. 252: 29-36, 2009). These findings were obtained during the time HRT was administered. Therefore, the end-result that these hormones have on the auditory system, i.e. effects of a recovery period, remains unclear. So, a primary motivation for the following investigation was to examine the recovery period after HRT was performed to see if hearing improved, worsened, or remained the same. Ovariectomized middle-aged female CBA mice were placed into 4 groups based upon treatment: E, P, E+P and placebo (Pb) via slow-release subcutaneous hormone pellets. All of the mice underwent Auditory Brainstem Response (ABR) Gap-in-noise (GIN) testing, longitudinally, where peak 1 (P1) for the second noise burst (NB2) was assessed to measure temporal processing deficiencies during the 6 months of HRT and 2-month recovery period. The results showed that mice treated with E+P had P1 amplitude levels that decreased and P1 latency values that increased significantly after 3 months of HRT. These values never quite recuperated during the recovery period. Decreased P1 latency values displayed by the E group indicated improvements in temporal processing. Mice treated with P showed little to no change of their temporal processing abilities during or after HRT. Meanwhile, the Pb group displayed typical age-related hearing loss (ARHL) changes, which diminished peripheral function throughout the study. Overall, there was no improvement observed in the hearing levels for any of the hormone treated mice during the recovery period, and the HRT groups all experienced some kind of decline in temporal processing by 3 months. For some groups, the decline in auditory function was noteworthy enough to consider HRT as harmful (the E+P group). Work supported by NIH Nat. Inst. on Aging grant P01 AG009524.

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Poster

529. Auditory Processing: Temporal, Frequency, and Spectral Processing-Perception

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 529.07/FF8

Topic: D.02. Auditory

Support: NIH-NIA P01 AG009524

Title: A mouse model of hormone replacement therapy reveals positive effects of estrogen on hearing

Authors: *A. S. HINTON[1,2], T. T. WILLIAMSON[2,3], X. ZHU[2,3], J. M. MANSOUR[2,3], R. D. FRISINA[1,2,3], J. P. WALTON[1,2,3]
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Abstract: Gonadal hormones play an important role in auditory development and they have recently been implicated in modulating age-related hearing loss. Estrogen and progesterone are reproductive hormones and influence excitatory activity of auditory nerve fibers, as well as help regulate neurotransmitters in the CNS. It has also been shown that rats given hormone replacement therapy (HRT) perform better on hippocampal tasks, yet they perform worse on prefrontal tasks. However, it is unknown how HRT affects behavioral measures of audition. In order to test the effects of estrogen and progesterone on auditory processing, mice received HRT using different combinations of gonadal hormones. Our goal was to determine if these hormones improve behavioral measures of hearing using pre-pulse inhibition (PPI) of the acoustic startle reflex (ASR). Middle-aged (15-17 mon) CBA/CaJ mice were randomly assigned to 5 groups. Male mice were assigned to a no-pellet control group. Female mice were assigned to placebo, estradiol (0.0056 mg/day), progesterone (0.39 mg/day), or estradiol + progesterone, groups. Hormones were administered by extended release pellets implanted subcutaneously. The ASR was measured using standard methods and stimuli were presented through speakers located 30 cm directly above the transducer platform. ASR input-output functions were collected by presenting the wideband startle elicitor at intensities ranging from 55-115 dB, in 10 dB increments. Approximately 30 minutes after startle testing, noise PPI testing occurred. Pre-pulse stimuli were presented at 20, 40, 55, and 75 dB SPL, 30 ms prior to the wideband startle elicitor. Baseline tests were followed by tests at 1, 2, and 3 months post hormone pellet treatment. We found that estrogen results in greater PPI as compared to the placebo, control, and progesterone groups at 1 month, as well as, at the 2 month test; while the progesterone HRT resulted in a reduction in startle amplitude. The progesterone group also showed significantly lower PPI

testing 3 months post-treatment at 20, 40, and 55 dB compared to control. A decrease in startle amplitude and PPI with progesterone treatment suggests that treatment may negatively affect sensorimotor reflexes and decrease hearing sensitivity. Increased inhibition with estrogen treatment at sub-threshold pre-pulse levels indicates increased salience for low level noise signals and demonstrates improvement in hearing sensitivity. These results indicate that reflex modification using acoustic PPI can assess the effects of HRT on auditory processing in mouse models.

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Poster

529. Auditory Processing: Temporal, Frequency, and Spectral Processing-Perception

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Topic: D.02. Auditory

Title: Phase coherence of auditory steady-state response (ASSR) reflects internal brain activation level modulated by a modified N-back task: an MEG study

Authors: *Y. YOKOTA, Y. NARUSE
NICT, Kobe, Hyogo, Japan

Abstract: The auditory steady-state response (ASSR) is oscillatory brain activity induced by repetitive auditory stimuli. Previous studies reported that the power and inter-trial phase coherence (ITPC) of ASSR could be modulated by the degree of brain activation level. For example, Griskova et al. (2011) compared ASSR activation between low and high-arousal conditions in order to examine the relationship between brain activation level and ASSR, resulting stronger in the low-arousal condition. Moreover, Roth et al. (2013) used a Tetris game as a visuospatial attention task, and reported that the difficult version of Tetris task reduced 40Hz auditory cortical responses compared with the easy version. However, those studies used different physical stimuli for different difficulty tasks, and the effect of the internal brain activation level itself has not been clarified enough. In this study, we employed modified N-back task as a visual working memory task in order to regulate the degree of difficulty with an identical physical stimulus. The experiment involved four types of tasks: no-load (NL), 1back, 2back, and 3back task. Images of two numbers (‘1’ or ‘2’) with two colors (white or red) were used as visual stimuli. One of two images (50% for each) was randomly presented for 0.5 s with

randomly selected color (80% for white and 20% for red). The number of trials was 200 in each task, and a blank image with a fixation point was presented with 2.0s duration between trials. In the NL task, participants did not perform the N-back task, however, they reported the number by pressing one of two buttons when the color of the stimulus was red. In contrast, in 1, 2, and 3 back tasks, participants reported whether the current number is same with the N-th before number. The 40Hz auditory stimulus was click, and transmitted to the participant continuously via plastic tubes. Sixteen healthy subjects participated in the present study and MEG responses were recorded using a 148-channel magnetometer system. The continuous MEG data was processed by a digital bandpass filter (butterworth 1-50Hz), and then divided into 1s epochs (0 to 1s from the onset of the auditory stimulus) with artifact rejection. Fourier analysis was performed, and ITPC of 40 Hz responses was calculated. Grand averaged phase coherence activities of fifteen showed the 40 Hz ASSR reductions accompanying with increase in the task difficulty. The phase coherence activities in 1-3 back tasks were significantly lower than the in NL task, and those in high load task (3back) were significantly lower than those in low load task (1back). The result suggests that ASSR reflects the internal brain activation level and can be a useful indicator for it.

Disclosures: **Y. Yokota:** None. **Y. Naruse:** None.

Poster

529. Auditory Processing: Temporal, Frequency, and Spectral Processing-Perception

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Support: PIFI-VIEP-BUAP-CONACYT-F1-153583 (EM)

Catedra Marcos-Moshinsky (EM)

Title: Internal stochastic resonance within the human brain elicited by binaural noises

Authors: ***A. GUTIERREZ**[1], N. HUIDUBRO[2], R. KRISTEVA[3], E. MANJARREZ[2]
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Abstract: The internal stochastic resonance is a phenomenon of non-linear systems in which the enhancement in the coherence, or synchrony, between two or more signals is produced by the

addition of a particular level of noise. Little is known about this phenomenon in the nervous system. The first experimental evidence of internal stochastic resonance in the central nervous system was reported by Manjarrez et al., (2002), who found that the coherence between spinal and cortical somatosensory neurons, in the cat, can be enhanced by the application of a particular level of tactile noise. Recently, we reported that the addition of an intermediate level of mechanical noise on the finger skin enhances the corticomuscular coherence during a visuomotor task (Trenado et al., 2014). However, in such studies only one source of external noise was applied, with the assumption that an internal noise source was also involved. The aim of the present study was to examine the electroencephalographic (EEG) coherence (between pairs of electrodes of the 10-20 system located over homologous auditory areas in the left and right cerebral hemispheres) during binaural hearing of two uncorrelated Gaussian noises. Experiments were performed in eight healthy subjects. The stimulation protocol was the following. One Gaussian noise of fixed intensity was delivered to the left ear, while a second Gaussian noise of variable intensity was delivered to the right ear. We found that all the subjects exhibited an enhancement in the beta and/or gamma band EEG coherence during this binaural noise protocol when an intermediate level of auditory noise was applied on the right ear. These results are the first demonstration that the internal stochastic resonance phenomenon can be evoked within the human brain by the interaction of two controlled noise sources.

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Poster

529. Auditory Processing: Temporal, Frequency, and Spectral Processing-Perception

Location: Halls A-C

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Program#/Poster#: 529.10/FF11

Topic: D.02. Auditory

Title: Differentiating phase-locked responses to the temporal envelopes of speech sentences

Authors: ***R. E. MILLMAN**, S. R. JOHNSON
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Abstract: The temporal envelopes of sounds contain important cues for auditory perception. In the context of speech comprehension, the role of neural activity phase-locked to the speech temporal envelope is controversial: It remains unclear whether linguistic content can enhance neural temporal envelope tracking for intelligible speech sentences. Whole-head

magnetoencephalography (MEG) and novel beamformer-based analyses of time series data were used to test the hypothesis that phase-locking to the temporal envelope of speech sentences is enhanced by linguistic information. The novel beamforming analyses were an extension of the Source Stability Index [Hymers et al., (2010). "Source Stability Index: A novel beamforming based localisation metric. NeuroImage 49, 1385-1397] but here analyses focussed on identifying *differences* (i.e., the Difference Stability Index, DSI) in phase-locked responses elicited by listening to intelligible and unintelligible speech sentences. Perceptual "pop-out" was used to change the percept of physically identical tone-vocoded speech sentences from unintelligible to intelligible. The use of pop-out dissociates changes in phase-locking to the speech temporal envelope arising from acoustical differences between unintelligible and intelligible speech from changes in speech intelligibility itself. In a 2 x 2 factorial design the DSI was calculated for speech sentences with 1) identical or different temporal envelopes and 2) identical or different percepts (i.e. unintelligible or intelligible). Differences in phase-locked responses elicited by listening to speech sentences with different temporal envelopes and identical/different percepts localised to the temporal lobes bilaterally. There were no functionally significant differences between the phase-locked responses to speech sentences with identical temporal envelopes and identical/different percepts. These results add to the evidence against a top-down amplification of speech envelope tracking to intelligible speech stimuli.

Disclosures: R.E. Millman: None. **S.R. Johnson:** None.

Poster

529. Auditory Processing: Temporal, Frequency, and Spectral Processing-Perception

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Topic: D.02. Auditory

Support: MEXT 23591494

Title: Differential oscillatory responses to aversive sounds in children with autism spectrum disorder showing auditory hypersensitivity

Authors: *K. SHIMONO[1], J. MATSUZAKI[2], H. SUGATA[3], I. HIRATA[4], R. HANAIE[2], F. NAGATANI[2], M. TACHIBANA[2], K. TOMINAGA[2], M. HIRATA[3], I. MOHRI[2], M. TANIIKE[2]
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Abstract: Aim: Although auditory hypersensitivity is the most common sensory impairment in autism spectrum disorder (ASD), its neurophysiological mechanism has not been known. We reported that auditory hypersensitivity in ASD revealed increased M50 dipole moment to sinusoidal tones using magnetoencephalography (MEG; Matsuzaki et al, 2012). In real life, however, ASD subjects with hypersensitivity are not always hypersensitive to all environmental sounds, but to specific ones. In this study, we studied oscillatory responses to aversive sounds and analyzed its correlation with hypersensitivity. Method: Oscillatory responses for two sounds were measured by 160 channel whole head MEG system (PQ1160C, Yokogawa Electric Corporation) in 18 boys (age: 8-12 years) with ASD (IQ≧75), consisting of 12 with and 6 without auditory hypersensitivity, and 9 age-matched typically developed (TD) controls. In all subjects, auditory hypersensitivity was assessed by the sensory profile. A neutral sound and an aversive sound were selected as auditory stimuli. The duration time was 4 seconds, and number of stimulation were 30 times of each. To determine the differentiation of cerebral oscillatory changes in each group, adaptive beamforming and group analysis were used. Result: α band event-related synchronizations (ERS) appeared in auditory cortex around 200ms after neutral sounds, followed by β and low γ band event-related desynchronization (ERD) in TD. However, in addition to β band and low γ band ERD, ERS also appeared after aversion sounds in TD. On the other hand, in ASD with hypersensitivity, delayed, wide and prolonged α band ERS was recognized for neutral sounds. In addition, instead of β band and low γ band ERD, high γ ERD were appeared. Furthermore, specific β and γ band ERS were activated after 400-1000 ms of aversive sounds. Discussion: Increased responsiveness in primary auditory cortex was previously reported in ASD children with hypersensitivity. In this study, although oscillatory difference between neutral sounds and aversive sounds were recognized even in TD. γ oscillation was gererated in ASD with hypersensitivity especially to aversive sounds. γ band synchronization was reported to play a crucial role in cognitive functions including perceptual and emotional processes. Thereafter, our data may suggest the basic mechanisms of emotional response to auditory stimuli in ASD with hypersensitivity.

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Poster

529. Auditory Processing: Temporal, Frequency, and Spectral Processing-Perception

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Program#/Poster#: 529.12/FF13

Topic: D.02. Auditory

Title: A model of theta band oscillation entrainment to speech

Authors: ***D. HARRELL**[1], O. GHITZA[2], E. LARGE[1]
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Abstract: Entrainment of cortical theta rhythms to the syllabic rhythm of natural speech is thought to engage neurodynamic mechanisms of temporal anticipation to segregate incoming information and organize spike timing. These neurodynamic mechanisms may also be critical for intelligibility of spoken language. Previous studies have demonstrated that the insertion of silent gaps into speech can be deleterious to intelligibility in certain circumstances and remedial to intelligibility in others. For example, when speech is temporally compressed by a factor of three, insertion of silent gaps can restore some intelligibility. However, beyond a certain duration of silent gap, intelligibility begins to deteriorate. One oscillator based model of speech perception, proposed to account for these results, tracks the artificial rhythm created by the insertion of silent gaps. However, this model requires the introduction of oscillatory bands beyond theta to explain the loss of intelligibility once gap length reaches a critical duration. This loss of intelligibility can be explained using only theta band processes by considering speech with artificially inserted silent gaps to be a stimulus with two rhythms: the syllable rhythm and the gap rhythm. Here, we present a model using two coupled theta band oscillators in which one oscillator tracks the syllable rhythm and the other tracks the rhythm of the silent gaps. For silent gaps less than a critical duration, the rhythms remain distinct. However, beyond that duration, both oscillators entrain to the rhythm of the silent gaps. These oscillator dynamics can account for the results shown in rhythmically interrupted speech. Implications for the role of theta oscillations in speech perception are discussed.

Disclosures: D. Harrell: None. **E. Large:** None. **O. Ghitza:** None.

Poster

530. Auditory Processing: Neural Coding, Animal Studies, and Computation

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Topic: D.02. Auditory

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NSF IOS-1257150

DFG Research Fellowship

Title: Influence of efferent innervation on lateral line sensing in larval zebrafish (Danio rerio)

Authors: *M. HAEHNEL[1], C. A. SMITH[2], J. C. LIAO[2]
[2]Whitney Lab., [1]Univ. of Florida, St Augustine, FL

Abstract: A swimming fish generates flow stimuli from its own motion, which it must distinguish from biologically relevant stimuli from the environment. It does this with the mechanosensory hair cells of the lateral line system, which receives direct efferent feedback from the octavolateral efferent nucleus (OEN) located in the hindbrain. In adult fishes, efferent feedback is thought to reduce or increase lateral line sensitivity. The mechanisms that regulate lateral line sensitivity likely already exist in larval fish as well, given that they are less likely to respond to a flow stimulus when swimming. To understand how lateral line sensitivity is regulated and coordinated during swimming in larval zebrafish we simultaneously recorded posterior lateral line sensory afferent and peripheral motor neuron activity during fictive swimming. We found that in about 30% of the cases spontaneous activity in sensory afferents decreased about five-fold, from 50 to 10 Hz during spontaneous swimming bursts (25-30 Hz). We hypothesize that efferent neurons provide inhibitory corollary discharge of the motor activity to lateral line hair cells. Our current work focuses on making direct recordings from the efferent neurons to test this hypothesis and to look at how efferent effects on afferent sensitivity may change with swimming speed. We also suspect a regional specialization of efferent feedback within the posterior lateral line system. There are two subpopulations of efferent neurons targeting the posterior lateral line, located rostral and caudal relative to each other. By carefully backfilling groups of efferent neurons with dye injection from their target neuromasts, we were able to identify a regional differentiation between the rostral efferent neuron (REN) which exclusively innervates the most rostral midline neuromast in the posterior lateral line and the caudal efferent neurons (CEN), of which one innervates all probed midline neuromasts, while the other innervates only the caudal portion of the midline neuromasts. We hypothesize that the functional significance of this subdivision might lie in providing regionally specific feedback, for example to offset self-stimulation induced by fin versus tail motion during swimming.

Disclosures: M. Haehnel: None. **J.C. Liao:** None. **C.A. Smith:** None.

Poster

530. Auditory Processing: Neural Coding, Animal Studies, and Computation

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 530.02/FF15

Topic: D.02. Auditory

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Title: Population coding for sound localization in the owl's midbrain

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Abstract: A major challenge in sensory neuroscience is to understand how populations of neurons capture the uncertainty in sensory information. Extracting the signal from unreliable input is essential in sensory processing. However, to what extent this is the result of averaging out noise or selecting the invariant parameters in the environment is a matter of debate. We study this question in the sound localization system of the barn owl using physiological, behavioral and modeling approaches. The interaural time difference (ITD) is a primary cue used to localize sound. Due to the filtering effect of the head, ITD varies depending on frequency and the presence of background noise. This statistically complex relationship between direction and ITD poses a problem to reliably localize sounds. We found that at each direction there is a frequency range within which ITD is most invariant. We show that neurons' preferred frequencies match this range. We are now exploiting the tight relationship between frequency and ITD to stimulate separately the units tuned to the front or to the periphery. We combine model predictions and behavioral results to investigate whether the direction estimate relies on the entire population of neurons. We monitor head saccades in response to sound stimulation and assess the consistency of the behavioral output with population decoding schemes such as population vectors or maximum a posteriori.

Disclosures: **F. Cazettes:** None. **J.L. Pena:** None. **B.J. Fischer:** None.

Poster

530. Auditory Processing: Neural Coding, Animal Studies, and Computation

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Title: Temporal sequence processing & natural song time-scales in Zebra finch auditory cortex

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Abstract: A highly developed auditory cortical network supports auditory-vocal behavior in songbirds. The hallmark of this cortical network is the ubiquitous presence of fine temporal coding in response to natural song (*1-3*). In this study we take advantage of the precise spike timing in songbird auditory cortex to ask how temporally patterned stimuli are transformed in the transition from primary to secondary cortical zones. We first demonstrate that in the transition from primary (L2a) to secondary (L2b) auditory cortex, neural responses to song become less synchronous across neurons and more informative about the identity of specific syllables. To zero in on temporal aspects of this process, we examined the same circuit transformation in response to click sequences defined by the temporal ordering of intervals between clicks. The interval distribution between clicks was chosen to produce, in area L2a, population firing patterns similar to the responses to natural songs. In L2a, each individual click elicited a synchronous response in all recorded neurons, regardless of the temporal context of the click. In secondary auditory cortex (L2b), neurons responded asynchronously and selectively, in a manner dependent on the temporal context of the click. We next tested whether songbirds could discriminate different click sequence patterns in a "restart-go" operant paradigm. In this training procedure, 24 zebra finches rapidly learned to discriminate between the different click sequences. However, when the click stimulus set was slowed by a factor two (no longer driving L2a in a manner similar to song), the sequence selectivity in the secondary auditory area (L2b) was significantly reduced, and zero of 15 zebra finches were able to perform the operant task. This work suggests that the zebra finch auditory cortex is capable of transforming temporal patterns to sequence-selective population responses or "spatial codes." We suggest that this transformation is tuned to the time-scale of sub-syllabic acoustic transitions in conspecific songs.
1. J. F. Prather, S. Peters, S. Nowicki, R. Mooney, *Nature* **451**, 305-310 (2008). 2. G. B. Keller, R. H. R. Hahnloser, *Nature* **457**, 187-190 (2009). 3. D. M. Schneider, S. M. N. Woolley, *Neuron* **79**, 141-152 (2013).

Disclosures: T.J. Gardner: None. **B. Shinn-Cunningham:** None. **Y. Lim:** None.

Poster

530. Auditory Processing: Neural Coding, Animal Studies, and Computation

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Title: Compensatory gain control partially restores midbrain and cortical sound coding following profound peripheral nerve degeneration

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Abstract: Peripheral deafferentation occurs routinely in the auditory system as a consequence of acoustic trauma, ototoxic insult, or aging. Central neurons corresponding to deafferented regions of the cochlea exhibit changes in sound-evoked tuning, wherein Hebbian mechanisms guide a shift in central responsiveness toward neighboring, intact regions of the periphery. In addition, central neurons maintain activity levels based on non-Hebbian homeostatic mechanisms involving synaptic or intrinsic changes to compensate for reduced sensory drive. The degree to which these processes are involved in recovery following hearing loss, as well as their consequences for perception, are unknown. We recorded from the inferior colliculus (IC) and primary auditory cortex (A1), in awake mice after 95% of Type-I auditory nerve fibers were eliminated unilaterally via administration of ouabain, a Na+/K+ ATPase pump inhibitor. Thresholds for auditory brainstem evoked potentials were substantially elevated. However, behavioral detection thresholds were near normal, indicating that neural activity at higher stations of the auditory pathway could support perception. We recorded central representations of tones and broadband pulse trains in order to address a three-fold hypothesis: First, we predicted that the rapid expansion of representations of the normal auditory nerve would occur in addition to a slower potentiation of the remaining inputs from the profoundly degenerated nerve, possibly by homeostatic mechanisms. Second, we predicted that the latter potentiation would occur to a greater extent in A1 than IC. Third, we predicted that this plasticity would increase the fidelity of a spike rate code, due to a preservation of firing rate magnitudes. We found that unit

thresholds were lower than those predicted from brainstem metrics, possibly indicating a central compensatory plasticity. A1 evoked firing rates with contralateral stimulation were preserved at near normal levels. Finally, using vector strength and classifier models to test the integrity of temporal encoding in IC and A1, the neurophysiological and behavioral categorization of rapidly modulated sounds was impaired while rate encoding mechanisms recovered to a greater extent over time. Ongoing studies compare single-unit responses to sound stimulation with optogenetic stimulation of the brainstem. These studies form a conceptual framework for understanding the contributions of competitive and non-competitive plasticity to recovery from hearing loss, as well as the limits of perceptual ability that can be preserved via the brain's intrinsic recovery mechanisms.

Disclosures: A.R. Chambers: None. **Y. Yuan:** None. **A.S. Edge:** None. **M.C. Liberman:** None. **D.B. Polley:** None.

Poster

530. Auditory Processing: Neural Coding, Animal Studies, and Computation

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Topic: D.02. Auditory

Title: Linear integration for perceptual behavior in mouse primary auditory and visual cortex

Authors: *M. H. HISTED, N. T. COMFORT, R. T. OHMAN, A. R. PERILLO, J. H. R. MAUNSELL
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Abstract: Many mammalian species can treat sensory information similarly whether it is distributed over time or concentrated over a short interval. Such near-perfect linear integration means that behavioral ability to perceive a stimulus is predicted by the product of stimulus duration and amplitude, a phenomenon often called Bloch's Law. By directly stimulating mouse visual cortex, we have previously shown that this linear integration can arise from computations in the cerebral cortex (Histed and Maunsell, 2014). Linear behavior of cortical circuits might have been unexpected because cortical neurons' spiking is a non-linear function of their inputs. However, behavioral linearity can be explained by a decoder that applies a threshold rule to a population spike count. Because near-perfect sensory temporal integration is seen in many species and different sensory systems, it is likely to be a computation that circuits in many cortical regions can perform. To determine if auditory cortex as well as visual cortex can use

linear integration to guide behavioral decisions, we trained mice to perform an auditory task in which we measured their ability to detect pure tones of different durations and amplitudes embedded in noise. We find that mice show linear integration for audition, following Bloch's law: detection threshold is related to the product of duration and tone amplitude (duration: 2-100ms, N=2 mice). We previously found using direct stimulation of visual cortex that small changes in neuronal activity can be detected and reported by mice. These small changes mean that individual neurons do not carry enough information for animals to do the task. Instead, a population of neurons must be decoded to achieve the observed behavioral performance. We find that neurons in mouse primary auditory cortex are also modulated only weakly by stimuli near detection threshold (median change in firing rate over interval before behavioral response: 0.5 spk/s; 75th percentile 1.7 spk/s; N=13 single units and 47 multiunits, recorded extracellularly in awake mice). Thus, despite the widely different types of sensory input processed in primary visual and auditory cortex, both areas can support linear integration. Linear behavior is useful under many behavioral circumstances, as when it is desirable to weight inputs equally regardless of their arrival time. The cerebral cortex might have evolved to support linear integration as one important computational regime.

Disclosures: **M.H. Histed:** None. **N.T. Comfort:** None. **R.T. Ohman:** None. **A.R. Perillo:** None. **J.H.R. Maunsell:** None.

Poster

530. Auditory Processing: Neural Coding, Animal Studies, and Computation

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Title: Brain state dependency of rate and temporal coding in the primary auditory cortex

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Abstract: The mammalian brain is always active, with different patterns of spontaneous activity corresponding to different behavioural states. For example, deep sleep is associated with large, slow oscillations in EEG and LFP, as cortical neurons alternate between firing and silence in a locally collective manner (inactivated state). Wakefulness, however, is associated with a suppression of such oscillations and desynchronisation of cortical neural activity, with neurons spontaneously firing near independently. Such differences in brain state may be indicative of different modes of processing, but this is not well understood. Here, we analyse brain state dependency of auditory processing using spiking data from medial geniculate body (MGB) and multiple layers of primary auditory cortex (A1) of five urethane anaesthetised adult rats. To quantify stimulus information conveyed, decoding and information theoretic procedures were applied to both rate and temporal measures of single unit evoked responses to different stimuli in two distinct brain states: the inactivated state, natural under urethane anaesthesia; and the activated state, induced through electrical stimulation of the nucleus basalis in the basal forebrain. Provisional results indicate that the most informative cortical neurons convey increased stimulus information in the activated state, whereas thalamic neurons show no clear difference between brain states, suggesting that auditory cortical processing is affected by brain state.

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Poster

530. Auditory Processing: Neural Coding, Animal Studies, and Computation

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Topic: D.02. Auditory

Support: New York University Provost Postdoctoral and Transition Program for Academic Diversity Fellowship

Title: The contribution of sensory and cortical processing to perceptual decision-making

Authors: *M. INSANALLY[1], I. CARCEA[1], B. ALBANNA[2,3], R. FROEMKE[1]
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Abstract: The process of perceptual decision-making requires the use of sensory information to guide action and consequently involves coordinated activity in multiple cortical regions. Little is known about the combined functional contributions of cortical sensory and frontal areas to perceptual decision-making in rodents. In this study, we investigated the role of auditory and frontal cortex in perceptual decision-making using electrophysiological recordings from animals performing an auditory cue-driven task. While traditionally it may be thought that auditory cortex acts as a feature detector for acoustic stimuli, there is growing evidence that it plays a role in perception and cognition as well. Activity in frontal cortical regions such as medial agranular cortex has been shown to reflect action selection, but it is unknown how activity in this region reflects other task related variables such as stimulus identity. We made 70 single-unit recordings from behaving rats trained to respond to 4 kHz tones and ignore tones of other frequencies (see Carcea et al. SfN abstracts 2014 for more details of the go/no-go task). The spike trains observed in this study are complex and highly variable from trial to trial. However, on a given trial, these responses are the only information available to downstream neurons. Accordingly, we used a novel analysis technique to assess how much information about task variables is encoded in the responses and the timing of the most informative responses during each trial. In this analysis, we first determined the probability of finding a given inter-spike interval (ISI) pattern conditioned on its occurrence during a particular task condition (e.g., go or no-go). Using this conditional probability, we inferred a task variable on a specific trial (e.g., is this a go or no-go trial?) by considering the responses on that trial as a sequence of ISI patterns and updating our certainty about the variable using Bayes' Rule as the trial progresses . Using this method, we find that (1) information about task variables is distributed throughout auditory and frontal cortex indicating that neurons in both regions have access to information regarding the stimulus (23/41 cells in AC and 18/29 cells in FC) and the future choice of the animal (23/41 cells in AC and 17/29 cells in FC), (2) responses related to the stimulus are most informative well after stimulus presentation has ended, and (3) accurate encoding of stimulus identity predicts improved behavioral performance. We are currently investigating how these representations vary across cortical regions and how robust these representations are across neurons and individual animals.

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Poster

530. Auditory Processing: Neural Coding, Animal Studies, and Computation

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Topic: D.02. Auditory

Title: Characterization of invariances to stimulus perception in auditory processing

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Abstract: Local probabilistic invariances, defined by the range of transformations that can be applied to a sensory stimulus without affecting the corresponding neural response, are largely unstudied in auditory cortex. We propose to assess these invariances at two stages of the auditory hierarchy using single neuron recordings from the primary auditory cortex (A1) and the secondary auditory cortex (PEG) of awake, passively listening ferrets. First, we hypothesize that neurons will show increasing degrees of local invariance at successively more central stages of the auditory hierarchy. To test this hypothesis, we considered two types of stimuli commonly known to evoke activity in the auditory system. The first set of stimuli was a sequence of isolated pure tones with randomly varying frequency, spanning 5-6 octaves and encompassing the best frequencies of the recorded neurons. The second was a sequence of bandpass noise bursts with varied durations, consisting of 20-30 bursts that logarithmically tile 5-7 octaves, thus achieving a bandwidth each of approximately 0.25 octave. Using these data sets, we have analyzed local invariance to frequency shifts by estimating the width of the tuning curve for the best responding neurons, and found that the corresponding 95% confidence interval was significantly larger in PEG than in A1. Similarly, preliminary analyses on invariance time shifts indicated that the invariance to time shift is higher in PEG than in A1. Second, we further hypothesize that parametric models having built-in invariance properties will provide a suitable basis to model spectro-temporal receptive fields (STRF). To confirm this hypothesis, we fitted low-parametric invariant kernels (9 parameters), in a boosting optimization setup using species-specific vocalization stimuli in A1. The performances of these models were then compared to similarly fitted non-parametric STRF (361 parameters). According to Akaike's Information Criterion, which corrects for model complexity, we found that the invariance-based models were significantly better models in A1. The generalization capability of the models, assessed with Pearson's correlation coefficient in a testing dataset, was also significantly better for the invariant kernels. We interpret this to mean that the constraints of invariant kernels capture well auditory neuronal dynamics. Overall, our result show that (a) stimulus invariance to frequency and time shifts are present at every stage and increase along the hierarchical auditory processing and (b) at least in the early stages, parametric models having invariance properties by design, are well-suited to describing biological functions.

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Poster

530. Auditory Processing: Neural Coding, Animal Studies, and Computation

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Topic: D.02. Auditory

Support: NIH Grant R01-DC009215

Title: Neural encoding of noisy vocalizations in primary auditory cortex of the marmoset monkey

Authors: *R. NI[1], N. M. LEDBETTER[1], J. R. GAMBLE[1], D. A. BENDER[2], D. L. BARBOUR[1]
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Abstract: The ability to extract behaviorally relevant sensory information from a cluttered environment is important for humans and animals to survive. Relatively little is known, however, about the single and population cortical neuron representation of noisy vocalizations in primates. To uncover how the neural code of behaviorally relevant signals is degraded when contaminated by noise, the neural representation of conspecific vocalizations embedded in noisy backgrounds was studied in marmoset monkey primary auditory cortex. Twenty different natural marmoset vocalizations were mixed with white noise to generate noisy vocalizations of different signal-to-noise (SNR) ratios (-15 dB to 20 dB SPL), and the corresponding responses of individual auditory cortical neurons were recorded in awake marmoset monkeys. Information theoretic analysis showed that the addition of white noise in vocalizations led to a monotonically decreasing relationship between total information content represented in neural code and SNR. This observation was consistent across different types of vocalizations. In addition, neural responses were quantified with an extraction index profile, which measures the similarity between neuron responses to distorted vocalizations (i.e., different SNRs) and responses to target vocalizations. About one-third of neurons displayed an extraction index profile with a critical SNR value around -5 dB SPL, below which the neural representation was more noise-like instead of vocalization-like. This trend agrees with studies in other species and supports the existence of noise-invariant neurons, while also revealing the overall variety in individual neuronal responses. Furthermore, by applying dimensionality reduction techniques to the pooled single neuron responses, we were able to visualize dynamic neural ensemble responses to noisy vocalizations as trajectories in low-dimensional space. The resulting trajectories showed a clear transition from pure vocalization to pure noise with a systematic change in trajectory shape,

demonstrating that the population of neurons encodes vocalizations more precisely than the individual neurons. The results of this study revealed the effect of white noise on the neural encoding of noisy vocalizations, a better understanding of which is critical for providing the link between cortical neural responses and auditory perception in primates in challenging acoustic environments.

Disclosures: R. Ni: None. **N.M. Ledbetter:** None. **J.R. Gamble:** None. **D.A. Bender:** None. **D.L. Barbour:** None.

Poster

530. Auditory Processing: Neural Coding, Animal Studies, and Computation

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Support: NIH Grant R01-DC009215

Title: Contribution of transient neural responses to stimulus discriminability in auditory cortex

Authors: *W. SUN, N. M. LEDBETTER, D. L. BARBOUR
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Abstract: In response to steady-state sound stimuli, neural responses are transiently spread across a large number of neurons following sound onset, then restricted to sustained firing in a small subset of neurons. To better understand the role of transient and sustained components in sensory coding, neural spiking responses to 500 ms tones were tested at six different frequencies across 1 to 32 kHz at 85 dB SPL for 99 neurons in the auditory cortex of awake marmoset monkeys. On average, the number of stimuli an individual neuron is responsive to is larger immediately following stimulus onset than during the later portion of the stimulus. This is consistent with previous observations of higher selectivity for sustained responses than onset responses. Then the ability to recover the stimulus frequency label from the responses of the neuron population was tested every 20 ms through the recording duration. Principal component analysis and linear discriminant analysis were used to construct a linear classifier for each time point. The frequency prediction accuracy quickly peaked to about 85% immediately following sound onset, and then gradually decreased to a plateau. Immediately following sound offset, prediction accuracy exhibited another, smaller second peak. Therefore, despite the low selectivity of onset responses of individual neurons, these responses when combined across a

neuron population are sufficient to achieve stimulus discriminability. To better understand the population response dynamics, firing rate response trajectories were examined next. The instantaneous firing rates of the neuron population were treated as a point in a high dimensional space, where each dimension is the response amplitude of one neuron. Connecting the firing rate points at different time points in temporal order produces a trajectory in the neural response space. Corresponding with the discriminability results, the population response trajectory quickly emerged from the locus of spontaneous firing after sound onset, finished a transient dynamical response process, and remained around a fixed point for the sustained portion of the stimuli before returning to the spontaneous locus after sound offset. This result reveals that in response to steady-state sound stimuli, the neural system begins responding with a dense code and quickly evolves to a stable state maintained with a sparser code. This response trajectory pattern and the distinct properties of onset and sustained responses resemble the behavior of the neurons in the olfactory system, potentially signifying a unified neuronal strategy for sensory coding.

Disclosures: W. Sun: None. **N.M. Ledbetter:** None. **D.L. Barbour:** None.

Poster

530. Auditory Processing: Neural Coding, Animal Studies, and Computation

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Title: Neural mechanisms underlying long lasting contextual modulations in auditory cortex of awake marmoset studied by intracellular recording

Authors: *L. GAO[1], X. WANG[2]
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Abstract: Extracellular recording studies have demonstrated long-lasting contextual modulations in spiking activity in auditory cortex of many species. For example, preceding sound stimuli with long duration could suppress or facilitate responses to succeeding sound stimuli for as long as a few seconds after the preceding stimulus ends in auditory cortex of awake marmosets (e.g. Bartlett and Wang 2005). Such long-lasting modulations are often specific to both preceding and following stimuli and likely form the basis for contextual processing in auditory cortex.

However, cellular mechanisms for the contextual modulations in auditory cortex are largely unknown. Using an intracellular recording technique developed in our laboratory for awake marmosets, we have studied neural mechanisms for contextual modulations in auditory cortex of the marmoset (callithrix jacchus), a highly vocal and social primate species. We found that there were long-lasting depolarization and hyperpolarization after sound termination in many cortical neurons. Furthermore, these long-lasting subthreshold events depended on parameters of sound stimuli, such as stimulus type, sound level and duration, carrier and modulation frequencies and so on. The duration of these subthreshold events after sound termination can last from hundreds of millisecond to more than one second, which is comparable to the duration of long-lasting contextual modulation observed in spiking activity. These findings suggest that long-lasting depolarization and hyperpolarization after the sound termination are possible neural correlates of long-lasting contextual modulations in auditory cortex under the awake condition.

Disclosures: L. Gao: None. **X. Wang:** None.

Poster

530. Auditory Processing: Neural Coding, Animal Studies, and Computation

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Support: NSF Grant 333-1131

Title: Frequency tagging and neural correlates of the cocktail party effect in the monkey inferior colliculus

Authors: *D. S. PAGES, V. C. CARUSO, S. TOKDAR, J. M. GROH
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Abstract: Humans and monkeys can perceive multiple simultaneous sounds in an auditory environment. Identifying the neural substrates supporting perception of each sound is challenging because it is difficult to determine from firing rates alone which of the multiple stimuli evokes a given spike. Here, we employ a frequency-tagging approach similar to that used in the EEG domain. Neurons in the inferior colliculus tend to phaselock their action potentials to the envelope of a sound. By evaluating phaselocking of spikes to each of two simultaneous sounds with different envelopes, we can infer which sound in the auditory environment triggered them. . We presented auditory environments containing two broadband sounds, each of which

originated from a different location and had a different AM envelope, to 2 monkeys who were passively listening. We found that most sites in our sample (N=17/27 recording sites) preferentially synchronized their spikes to the envelope of one of the two sounds (circular K test, $p<0.00001$, p value corrected for two conditions per site). Usually the more contralateral sound induced more phaselocking, regardless of carrier frequency (sign test, N=27, $p<0.001$). This was true even if the more contralateral sound evoked fewer spikes overall than the more ipsilateral sound (when each was presented separately). For example, even in cases where the ipsilateral sound was a better match to a neuron's frequency response area, neurons exhibited greater phaselocking to the contralateral sound. Furthermore, neurons reliably phaselocked to the ipsilateral sound when it was presented in isolation, so the preferences in phaselocking we observe are specific to the dual-sound condition. Together, these findings suggest the presence of a neural assembly "sorting" mechanism that is active when the animal is exposed to more than one sound at the same time, but weaker or absent when the animal is exposed to a single sound. . Our results implicate phaselocking as part of the mechanism contributing to maintaining information about multiple sound sources, and suggest that this mechanism exerts effects at the level of the inferior colliculus.

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Poster

531. Auditory Processing: Neural Coding, Human Experiment, and Theory

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Title: Specialized auditory temporal processing in the theta and gamma bands revealed by priming

Authors: *X. TENG, D. POEPPEL
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Abstract: Abstract The auditory system extracts acoustic information at different timescales to form a global percept, and cortical oscillations are argued to play an important role in chunking acoustic information. It has been proposed that acoustic dynamics at a longer timescale (~ 200 ms) are tracked by the theta oscillations (4-7 Hz) and at a shorter timescale (~30 ms) by low

gamma oscillations (here: 30-45 Hz). We test to what extent theta and gamma oscillations are specialized for processing information at the corresponding timescales and whether an intermediate timescale (~ 100 ms) is tracked by a corresponding oscillation band (alpha). Here we use a priming paradigm in the context of MEG recording to address this question. After being primed by sounds with modulation rates in the theta and gamma band ranges, we find that the power is maintained at the theta and gamma bands, but is decreased at other bands. Priming with sounds of different modulation rates does not cause corresponding power decreases. Interestingly, the sound with the modulation rate in the alpha band range does not induce such a priming effect. Furthermore, the priming effect is largely observed in the right hemisphere. These results suggest that the theta and gamma band oscillations are specifically driven by the sounds of corresponding modulation rates and that there are specialized processing modes for acoustic information at the long and short timescales (~200 and ~30 ms), but a different processing regime at an intermediate scale (~ 100 ms).

Disclosures: X. Teng: None. **D. Poeppel:** None.

Poster

531. Auditory Processing: Neural Coding, Human Experiment, and Theory

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NSF

Title: Influence of aging on cortical auditory temporal processing of speech in noise

Authors: *A. PRESACCO[1], S. ANDERSON[2], J. SIMON[3]
[1]Hearing and Speech Sci., Univ. of Maryland, College Park, MD; [2]Hearing and Speech Sci., [3]Electrical and Computer Engin., Univ. of Maryland, College Park, MD

Abstract: Older adults frequently report that they can hear what they have been told, but cannot understand the meaning, particularly in a noisy environment. The use of hearing aids is beneficial in quiet, but less so in noise, suggesting that weakening of signal amplification is not the main problem. Earlier results have demonstrated that low frequency auditory cortical activity is so reliably synchronized to the slow temporal modulations of speech that it can be used to

reconstruct the speech envelope, even when the background noise is significantly stronger than the speech signal. This has been shown only in young adults, however. We hypothesize that the neural encoding of the speech envelope in background noise is degraded in older adults compared to younger adults, even when the older adults have “normal hearing” based on commonly used clinical assessments. Thus, in the presence of noise, accurate cortical temporal encoding would be a prerequisite for speech recognition and would affect the performance of listeners for speech-in-noise recognition. Magnetoencephalography (MEG) recordings were made from 8 younger (20 - 28 years) and 8 older adults (60 - 68 years old), all with normal hearing, while listening to two simultaneous speech streams but attending to only one stream. Participants’ sentence recognition in noise was behaviorally assessed using the Quick Speech-in-Noise test (QuickSIN). The MEG responses in the 1 - 8 Hz frequency band were used to reconstruct the envelope of the speech stimulus to which the participant was instructed to attend, with success measured by the linear correlation between the reconstructed and actual speech envelope. Results show that both younger and older adults’ neural responses can reconstruct the envelope of the target speech, even at 0 dB SNR. The two groups are differentiated, however, by the inability of older adults to efficiently suppress the competing speech, as demonstrated by their reduced contrast in decoding accuracy between the target and competing speech. Moreover, younger adults had significantly lower thresholds on the QuickSIN than older adults. Our findings suggest that aging may indeed cause deterioration of auditory temporal processes at the cortical level, a potential key explanation for why normal hearing older adults have problems understanding speech in noise.

Disclosures: **A. Presacco:** None. **S. Anderson:** None. **J. Simon:** None.

Poster

531. Auditory Processing: Neural Coding, Human Experiment, and Theory

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 531.03/FF28

Topic: D.02. Auditory

Support: ERC "Predispike" ERC-2012-StG_312227

James S. McDonnell Foundation award: "Understanding Human Cognition“

ANR-10-LABX-0087 IEC

Title: Nonlinearities of auditory neurons explained by predictive coding

Authors: *I. B. YILDIZ, S. DENEVE
Ecole Normale Superieure, Paris, France

Abstract: The response properties of neurons in the auditory system are usually characterized by the spectrotemporal receptive fields (STRF). Despite its descriptive power, the STRF performs poorly when used to predict auditory neuronal responses because of the nonlinearities in the responses that are functionally important. Moreover, response properties of auditory neurons are subject to significant variability depending on the context, percept, and task. Here, we propose that auditory neurons are predictors rather than filters of their input and we hypothesize that they have a "true selectivity" independent of stimulus context. We build a dynamic Bayesian inference model and train it on a large database of natural speech to reveal this invariant selectivity, i.e. predictive fields. We show that the model can account for nonlinear contextual effects such as two-tone and forward suppression. We also demonstrate that the model neurons can adapt their predictive fields to recent input statistics such as behaviorally relevant tones in an online fashion. Moreover, simulations reveal how the STRF of a neuron depends on the selectivity and dynamics of other neurons in the network and point out the importance of analyzing neuronal data collectively instead of describing each neuron's response separately. Furthermore, we employ the model to analyze neuronal responses to natural speech obtained from the primary auditory cortices of ferrets. Following the hypothesis that auditory neurons aim to reconstruct their stimuli, we compute the decoding filters of auditory neurons as an approximation to their predictive fields and then employ the model to quantitatively describe neuronal responses. As hypothesized by the model, the decoding filters of auditory neurons differ significantly from the encoding filters (STRFs) and they provide a basis for explaining nonlinear interactions between neurons which cannot be explained by classical receptive fields. This confirms the view that auditory responses are not only shaped by the stimulus itself but also by other neurons' responses and selectivities. In conclusion, the proposed model (i) provides mechanistic explanations to several nonlinear phenomena in auditory processing, (ii) introduces a state-of-the-art analysis tool for novel interpretations of neuronal data, (iii) produces testable hypotheses and measurable quantities to design and analyze future auditory experiments.

Disclosures: I.B. Yildiz: None. **S. Deneve:** None.

Poster

531. Auditory Processing: Neural Coding, Human Experiment, and Theory

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Topic: D.02. Auditory

Support: NIH Grant DC02260

NIH Grant DC011874

Title: Maximum noise entropy models reveal multidimensional auditory cortical receptive fields

Authors: *C. A. ATENCIO[1], T. O. SHARPEE[2], C. E. SCHREINER[1]
[1]Otolaryngology, UCSF, SAN FRANCISCO, CA; [2]Computat. Neurobio. Lab., The Salk Inst. for Biol. Studies, La Jolla, CA

Abstract: The receptive fields of many auditory cortical neurons are multidimensional. However, standard methods that are often used to characterize multidimensional stimulus selectivity, such as spike-triggered covariance (STC) or maximally informative dimensions (MID), are either limited to Gaussian stimuli or are only able to recover a few stimulus features due to data limitations. To overcome these limitations, we applied an information theoretic extension of STC, the maximum noise entropy (MNE) model. The model assumes a logistic nonlinear input/output function and can be used with non-Gaussian stimulus distributions to find an arbitrary number of stimulus dimensions. For auditory cortical neurons, we often found more than two stimulus dimensions that influenced neuronal firing. Additionally, we employed two stimuli that differed only in their short-term correlation structure and found that cortical neurons were sensitive to these stimulus statistics. Our results suggest that the MNE approach is a principled method that can be used to recover the multidimensional receptive fields of auditory neurons.

Disclosures: C.A. Atencio: None. **T.O. Sharpee:** None. **C.E. Schreiner:** None.

Poster

531. Auditory Processing: Neural Coding, Human Experiment, and Theory

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Program#/Poster#: 531.05/FF30

Topic: D.02. Auditory

Support: NIH Grant DC010439

Title: Combinatorial analysis of models for high-order neural representation of natural sounds

Authors: *I. L. THORSON[1], J. F. LIÉNARD[2], A. G. DIMITROV[2], S. V. DAVID[1]
[1]Oregon Hearing Res. Ctr., Oregon Hlth. & Sci. Univ., Portland, OR; [2]Mathematics Dept., Washington State Univ., Vancouver, WA

Abstract: Despite the central role of the auditory system in guiding behavior, neural computations underlying the transformation of raw auditory inputs into meaningful high-level representations remain poorly understood. Filter models derived from theories of signal processing, such as the Spectro-Temporal Receptive Field (STRF), provide powerful tools for studying representations of complex dynamic stimuli, including vocalizations and other behaviorally relevant sounds. We have recently developed multiple model architectures, extending the classic linear STRF with models that incorporate two nonlinear mechanisms widely observed in the brain: synaptic depression (DEP STRF) and co-tuned excitatory and inhibitory currents (EI STRF). The goal of this work is to assess the ability of these new plausible models to describe the representation of natural sounds at successive stages of the auditory processing hierarchy (primary and secondary cortical areas). The experimental data used to constrain the models consist of single-unit recordings collected during the presentation of natural stimuli from across the auditory hierarchy of awake ferrets. We first used boosting methods to fit each model to a training dataset. We then performed an exhaustive combinatorial analysis of models using the same dataset, employing a stacking model combination paradigm. Finally, we assessed the performance of individual and combined models on a reserved validation dataset. In our preliminary analysis of A1 recordings, the combined models performed significantly better in predicting the validation dataset than the best individual model for 76% of neurons, confirming the existence of unexplored degrees of freedom. We further report that models including both EI STRF and DEP STRF are selected significantly more frequently in model combination than all other models, even when their individual performance in training was not significantly different. This leads to the hypothesis that the specific interplays between EI and DEP STRFs allow for better characterization of neural dynamics than the classic linear STRF. Critically, our analysis also distinguished clusters of cells that were best fit by different single models, suggesting the presence of functionally different groups of neurons. Despite being widely employed in statistical and ecological sciences, model combination techniques have been barely explored in computational neuroscience, and their applications have been mostly limited to neuroimaging. Extensions of our work at other levels of the auditory hierarchy, will allow to further testing a very large number of hypotheses about auditory coding.

Disclosures: I.L. Thorson: None. **S.V. David:** None. **J.F. Liénard:** None. **A.G. Dimitrov:** None.

Poster

531. Auditory Processing: Neural Coding, Human Experiment, and Theory

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Topic: D.02. Auditory

Support: Action on Hearing Loss

Capita Foundation

Title: Neural computations underlying temporal and rate coding in auditory cortex

Authors: *D. A. BENDOR
Psychology and Language Sci., UCL, London, United Kingdom

Abstract: When a brief sound is slowly repeated, the resulting percept is a stream of discrete events, referred to as acoustic flutter. If the sound's repetition rate increases above ~40-45 Hz, the percept changes from flutter to fusion; the sensation of discrete events is transformed into a fused percept, with a pitch equal to its repetition rate. Within auditory cortex, repetitive acoustic stimuli are encoded by two main types of responses, synchronized and non-synchronized. Synchronized neurons represent a repeated sound temporally by virtue of their ability to stimulus lock to the onset of each repeated sound for repetition rates within the perceptual range of flutter. Non-synchronized neurons form a complimentary neural code, increasing their firing rate monotonically with repetition rate over the perceptual range of fusion. To explore the underlying mechanisms that could generate these dichotomous neural coding regimes, I created a leaky integrate and fire (LIF) computational model of an auditory cortical neuron. Using this model, I find that strong, delayed inhibition (relative to excitation) leads to stimulus synchronization while non-synchronized responses can be generated by excitation occurring in close temporal proximity with weaker inhibition. To help validate this model, I recorded single unit activity in the auditory cortex of four awake marmosets, and tested several predictions made by this computational model, including the existence of additional neural coding regimes and the ability of some neurons to switch between synchronized and non-synchronized response modes. These results suggest that the underlying mechanism responsible for temporal and rate coding in auditory cortex can be parsimoniously explained as a byproduct of inhibition varying in strength and timing.

Disclosures: D.A. Bendor: None.

Poster

531. Auditory Processing: Neural Coding, Human Experiment, and Theory

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 531.07/FF32

Topic: D.02. Auditory

Title: Locally competitive networks mediated by inhibition as a basis for the development of cortical processing of natural auditory stimuli

Authors: *O. ROURKE, D. A. BUTTS
Univ. of Maryland, College Park, MD

Abstract: Connectivity to and within sensory cortices are guided by exposure to natural stimuli, but the underlying principles that might explain how cortical networks are shaped by their inputs is still not understood. We hypothesize that a fundamental property of such networks is inhibition-mediated competition between neurons, which can govern both how natural stimuli are represented by a given cortical area, as well as how the receptive field of each neuron develops. We consider cortical networks governed by excitatory- and inhibitory-specific learning rules based on spike timing, and model the development of selectivity in these layers in response to natural auditory stimuli. The inputs and outputs of the network are in population averaged firing rates, allowing networks to be stacked so that selectivity to more complex stimulus features may develop in higher layers. Furthermore, this network can be applied to visual stimuli as well, building on previous work studying sparse representation of natural visual stimuli. This work helps distill how the interacting excitatory and inhibitory networks allow for the collective processing of a variety of inputs, with implications to both experimental work and to machine learning.

Disclosures: O. Rourke: None. **D.A. Butts:** A. Employment/Salary (full or part-time):; University of Maryland.

Poster

531. Auditory Processing: Neural Coding, Human Experiment, and Theory

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Program#/Poster#: 531.08/GG1

Topic: D.02. Auditory

Support: NSF BCS-1027761

AFOSR FA9550-12-10388

Title: A neurodynamic model of pitch perception

Authors: *K. D. LERUD, J. C. KIM, E. W. LARGE, III
Psychology, Univ. of Connecticut, Storrs, CT

Abstract: All levels of the auditory system are known to respond to the amplitude envelope of complex sounds, and amplitude modulation is important for the perceived pitch of these sounds. However, the calculated envelopes of certain sounds can be identical while the sounds elicit different pitches. This can result, for example, from complex sounds consisting of multiple equally spaced frequency components when the absolute frequencies in the sounds differ. This is the case if one starts with multiple higher harmonics of a missing fundamental, and then shifts these harmonics linearly up or down. The perceived pitch then shifts in the same direction of the frequency shift, but to a lesser degree that is a function of harmonic number. The perceived low pitch of higher complexes such as these is called residue pitch, and this specific phenomenon is called “pitch shift of the residue”. Increasing amounts of this shift can result in ambiguity of pitch, in which the perceived frequency may vary depending on context. Since sounds with this relationship have identical amplitude envelopes, their temporal fine structure clearly plays a role in their perceived pitches. Here we develop an account and a model of these phenomena with canonical nonlinear oscillators. Organized in gradient-frequency neural networks, they model essential signal processing characteristics of the auditory system. The networks correctly predict the multistable pitch correlates of the stimuli. Dynamical analysis shows, as predicted, that the fine structure of these stimuli plays a crucial role in the development of regions of network resonance corresponding to the pitch perceptions. These results arise naturally in highly nonlinear systems, of which the auditory system is a prime example.

Disclosures: K.D. Lerud: None. **J.C. Kim:** None. **E.W. Large:** None.

Poster

531. Auditory Processing: Neural Coding, Human Experiment, and Theory

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Topic: D.02. Auditory

Support: K18 DC011602

ERC ADAM E-138

R01 DC007657

ERC295603

Title: Facilitated inhibition biases tritone comparison in a network model

Authors: *C. HUANG[1], B. ENGLITZ[3,4], S. SHAMMA[4], J. RINZEL[1,2]
[1]Courant Inst. of Mathematical Sci., [2]Ctr. for Neural Sci., New York Univ., New York, NY; [3]Auditory Behavior & Computat. Neurosci. Group, Equipe Audition, École normale supérieure, Paris, France; [4]Dept. of Electrical and Computer Engin., Univ. of Maryland, College Park, MD

Abstract: Perception can be strongly influenced by preceding context. Ambiguous stimuli are well suited to study the contextual effect. Shepard tones are comprised of octave-spaced pure tones, represented by their base pitches within one octave. A sequentially played pair of Shepard tones can be perceived as ascending or descending if the second tone, T2, is less than one-half octave above or below, respectively, the first tone, T1 [Shepard 1964]. The comparison task is ambiguous when two Shepard tones are separated by a half octave (tritone). However, a pre-test sequence of Shepard tones with pitch classes between the tritone pair can bias the perception toward ascending/descending for biasing tones above/below the first test tone [Chambers and Pressnitzer, 2011]. Results of the two-tone comparison with biasing implies a shorter perceived distance between T1 and T2 across the biasing region while a pitch-based decoding algorithm of extracellular recordings in A1 of awake ferrets indicates a larger distance [Englitz et al., 2013]. To investigate potential neural mechanisms of the Shepard tone comparison, we consider two tonotopically arranged direction-selective populations (UP and DOWN) that drive a common inhibitory array that provides recurrent feedback but with oppositely directed asymmetric footprints. Inhibitory synapses slowly facilitate in the biasing region. When the biasing tones are below T2, DOWN units tuned to T2, which receive inhibition from where facilitation has built up, are more suppressed than UP units. Hence, competition is favored toward UP units. Similarly, when biasing tones are above T2, DOWN units will win over UP units. The biasing effects gradually build up with the number of bias tones, reaching above 90% after 5 tones. Moreover, we found that pitch class of the most effective bias tone depends on the tuning width of the inhibitory population; tones near T2 have strongest effects if tuning is narrow, while tones at center between T1 and T2 is most effective if tuning is broad. Our model also accounts for the variation among subjects in the tritone comparison when non-uniform inhibitory synaptic strengths are incorporated. A simplified winner-take-all model is analyzed to explain the mechanisms of biased competition. Lastly, we compare alternative mechanisms for adaptation, such as feedforward depression and spike-frequency adaptation, with our model with the same architecture. In sum, our model is an idealized mean-field model, designed to account for trends

in behavior data. We utilize frequency shift detectors to account for the results of Shepard tones and predict that facilitation of inhibitory synapses underlies the context effects.

Disclosures: **C. Huang:** None. **B. Englitz:** None. **S. Shamma:** None. **J. Rinzel:** None.

Poster

531. Auditory Processing: Neural Coding, Human Experiment, and Theory

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Topic: D.02. Auditory

Support: NSF grant IIS-1116530

Title: Noise-robust automatic speech recognition by detecting syllables and phonetic features

Authors: ***P. SCHAFER**, D. Z. JIN
Pennsylvania State Univ., University Park, PA

Abstract: Despite decades of technological development, systems for automated speech recognition still underperform human listeners. This performance disparity is particularly large in noisy acoustic environments. Previously, we demonstrated that sparse spike representations modeled from those seen in auditory cortex can robustly represent the phonetic content of speech, and that sequences of spikes can be used to recognize isolated words in noise. However, in the case of continuous speech, where the word boundaries are not known, we find that noisy corruption of the spike code leads to insertion errors. Here we present a new, perceptually-motivated method for identifying syllable locations in continuous speech, which constrains the alignment of spike patterns to words and eliminates these errors. We train a population of spiking neural units to detect acoustic patterns corresponding to the nuclei of syllables, and integrate the activity of these neurons to generate candidate syllable locations and durations. Using the Aurora-2 noisy digits database, we show that the method performs well in varied noise conditions without fine-tuning parameters to any one condition. To identify words, we train a separate population of feature-detecting neurons to determine the phonetic content of the speech during short time windows within each syllable candidate. A dynamic programming search finds the best alignment of feature detector spikes to the syllable candidates, thereby robustly recognizing the speech.

Disclosures: **P. Schafer:** None. **D.Z. Jin:** None.

Poster

532. Striate Cortex: Intracortical Circuitry

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Topic: D.04. Vision

Support: NIH Grant EY10115

Allen Institute for Brain Science

Lefler Foundation

NIH Grant NS085320

Title: Exploring the network anatomy of visual cortical processing

Authors: *W.-C. A. LEE[1], V. BONIN[1], M. REED[1], K. GLATTFELDER[2], J. HOHMANN[2], R. C. REID[1,2]
[1]Neurobio., Harvard Med. Sch., Boston, MA; [2]Allen Inst. for Brain Sci., Seattle, WA

Abstract: Understanding how information is processed in neuronal circuits is a central question in neuroscience. A neuron's function is fundamentally dependent on how it is connected within its network. Therefore, understanding the relationship between connectivity - circuit structure - and cellular function will help us understand how neurons and networks transform information to bring about perception and behavior. Recent advances in non-linear light and high-throughput electron microscopy (EM) allow detailed mapping of neuronal sensory physiology and network anatomy. To examine the sparsely connected pyramidal cell network, we used volumetric *in vivo* two-photon calcium imaging of a genetically-encoded calcium indicator to measure the time-resolved responses of a large population of identified neurons to a wide array of stimuli. We then used large-scale EM of serial sections to reconstruct the local neuronal network. In contrast to local connectivity of the inhibitory circuit, we find that the local connectivity between excitatory neurons exhibits functional specificity. First, we find that excitatory neurons with similar sensory physiology converge onto downstream neuronal targets. Interestingly, downstream, interlaminar targets have dissimilar selectivities. Finally, we observe topological organization of synaptic input onto dendrites from individual axons and from axons of presynaptic cells with similar tuning. This wiring specificity may act as a substrate for computations underlying cortical sensory processing.

Disclosures: **W.A. Lee:** None. **V. Bonin:** None. **M. Reed:** None. **K. Glattfelder:** None. **J. Hohmann:** None. **R.C. Reid:** None.

Poster

532. Striate Cortex: Intracortical Circuitry

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NSF GRF DGE-0909667

Title: Resting state correlations in visual cortex reflect fluctuations of cortical arousal

Authors: ***B. MOORE**, M. A. COX, K. DOUGHERTY, M. S. YOUNG, A. MAIER
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Abstract: The spatial structure of spontaneously occurring neural correlations, termed functional connectivity, is highly organized and often reflects known anatomical connections at multiple scales. These spontaneously generated neural activity patterns are typically recorded with subjects resting idly rather than engaging in a task. Even in the absence of explicit stimulation, however, a subject's internal state is constantly changing. As resting state studies typically collapse data across several minutes of time, the influence of these internal dynamic variables on spontaneous neural activity is largely uncharacterized. In order to determine whether cortical functional connectivity during rest is constant or time-varying, we examined dynamic changes of spontaneous neural correlations across the laminae of primary visual cortex. Local field potentials (LFPs) were recorded from two linear multi-electrode arrays that were simultaneously placed in nearby (1-5mm) locations in V1 of two macaque monkeys that were at rest in a dark room. We first examined LFP coherence between all electrode positions across the cortical laminae and found that for all electrode pairs, interlaminar coherence varied greatly throughout our recording period. We then compared these dynamic changes in interlaminar functional

connectivity with a time-varying measure of cortical arousal. More specifically, we related the pair-wise measures of LFP coherence to fluctuations of an arousal-related index that was derived from the ratio between high frequency (60-100Hz) and low frequency (5-10Hz) LFP power. We found that interlaminar coherence correlated inversely with this index, suggesting that periods of low arousal are marked by periods of high coherence between laminae. Coherence between electrode locations within the same laminar compartment covaried less with the state of cortical arousal than coherence between electrode contacts spaced farther (>1mm) apart. These findings add to the body of evidence suggesting that resting state functional connectivity is not a static condition but rather a dynamic process that is linked to changes in cortical arousal.

Disclosures: B. Moore: None. **M.A. Cox:** None. **K. Dougherty:** None. **M.S. Young:** None. **A. Maier:** None.

Poster

532. Striate Cortex: Intracortical Circuitry

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NWO VENI grant to M.R. (#451-09-025)

Title: Orientation tuning of Gamma band measured in human MEG and across multiple scales of measurement in macaque V1 reveals network mechanisms

Authors: *M. J. ROBERTS[1,2], E. LOWET[1,2], A. HADJIPAPAS[3], A. PETER[4], P. DE WEERD[1,2]
[1]Dept. of Cognitive Neuroscience, Fac. of Psychology and Neurosci., Univ. of Maastricht, Maastricht, Netherlands; [2]Donders Institue for Brain, Cognition and Behavior, Radboud Univ. Nijmegen, Nijmegen, Netherlands; [3]Univ. of Nicosia Med. Sch., Nicosia, Cyprus; [4]Ernst Strungmann Inst. for Neurosci. in cooperation with Max Planck Society, Frankfurt, Germany

Abstract: Studying the properties of gamma band oscillations offers a route to understanding their generative mechanisms; however different measurement techniques may be sensitive to different generative mechanisms. Single unit spikes and network (gamma band) activity in primate visual cortex are sensitive to stimulus orientation. However, while the preferred

orientation of single units is variable between orientation columns, gamma band power and frequency have more uniform preferred orientations across the population. One possible explanation for this discrepancy may be that most measures of gamma band activity combine activity over large volumes of cortex typically encompassing many orientation columns. Orientation tuning of network gamma activity may therefore reflect the summation of activity over a volume of cortex in which there is an overrepresentation of specific orientation domains (summation hypothesis). Alternatively, the gamma band may reflect the temporal coordination of activity within a large synchronous network encompassing many orientation columns, orientation tuning may here reflect network properties in which all units have the same behavior (network hypothesis). To dissociate these hypotheses we measured gamma band orientation tuning using a range of measurement techniques sensitive to various spatial scales from single unit activity, Current Source Density, Local Field Potential and surface ECoG in Macaques to MEG in humans. In all experiments, gratings of varying orientation where presented parafoveally while the subject performed a color change detection task at fixation and ignored the grating. We reasoned that under the summation hypothesis orientation preference diversity and closeness to spike rate orientation preference would increase for the more local signals. However, in line with the network hypothesis, we found that even for the most local signals gamma band orientation tuning was homogonous over the population and was not associated with spike rates. We conclude that 1) gamma band activity and spike rate tuning do not reflect the same process. 2) Gamma band activity recorded over a range of measurement scales reflect largely the same process. This work highlights the major contribution of basic stimulus features in determining gamma power and frequency in addition to high level factors such as grouping or attention.

Disclosures: **M.J. Roberts:** None. **E. Lowet:** None. **P. De Weerd:** None. **A. Hadjipapas:** None. **A. Peter:** None.

Poster

532. Striate Cortex: Intracortical Circuitry

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Helmholtz Association: HASB and portfolio theme SMHB

Jülich Aachen Research Alliance (JARA)

Title: Computing local-field potentials based on a point-neuron network model of cat V1

Authors: *D. DAHMEN[1], E. HAGEN[1,2], M. L. STAVRINOU[2], H. LINDÉN[3,4], T. TETZLAFF[1], S. VAN ALBADA[1], M. DIESMANN[1,5], S. GRÜN[1,6], G. T. EINEVOLL[2,7]
[1]Inst. of Neurosci. and Med. (INM-6)&Inst. for Advanced Simulation (IAS-6), Jülich Res. Ctr., Jülich, Germany; [2]Dept. of Mathematical Sci. and Technol., Norwegian Univ. of Life Sci. (NMBU), Ås, Norway; [3]Dept. of Neurosci. and Pharmacol., Univ. of Copenhagen, Copenhagen, Denmark; [4]Dept. of Computat. Biol., Royal Inst. of Technol. (KTH), Stockholm, Sweden; [5]Med. Fac., [6]Theoretical Systems Neurobio., RWTH Aachen Univ., Aachen, Germany; [7]Dept. of Physics, Univ. of Oslo, Oslo, Norway

Abstract: While recordings of extracellular potentials are common in monitoring neural activity, the interpretation of the low frequency part, the local field potential (LFP), remains ambiguous. Several studies have shown that the LFP depends on the electrode position, tissue volume conduction, neuronal morphology, membrane properties, synaptic locations and -input correlations [1-4]. To gain insight into the relation of experimentally measured LFPs to the underlying spiking network activity, we have developed a hybrid modeling scheme. In this framework, spike trains generated by a network of single-compartment leaky integrate-and-fire neurons are used as synaptic input onto equivalent multi-compartment neurons with reconstructed morphologies, which in turn generate the LFP. Here, we apply this scheme to a multi-layered excitatory-inhibitory (E-I) point-neuron network model (implemented in NEST [5]) describing 1 mm^2 of cat primary visual cortex (modified from [6], Fig. 1a,b). For the LFP model (Fig. 1c,d), unconnected neurons of sixteen cell types with passive membranes (implemented with LFPy [7] / NEURON [8]) receive type- and layer-specific inputs derived from the network model and anatomical data [9]. In both models, a total of 78000 cells are distributed across layers 2/3 to 6. We show that both spontaneous and stimulus-evoked LFPs (ERPs) depend critically on the underlying network state (local recurrent dynamics). Further, we demonstrate that the effect of synaptic-input correlations on LFP power can only be accounted for in a full-scale model with realistic neuron density.

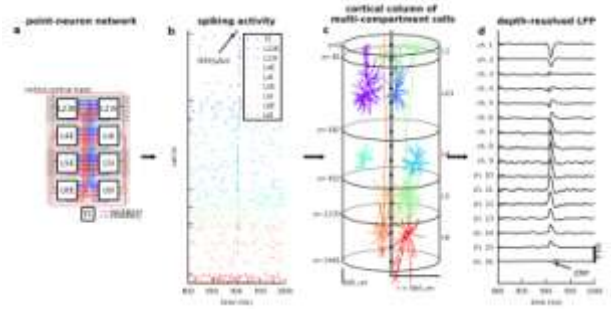

Figure 1: Sketch of the hybrid model. Thalamic stimulus at t=900ms. References: 1. Lindén et al. (2011). Neuron. 72:859 2. Einevoll et al. (2013). Nat Rev Neurosci. 14:770 3. Bedard et al. (2004). Biophys. J. 86:1829 4. Reimann et al. (2013). Neuron. 79(2):375 5. Gewaltig & Diesmann (2007). Scholarpedia 2(4):1430 6. Potjans & Diesmann (2012). Cereb Cortex. 24:785 7. Lindén et al. (2014). Front Neuroinform. 7:41 8. Hines et al. (2009). Front Neuroinform. 3:1 9. Binzegger et al. (2004). J Neurosci. 24:8441

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Poster

532. Striate Cortex: Intracortical Circuitry

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Title: Stimulus dependent spatial distribution of synaptic inhibition in simple cells of cat striate cortex

Authors: ***M. SEDIGH-SARVESTANI**, L. VIGELAND, L. A. PALMER, D. CONTRERAS
Univ. of Pennsylvania, Philadelphia, PA

Abstract: Our goal is to understand visually evoked synaptic inputs to thalamorecipient simple cells in primary visual cortex. The push-pull model of simple cell receptive fields (RF) based on extracellular recordings (Palmer and Davis, 1981) suggests excitatory and inhibitory synaptic inputs with reciprocal spatial arrangement. In contrast, the lateral inhibition model suggests

broad inhibitory synaptic input covering all regions of the receptive field (Priebe and Ferster, 2008). Both models assume that the spatial distribution of synaptic excitation and inhibition is fixed and independent of the visual stimulus. We conduct measurements of excitatory and inhibitory synaptic input to simple cells in the cat striate cortex. Our preliminary data suggests that the spatial distribution of synaptic inhibition in simple cells depends on the spatiotemporal properties of the stimulus and is not a fixed property of the RF. We use sharp electrodes to obtain intracellular recordings of simple cells in layer 4 of the primary visual cortex of the anesthetized and paralyzed adult cat. We use two different stimulus sets: (i) individual bars of light of optimal orientation at 2 different contrasts (bright/dark) and 8 positions across the cell's RF and, (ii) the same 8 optimally oriented bars presented simultaneously, with bright or dark contrasts. We inject 5-7 levels of current into the cell during visual stimuli to estimate the excitatory and inhibitory synaptic conductance and combined reversal potential. Some pipettes include QX-314 solution in order to block action potential generation and allow for more accurate conductance estimates. We observe both push-pull and spatially broad inhibition in simple cells. Single bar stimuli usually result in spatially broad inhibition whereas multiple bar stimuli result in push-pull type inhibition. The spatial distribution of excitation is similar under both stimulus conditions. Thus our results indicate that the spatiotemporal properties of the stimulus modulate the spatial distribution of intracortical inhibition. Works Cited: Palmer LA, Davis TL. J Neurophysiol 1981; 46(2):260-76. Priebe NJ, Ferster D. Neuron 2008, 57(4):482-97.

Disclosures: **M. Sedigh-Sarvestani:** None. **L.A. Palmer:** None. **D. Contreras:** None. **L. Vigeland:** None.

Poster

532. Striate Cortex: Intracortical Circuitry

Location: Halls A-C

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Program#/Poster#: 532.06/GG9

Topic: D.04. Vision

Title: Modeling optogenetic manipulation of neural circuits

Authors: ***M. AVERY**, J. H. REYNOLDS, J. J. NASSI
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Abstract: In recent years, a host of optogenetic tools have been developed, enabling experimentalists to manipulate neuronal activity with high temporal precision and cell-type specificity. This makes it possible to causally perturb the cortical circuit in ways that, when

linked with computational modeling techniques, enable the testing of specific hypotheses regarding how optogenetic activation of excitatory pools influences neurons embedded in a network. Using optogenetics in the alert macaque, we have found that activation of excitatory neurons results in a variety of different forms of facilitation and suppression of visually-evoked responses. These include additive, multiplicative, and more complex changes in the contrast response function, including suppression, even under conditions when the laser alone evokes an excitatory response. We showed that many of these changes can be accounted for by a normalization model in which optogenetic stimulation recruits both excitation and inhibition in the local cortical circuit. This model, however, is abstract, and does not adequately account for the dynamics of the network response. We have thus developed a neural network model that incorporates optogenetic stimulation as a light activated conductance expressed on excitatory neurons. We use this model to make testable predictions about how network dynamics will change under laser optogenetic stimulation conditions, including sustained laser activation and laser activation across a range of biologically relevant temporal frequencies.

Disclosures: **M. Avery:** None. **J.H. Reynolds:** None. **J.J. Nassi:** None.

Poster

532. Striate Cortex: Intracortical Circuitry

Location: Halls A-C

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Program#/Poster#: 532.07/GG10

Topic: D.04. Vision

Support: Swartz Foundation

Title: How SOM+ and PV+ inhibitory neurons could differentially modulate surround suppression of cortical neurons

Authors: ***M. JADI**, T. J. SEJNOWSKI
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Abstract: Surround suppression in the cortex can be explained by normalization models in which the output is modulated by the summed local activity. In these models, the region of the sensory space that is pooled to produce suppression to a neuron is larger than that for summation. The neural implementation of normalization in the visual cortex is thought to involve inhibitory neurons that contribute the suppressive field by summing over a larger visual space compared to the local pyramidal neurons. There are two competing models for the action of inhibitory

neurons in the neural implementation of surround suppression: The lateral inhibition model proposes that local inhibitory neurons increase their activity in response to stimulation of a larger visual space. On the other hand, the inhibition-stabilized network model predicts the activity of inhibitory neurons to decrease in response to stimulation of a larger visual space (Tsodyks et al., 1997; Ozeki et al., 2009). Empirical evidence from the visual cortex suggests both an increase (Haider et al., 2010) and a decrease in inhibitory activity (Ozeki et al., 2009) in response to surround suppression. Activity of Somatostatin-expressing (SOM+) inhibitory neurons increases during surround suppression and their optogenetic silencing weakens the suppression, thus supporting the lateral inhibition model (Adesnik et al., 2012). However, the contribution and operating regime of other subclasses of inhibitory neurons, for example the Parvalbumin-expressing (PV+) inhibitory neurons, during surround suppression is not well understood. Since the exquisite sensitivity of the cortical circuit to the manipulation of PV+ inhibitory neurons offers significant experimental challenges, we explore the two competing proposals with a computational approach. We propose a model for the surround suppression circuitry consisting of both SOM+ and PV+ inhibitory neurons in which the differences in experimental observations are accounted for by differences in the inhibitory neuron subclasses in terms of their network connectivity and post-synaptic targets.

Disclosures: **M. Jadi:** None. **T.J. Sejnowski:** None.

Poster

532. Striate Cortex: Intracortical Circuitry

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Topic: D.04. Vision

Support: MRC

Title: Spatial summation induce layer-specific gamma oscillations in the local field potential in primate V1

Authors: ***M. A. GIESELMANN**, J. CLARKSON, A. THIELE
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Abstract: Neurons of primary visual cortex (V1) typically show a lower response to visual stimuli when the stimuli are covering the neurons' receptive field (RF) as well as areas of the visual field surrounding the RF (surround suppression). We have shown earlier (Gieselmann and

Thiele, 2008) that simultaneous to the decrease in firing rate, the local field potential (LFP) recorded from the same electrode shows an increase in oscillatory activity in the gamma range, as stimuli extend into the inhibitory surround. To identify to what extent these effects differ between cortical layers and to identify the networks involved in the generation of gamma oscillations we used multi-contact laminar recordings of the LFP and current-source density analysis (CSD). We recorded from 16-channel laminar electrodes inserted perpendicularly into the primary visual cortex. Electrode contacts we arranged vertically along the probe with a distance of 150 μm between them. We trained two awake monkeys on a passive fixation task during which they were presented with a series of square-wave gratings of different sizes. We analysed 23 experiments (17 in monkey, 5 in monkey 2). For all experiments recording channels were aligned in depth to the channel closest to the border between layer 4c and layer 5. We then calculated the LFP signal generated locally at each channel by estimating the CSD on a trial-by-trial basis. Large grating stimuli (>7° diameter) consistently induced strong sustained gamma oscillations in the LFP with a peak around 40 Hz in both monkeys. However, the amplitude of the gamma peak varied strongly across layers. In both monkeys the amplitude of the gamma-peak dropped below a z-score of 3 (normalized to spontaneous activity) in channels equivalent to the granular layers (monkey 1, 150 μm below layer 4c; monkey 2, 150 μm above layer 4c alignment). In supra-granular layers the strongest gamma-oscillations were found 600 μm (monkey1, z-score=9.4) and 750 μm (monkey2, z-score=27.1) above the layer 4c alignment, equivalent to cortical layer 2/3. Compared to small stimuli (<1°) this constitutes a 1.8 fold and 4 fold increase for monkey 1 and 2 respectively. In infra-granular layers the strongest gamma-oscillations were found 450 μm below the layer 4c alignment in both monkeys (monkey1, z-score=7.5; monkey2, z-score=20.3), which is equivalent to cortical layer 5/6. This corresponds to a 1.7 fold (monkey 1) and 3.8 fold (monkey 2) increase in gamma activity compared to small stimuli. Thus, spatial summation and surround suppression as measured by the gamma power CSD, does not manifest in granular layers of V1, but is prominent in supra and infragranular layers.

Disclosures: **M.A. Gieselmann:** None. **J. Clarkson:** None. **A. Thiele:** None.

Poster

532. Striate Cortex: Intracortical Circuitry

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Topic: D.04. Vision

Support: Australian Research Coucil FT100100502

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National Health and Medical Research Council APP1004575

Title: Intra-cortical excitatory circuitry innervating layer 6 pyramidal neurons of the rat primary visual cortex

Authors: *F. COTEL[1], J. APERGIS-SCHOUTE[2,3], S. R. WILLIAMS[1,3]
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Abstract: Layer 6 (L6) is a key output layer of the visual cortex. L6 pyramidal neurons receive direct thalamo-cortical input, which is integrated together with intra-cortical circuit activity to form unique visual receptive fields (RFs). However, the source(s) of the intra-cortical excitatory circuitry that drive L6 pyramidal neurons are poorly determined. Functionally, L6 pyramidal neurons can be divided into two classes: L6-corticothalamic neurons (L6-ct), which possess end-stopped RFs, and corticoclaustral pyramidal cells (L6-cl) which display elongated RFs. In order to determine the pattern of intra-cortical excitatory input sampled by L6 pyramidal neurons we performed paired whole-cell recordings between morphologically identified L6 pyramidal neurons and excitatory neurons in all layers of the visual cortex maintained in acute rat brain-slices (P22-P30). We first confirmed the morphological division of L6-ct and L6-cl pyramidal neurons by recording from neurons retrogradely labeled from the thalamus and claustrum. The dendritic arbor of L6-ct terminates in L4 where it arborizes densely where as the apical dendrite of L6-cl extends to layer 1 with limited bifurcations. To our surprise we found that L6-cl and L6-ct neurons exhibited similar electrical properties. Paired recordings revealed that L6-ct and L6-cl pyramidal neurons were reciprocally inter-connected. In contrast, paired recording between L4 excitatory neurons and L6 pyramidal neurons revealed unitary excitatory connections only in L6-cl neurons. Strikingly and despite numerous attempts (n= 728 putative pairs) we failed to detect any connection from L2/3 and 5 pyramidal neurons to either L6-cl or L6-ct neurons. However, minimal extracellular stimulation in layer 1 evoked EPSPs in L6-cl, but not L6-ct, neurons with slow rise times (7.24±0.27ms), thus likely to result from remote, distal synapses. The use-dependent dynamics of synaptic transmission were connection-specific. L6-ct neurons evoked uEPSPs in L6-cl or L6-ct neurons with high failure rate (64%), and facilitated on repetitive activation (mean paired pulse ratio, PPR (40Hz): 4.06±0.91). In contrast, connections from L6-cl and L4 were more reliable (L6-cl: 23%; L4: 26%) and uEPSPs mostly depressed (PPR L6-cl: 0.88±0.08; L4: 0.78±0.02). Our results suggest that the receptive field properties of L6 pyramidal neurons emerge as a consequence of the intra-laminar reciprocal innervation between L6 pyramidal neurons. Uniquely we find that L6-cl pyramidal neurons receive both local L4 intra-cortical excitatory input and long-range input conveyed in layer 1, which may help to explain their elaborate receptive field properties.

Disclosures: **F. Cotel:** None. **J. Apergis-Schoute:** None. **S.R. Williams:** None.

Poster

532. Striate Cortex: Intracortical Circuitry

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Topic: D.04. Vision

Support: Wellcome Trust

ERC

Title: Probing the synaptic effects of distal connectivity in mouse visual cortex

Authors: ***T. SATO**[1], B. HAIDER[1], M. HAUSSER[2], M. CARANDINI[1]
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Abstract: Neurons in primary visual cortex (V1) receive inputs from remote cortical sites through networks of distal intracortical connectivity, which include horizontal connectivity within V1 and feedback connectivity from higher areas. The impact of this distal connectivity is flexible, and obeys divisive normalization (Sato et al, Nature Neurosci, 2014): When a neuron is at rest or weakly active, distal connectivity provides drive (summation), whereas when the neuron is strongly active, distal connectivity provides suppression (division). A possible cellular explanation for these findings is that distal connectivity causes depolarization or hyperpolarization in the absence or the presence of sensory cortical activation. Hyperpolarization, in turn, may be mediated by somatic inhibition. To test these hypotheses, we have used *in vivo* patch-clamp recording in area V1 of anesthetized mice to measure the membrane potential (Vm) of neurons in layer 2/3 in response to visual stimulation and to distal activation. We activated the binocular zone (BZ) of area V1 using optogenetic antidromic stimulation (Sato et al, 2014), which allows us to impose spiking activity regardless of subthreshold influences. We then performed patch-clamp recordings in the distal monocular zone (MZ). Preliminary results indicate that distal activation in the BZ caused depolarization in the MZ when neurons were not visually driven, and hyperpolarization during high-contrast visual stimulation. The depolarization is likely to be mediated by synaptic excitation. As for the hyperpolarization, current experiments seek to establish whether it is due to somatic inhibition. To assess whether we can measure somatic inhibition using a conventional current-clamp mode,

we performed experiments in a mouse line expressing Channelrhodopsin-2 in parvalbumin-positive (PV) interneurons, which are known to provide somatic inhibition. Optogenetic activation of PV interneurons caused hyperpolarization in pyramidal cells when Vm was at rest (68.3 ± 1.7mV), and depolarization when Vm was more negative than the reversal potential for chloride (~ -80mV, 6mM intracellular chloride). These results confirm that our somatic recording electrodes can measure the chloride-mediated hyperpolarization or depolarization of Vm caused by somatic inhibition. Ongoing studies use this method to probe the role of somatic inhibition in the hyperpolarization caused by distal connectivity during visual stimulation. These experiments will provide a better understanding of how intracellular mechanisms implement flexible effects of distal connectivity as divisive normalization.

Disclosures: **T. Sato:** None. **B. Haider:** None. **M. Hausser:** None. **M. Carandini:** None.

Poster

532. Striate Cortex: Intracortical Circuitry

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McDonnell Center for Systems Neuroscience

Title: Distinct excitatory/inhibtory balance in subnetworks within mouse primary visual cortex (v1)

Authors: P. BISTA, *A. H. BURKHALTER
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Abstract: Mouse visual cortex is subdivided into multiple, functionally distinct, hierarchically organized areas that are interconnected through feedforward (FF) and feedback (FB) pathways (Dong et al., 2004; Wang and Burkhalter, 2007). Within cerebral cortex the vast majority of interareal FF and FB connections are formed by excitatory pyramidal cells (Pyr), which in the target area synapse onto Pyr and, most commonly on parvalbumin-expressing (PV) GABAergic neurons (Gonchar and Burkhalter, 1999; 2003). Thus, in both pathways monosynaptic excitation of Pyr is followed by disynaptic PV neuron-mediated inhibition of activated Pyr cells (Dong et

al., 2004). The strength of this, so called, feedforward inhibition is likely to play a key role in the selection and top-down modulation of inputs as well as the dynamics of communication across the cortical hierarchy. Recently, we have found that FF input to PV neurons is stronger than to Pyr, whereas FB inputs to both types of neurons are balanced (Yang et al., 2013). Here, we report that FF geniculocortical and FB projections from higher visual areas to layers 1 and 2 of V1are patchy, raising the question whether the E/I balance within and outside patches is different. To study the issue, we used subcellular Channelrhodopsin-2 assisted circuit mapping (Petreanu et al., 2009) in tangential slices of V1 in which PV neurons were genetically labeled with tdTomato. We found that inside and outside of geniculocortical patches inputs to PV neurons were stronger than to Pyr cells, but the overall excitation within patches was 2-3 more powerful. Similar to FF connections, FB inputs to labeled patches were stronger to PV than Pyr. In contrast, FB inputs to unlabeled patches were balanced. These results suggest that feedforward inhibition depends on both the bottom-up and top-down direction of pathways as well as the subnetwork within the circuit.

Disclosures: P. Bista: None. **A.H. Burkhalter:** None.

Poster

532. Striate Cortex: Intracortical Circuitry

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Support: Knights Templar Eye Foundation

Title: In-vivo GABAergic parvalbumin interneuron contribution to cortical transforms is laminar dependent

Authors: *D. E. PAFUNDO, B. D. FEESE, S. J. KUHLMAN
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Abstract: Gain control of neuronal networks is achieved by divisive normalization mechanisms, likely mediated by local inhibition and allows networks to encode a wide dynamic range of input values (Carandini and Heeger, NatRevNeurosci 2012). In the visual system, a significant part of stimulus driven gain control takes place in layer 4 of primary visual cortex (V1) where the contrast invariant tuning of excitatory neurons is mediated by stimulus-dependent broadening of inhibition produced by parvalbumin positive (PV) GABAergic neurons (Li et al, J Neurosci

2012). The processed visual information is then forwarded to layer 2/3 pyramidal neurons (PC) and PV neurons. Given that transformation of the visual stimulus takes place in layer 4 and that layer 2/3 PC and PV neurons do not receive strong direct thalamic stimulus dependent drive we hypothesize that there are laminar differences in how PV neurons mediate contrast invariant tuning of excitatory neurons. We used 2-photon guided electrophysiological cell-attached recordings of PV neurons to assay PV neuron activity in primary visual cortex in response to visual stimuli of different intensities. Consistent with previous reports, we find that the tuning curves of layer 4 PV neurons are contrast modulated, displaying a two fold increase in the orientation selectivity index when visual stimulation contrast was reduced from 100% to 25%. However, in layer 2/3 we find tuning curves of PV neurons are largely contrast independent, similar to excitatory neurons. Notably, preliminary whole cell current clamp experiments show that the tuning curves of action potential firing and subtreshold potentials of layer 2/3 PV neurons are similar. These results indicate that there are significant laminar differences in stimulus driven gain control mediated by PV neurons. Interestingly, the feedback connections from secondary visual cortex (V2) to V1 that target PCs and PV neurons in supra and infragranular layers seemingly avoid layer 4. Consequently, in layer 2/3, contextual gain control may be engaged by V2 feedback onto PV neurons, a modulation which would be absent in layer 4. Future experiments will address the contribution of V2 feedback to PV neuron mediated gain control in layer 2/3 of V1.

Disclosures: D.E. Pafundo: None. **B.D. Feese:** None. **S.J. Kuhlman:** None.

Poster

532. Striate Cortex: Intracortical Circuitry

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Topic: D.04. Vision

Title: Very long-range disynaptic connections through layer 6 pyramidal neurons in macaque monkey V1

Authors: *D. C. LYON, Y.-J. LIU, M. ARREOLA
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Abstract: Neurons in primary visual cortex (V1) integrate across the representation of the visual field through networks of long-range projecting pyramidal neurons. These projections which originate from within V1 and through feedback from higher visual areas are likely to play a key

role in such visual processes as low contrast facilitation and extraclassical surround suppression. The extent of the visual field representation covered by feedback is generally much larger than that covered through monosynaptic horizontal connections within V1; and, although it may be possible that multisynaptic horizontal connections across V1 could also lead to more widespread spatial integration, nothing is known regarding such circuits. Here we used injections of the CVS-11 strain of rabies virus to examine disynaptic long-range horizontal connections within macaque monkey V1. Injections were made around the representation of 5° eccentricity in the lower visual field. Along the opercular surface of V1, we found that the majority of connected neurons extended up to 8 mm in layers 3, 4A, 4B, 5 and 6, consistent with twice the typically reported distances of monosynaptic connections. In addition, mainly in layer 6, a steady presence of connected neurons within V1 was observed up to 16 mm away. A relatively high percentage of these connected neurons had large diameter somata characteristic of Meynert cells, which are known to project as far as 8 mm individually. Several neurons, predominantly in layer 6, were also found deep within the calcarine sulcus reaching as far as 20° of eccentricity, based on estimates, and extending well into the upper visual field representation. Thus, our anatomical results provide evidence for a wide-ranging disynaptic circuit within V1, mediated largely through layer 6 that accounts for integration across a large region of the visual field.

Disclosures: D.C. Lyon: None. **Y. Liu:** None. **M. Arreola:** None.

Poster

532. Striate Cortex: Intracortical Circuitry

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Title: The sparseness of excitatory lateral connections can account for the formation of species-dependent orientation representation in the mammalian primary visual cortex

Authors: *M. MIYASHITA[1], S. TANAKA[2]
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Abstract: In the primary visual cortex of monkeys, cats and ferrets, preferred orientations are arranged along the cortical surface in an orderly manner, forming orientation maps. Recently, 2-

photon calcium imaging has revealed that preferred orientations are randomly distributed in the rodent visual cortex. This finding raised a question of why orientation maps exist in some species and not in others. To address this question, we examined the activity-dependent self-organization of orientation representation for different degrees of sparseness of excitatory lateral connections in the visual cortex. We assumed that cortical cells send excitatory connections to neighboring cells with the connection probability p, whereas inhibitory connections are long-range and nonspecific. In addition, we normalized each synaptic weight to be proportional to 1/p, because without the normalization, small connection probability leads to relatively strong lateral inhibition and cortical cells tend to be silent. Computer simulations were conducted by changing the value of p. As a result, we found a certain value p* such that salt-and-pepper-like orientation representations emerge for $p < p^*$ and orderly orientation maps emerge for $p > p^*$. For any value of p, model cortical cells exhibited simple-cell-like receptive fields. Note that without the normalization of synaptic weight, receptive fields were disrupted. The value p* depended on the "temperature" that is interpreted to indicate the magnitude of fluctuation in synaptic modification within our mathematical framework. Although we cannot determine the exact value of the temperature in real systems, a fairly sharp transition between an orderly map representation and a salt-and-pepper-like representation occurred at $p=p^*$ for lower temperatures. Combining these theoretical results with species-dependent orientation representations, it is suggested that excitatory lateral connections are sparse and each synaptic weight is large in the rodent visual cortex, whereas excitatory connections are relatively dense and each synaptic weight is small in the visual cortex of monkeys, cats and ferrets.

Disclosures: **M. Miyashita:** None. **S. Tanaka:** None.

Poster

532. Striate Cortex: Intracortical Circuitry

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Topic: D.04. Vision

Title: GABAergic interneurons form spatial clusters in the mouse visual cortex to enhance inhibitory actions on excitatory neurons

Authors: ***T. EBINA**[1], K. SOHYA[1], I. IMAYOSHI[2], S.-T. YIN[1], R. KIMURA[1], Y. YANAGAWA[3], H. KAMEDA[4], H. HIOKI[4], T. KANEKO[4], T. TSUMOTO[1]
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Abstract: Neocortical neurons with similar functional properties assemble into spatially coherent circuits, but it remains unclear how inhibitory interneurons are organized in the spatial domain of the cerebral neocortex. Recent *in vitro* studies revealed the clusters of inhibitory interneurons originated from the same progenitor cells in the developing neocortex. However, it is still not clear how long such a clustered distribution persists during postnatal development, and even if it persists to the adulthood, it remains unknown what is the functional significance of spatial clustering of GABAergic interneurons in operation of cortical circuits. To address these questions, we applied *in vivo* two-photon functional Ca2+ imaging and whole-cell recording of synaptic currents to record visual responses of cortical neurons, and analyzed their spatial arrangements. GABAergic interneurons were clustered in the three-dimensional space of the adult visual cortex, and the three subtypes of interneurons, parvalbumin-, calretinin- and somatostatin-positive neurons also formed clusters with slightly overlapping each other. Excitatory neurons located within the clusters (insiders) had a lower amplitude and sharper orientation tuning of visual responses than outsiders, whereas the neurons near and far from non-clustered interneurons did not show the location-tuning/amplitude relationship. Furthermore, inhibitory synaptic currents recorded from the insiders were larger than those of the outsiders. These findings suggest that GABAergic interneurons form spatial clusters to make their inhibitory function more effective than isolated interneurons.

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Poster

532. Striate Cortex: Intracortical Circuitry

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Title: Layer 6 corticothalamic neurons activate layer 5a pyramidal neurons

Authors: J. KIM[1], C. J. MATNEY[1], A. BLANKENSHIP[2], S. HESTRIN[2], *S. P. BROWN[1]
[1]Dept. of Neurosci., Johns Hopkins Sch. of Med., Baltimore, MD; [2]Dept. of Comparative Med., Stanford Sch. of Med., Stanford, CA

Abstract: Layer 6 corticothalamic (L6 CT) neurons are thought to modulate sensory processing through feedback projections to the thalamus and feedforward projections to the main input layer of the cortex, layer 4 (L4). Anatomical reconstructions of individual L6 CT neurons, however, have shown that their axonal processes ramify not only in L4 but also in layer 5 (L5), the major output layer of the cortex, raising the question of the relative synaptic impact of L6 CT neurons on L4 and L5. Using a combination of anatomical, electrophysiological and optogenetic approaches, we show that the intracortical axons of L6 CT cells densely ramify within layer 5a (L5a) in both visual and somatosensory cortex and activate L5a pyramidal neurons. Following injections of retrograde tracers into the thalamus of Ntsr1-Cre mice, we show that Cre expression is largely restricted to L6 CT neurons. By crossing Ntsr1-Cre mice with Cre-dependent reporter lines combined with injections of viral and retrograde neuronal tracers, we show that the intracortical axons of L6 CT neurons are primarily located in L5a rather than in L4. We next compared the relative synaptic impact of L6 CT neurons on L4 and L5a excitatory neurons. By selectively expressing channelrhodopsin-2 (ChR2) in L6 CT neurons while performing whole-cell recordings from a L4 and a L5a excitatory neuron simultaneously, we show that photostimulation of L6 CT neurons elicits strong, short-latency excitatory postsynaptic potentials (EPSPs) in L5a pyramidal neurons while eliciting weak EPSPs or disynaptic inhibitory responses in L4 cells. We next compared this input with L6 CT input to inhibitory neurons. We performed similar experiments with pairs composed of a L5a fast-spiking (FS) interneuron and a pyramidal neuron or a L4 FS cell and excitatory neuron. We found that L6 CT photostimulation evokes strong, short-latency EPSPs in FS cells in both L4 and L5a. Interestingly, L6 CT photostimulation evokes action potentials in L5a pyramids despite the strong input to FS cells. We next asked whether L6 CT neurons provide input selectively to FS cells input or to inhibitory neurons more generally. By recording from pairs composed of a L5a somatostatin-expressing (SOM) interneuron and a L5a pyramidal cell, we show that SOM cells receive only weak L6 CT cell input. Taken together, our results suggest that L6 CT neurons activate pyramidal cells and FS cells in L5a, an output layer of the cortex, via feedforward excitation, while inhibiting L4, the major input layer of the cortex, via feedforward inhibition.

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Poster

532. Striate Cortex: Intracortical Circuitry

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Topic: D.04. Vision

Support: Paul and Jody Allen

Title: Cellular imaging of genetically defined populations in layer 4 of mouse primary visual cortex reveals functional diversity

Authors: *A. R. GARNER, A. CHENG, L. MADISEN, H. ZENG, R. C. REID
Allen Inst. For Brain Sci., Seattle, WA

Abstract: Pyramidal cells of primary visual cortex are a genetically and physiologically heterogeneous population. We have employed *in vivo* 2-photon calcium imaging using GCaMP6 in awake, active transgenic mice to examine the functional diversity of genetically defined populations of pyramidal cells during visual stimulation. We focused on layer 4 (L4) because in addition to its role as the primary cortical recipient layer of LGN afferent fibers, anatomical studies have shown that most excitatory input is from recurrently connected cortical neurons. These circuit features make L4 an intriguing candidate for investigating information processing on input signals from the external world en route for higher order downstream cortical computations. Our results suggest that in addition to processing of visual sensory input, the information content of a layer 4 pyramidal cell's output is highly dependent on its spatiotemporal relationship with the activity of the assembly in which the cell is embedded as well as the behavioral state of the animal.

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Poster

532. Striate Cortex: Intracortical Circuitry

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ANR 10-BLAN-1402 (Complex-V1)

Title: A comprehensive large-scale spiking model of cat primary visual cortex

Authors: J. ANTOLÍK, C. MONIER, Y. FRÉGNAC, *A. P. DAVISON
UNIC, Ctr. Nationale De La Recherche Scientifique (CNRS), Gif sur Yvette, France

Abstract: Neuroscience has produced a large body of data on the function and anatomy of early visual areas but the transformation of this knowledge into a general coherent understanding has been limited. Computational modeling can integrate such fragmented data into comprehensive models of brain structures that satisfy the broad range of constraints imposed by experiments, thus advancing our understanding of their computational role, and their implementation in the neural substrate. Unfortunately most studies have so far focused on small isolated models targeting only a few properties at a time. Here we introduce a comprehensive multiscale spiking model of cat primary visual cortex (V1/area 17) which satisfies a range of anatomical, statistical and functional properties. The model considers cortical layers 4 and 2/3, corresponding to a 5x5 mm patch of parafoveal V1. Thalamocortical connections are seeded based on orientation maps obtained in a developmental simulation of V1 (Antolík et al. 2010; Front Comp Neurosci 5:1-19). The inter and intra-layer connectivities are based on rules extracted from anatomical and functional studies (Binzegger et al. 2004; J Neurosci 24:8441-53): the thalamocortical pathways and local layer 4 connectivity follow a push-pull organization (Troyer et al. 1998; J Neurosci 18:5908-27), while the long distance horizontal connection probability falls off as a Gaussian curve with parameters derived from Stepanyants et al. (2008; Cereb Cortex 18:13-28). We assumed a slow propagation speed for horizontal connectivity and fast synaptic depression in both thalamocortical and corticocortical connections. We have subjected the model to a range of visual stimulation protocols including gratings and natural scenes animated with eye-movements (Baudot et al. 2014; Front Neural Circuits 7:206). The model expresses over multiple scales a large number of statistical and functional properties including: spontaneous activity with a physiologically plausible resting conductance regime; contrast-invariant orientation-tuning width; realistic interplay between visually evoked excitatory and inhibitory conductances, including the experimentally observed variations among neurons; surround modulation effects including size and orientation-contrast tuning; stimulus-dependent changes in firing precision; and a realistic distribution of Simple and Complex receptive fields. Overall we believe this

model offers the most comprehensive description of V1 to date from conductances to spikes. It offers numerous insights into how the properties studied interact, and thus contributes to a better understanding of visual cortical dynamics.

Disclosures: **J. Antolík:** None. **A.P. Davison:** None. **C. Monier:** None. **Y. Frégnac:** None.

Poster

532. Striate Cortex: Intracortical Circuitry

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Program#/Poster#: 532.19/GG22

Topic: D.04. Vision

Support: EP/F500385/1

BB/F529254/1

Title: Different cortical layers in V1 encode different visual information in different frequency bands

Authors: ***S. C. LOWE**[1], D. ZALDIVAR[2,3], Y. MURAYAMA[2], M. C. W. VAN ROSSUM[1], N. K. LOGOTHETIS[2], S. PANZERI[4]
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Abstract: We previously reported that the activity of primary visual cortex (V1) transmits information about complex naturalistic video stimuli in two distinct frequency bands: a low frequency band (1-24 Hz), and a high frequency (60-100 Hz) gamma oscillation range, with each range carrying its own independent information about the visual stimuli [1]. Here we ask whether these independent frequency channels originate from distinct cortical laminae. We used laminar electrodes with 150 micron spacing spanning the whole cortical depth to record extracellular field potentials from the primary visual cortex of opiate-anaesthetised macaques during presentation of a 2 minute long Hollywood colour movie clip. Using the recorded Local Field Potential (LFP), we computed the Current Source Density (CSD) for each trial. From the time-resolved power of the CSD in each trial, we estimated the mutual information that the power at each frequency carries about which section of the movie is being presented, and how

much information there is in frequency bands about different spatial resolutions of changes in luminance. We found, across depth and frequency, two distinct regions carrying large amounts of independent information about the movie stimulus: the low frequency (4-16 Hz) band had high information at depths corresponding to layers 4-6, whereas the high frequency (64-250 Hz) band had high information in layers 1-3. This suggests that different laminae of cortical circuits generate independent information channels that code information in separate frequency ranges. Furthermore, we found the low frequency band contained information about low spatial frequencies changes in luminance (<1 cycle per degree), whilst the high frequency band contained information about finer spatial details (1-6 cycles per degree). This suggests information about these two spatial frequency components arises through two different cortical mechanisms within V1, and information about them is encoded separately in two different frequency bands. References [1] Belitski, A., et al. (2008). J Neuroscience, 28(22), 5696-709. doi:10.1523/JNEUROSCI.0009-08.2008

Disclosures: **S.C. Lowe:** None. **D. Zaldivar:** None. **Y. Murayama:** None. **M.C.W. van Rossum:** None. **N.K. Logothetis:** None. **S. Panzeri:** None.

Poster

532. Striate Cortex: Intracortical Circuitry

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Support: National Institute of Mental Health of the National Institutes of Health under Award Number R01MH101547

donation by Dean and Brenda Proctor

Title: Dynamic processing of visual information by layer-specific functional interaction between the pulvinar and primary visual cortex

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Abstract: The pulvinar plays an important role in neural information processing, especially in facilitating the communication between cortical areas in response to attention demands (Saalmann et al., 2012). However, its functional connectivity with primary sensory cortices has remained unknown. We hypothesized that the different layers of primary visual cortex (V1) form distinct, frequency-specific functional connections with the pulvinar. To test this hypothesis, we presented artificial and naturalistic visual stimuli to anesthetized ferrets (Mustela putoris furo, 16-20 weeks, female, n=8) and simultaneously recorded multiunit activity (MUA) and local field potential (LFP) from the pulvinar and V1. We determined functional connectivity patterns between pulvinar and V1 cortical layers (supragranular, granular and infragranular layers) both for spontaneous network activity in absence of sensory input and for sensory processing of full-field visual stimuli. We found that naturalistic visual input caused most pronounced functional connectivity between the pulvinar and V1 measured by MUA spike correlations (ANOVA, main effect of stimulus type, $p=0.01$; no effect by layer, $p>0.05$). In agreement with our hypothesis, we found that the pulvinar and all three cortical layers exhibited highest correlations of LFP spectral power in the alpha frequency band (8-12 Hz) for spontaneous activity (main effect of frequency band, $p<0.001$). Furthermore, LFP coherence during visual stimulation exhibited layer- and stimulus-specific structure in distinct frequency bands: (1) supragranular and granular layers displayed higher coherence with the pulvinar than the infragranular layer in the delta (0.5-4 Hz) and alpha bands (main effect of layer, $p<0.001$) and (2) gamma coherence was suppressed by both artificial and naturalistic visual input (main effect of stimulus type, $p=0.003$). Cross-area coupling determined by phase preference of MUA as a function of LPF phase further confirmed layer-specific functional connectivity between pulvinar and V1. MUA in infragranular layers exhibited the strongest phase preference in the delta band of the pulvinar LFP. Differently, pulvinar MUA exhibited the strongest phase preference for all frequency bands of the supragranular V1 LFP. Together, our data suggest that, at the level of the LFP, several communication channels exist between the pulvinar and specific layers in V1 that are enabled by frequency-specific oscillatory activity patterns. Saalmann YB, Pinsk MA, Wang L, Li X, Kastner S (2012) The pulvinar regulates information transmission between cortical areas based on attention demands. Science 337:753-756.

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Poster

532. Striate Cortex: Intracortical Circuitry

Location: Halls A-C

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Program#/Poster#: 532.21/GG24

Topic: D.04. Vision

Support: NSERC

Title: Modulation of spike rate by optogenetic control of GABAergic neurons alters contrast adaptation in mouse primary visual cortex

Authors: *N. A. CROWDER, K. R. STOVER, J. L. KING, K. M. GORDON
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Abstract: Prolonged viewing of high contrast gratings alters perceived stimulus contrast, and produces characteristic changes in the contrast response functions of neurons in the primary visual cortex (V1). This phenomenon is referred to as contrast adaptation. The underlying cellular and network mechanisms mediating contrast adaptation are largely unknown. Therefore, having previously established broad similarities in V1 contrast adaptation between mice and higher mammals, we sought to use a mouse model of optogenetic perturbation to study the possible roles of intrinsic firing rate and/or GABAergic innervation in this form of short-term plasticity. We performed extracellular recordings from V1 neurons in transgenic mice that express channelrhodopsin-2 (ChR2) in GABAergic neurons, and coupled contrast adaptation stimulus protocols with V1 photostimulation. We found that optogenetic activation of GABAergic neurons when the adapting grating was present caused a decline in activity during this period that consistently altered the shape of adapted contrast response functions that were collected following cessation of photostimulation. The precise temporal control allowed by optogenetics shows promise in helping to unravel the mechanisms of visual adaption.

Disclosures: N.A. Crowder: None. **K.R. Stover:** None. **J.L. King:** None. **K.M. Gordon:** None.

Poster

532. Striate Cortex: Intracortical Circuitry

Location: Halls A-C

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Topic: D.04. Vision

Support: NWO VIDI

Title: No functional specialization of Calretinin-expressing neurons in the mouse visual cortex

Authors: D. CAMILLO[1], C. N. LEVELT[2], *J. A. HEIMEL[3]
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Abstract: Calretinin is a calcium-binding protein often used as a marker of a subset of inhibitory interneurons in the cerebral cortex. Using Calb2-ires-cre mice, we show that calretinin is also likely to be transiently expressed in cortical pyramidal neurons during development. In the mature visual cortex of the mice, only a minority of the cells that have expressed Cre is gabaergic and only about 13% stain positive for the Calretinin protein. To determine whether this group of cells share any functional characteristics, we recorded their visual response properties using GCaMP6s calcium imaging. The average orientation selectivity, size tuning, and temporal and spatial frequency tuning of this group of cells, however, match the response profile of the general neuronal population, revealing no functional specialization.

Disclosures: D. Camillo: None. **J.A. Heimel:** None. **C.N. Levelt:** None.

Poster

532. Striate Cortex: Intracortical Circuitry

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Title: Inhibition-stabilized balanced dynamics account for stimulus-induced changes of noise variability in the cortex

Authors: Y. AHMADIAN[1], G. HENNEQUIN[2], D. B. RUBIN[1], K. D. MILLER[1], *M. LENGYEL[2]
[1]Columbia Univ., New York, NY; [2]Univ. of Cambridge, Cambridge, United Kingdom

Abstract: Hypotheses about the dynamical regime in which cortical circuits may operate have traditionally relied on data about trial average responses and typically ignored across-trial variability, or at best considered its most basic aspect, the Poisson-like variability of single cell spike counts. However, there is now a growing body of data on more detailed patterns of variability and co-variability of neural responses, and how they change in a stimulus-dependent manner, both at the level of membrane potentials and spike counts. These data could be used to further constrain theories of cortical dynamics, but establishing a quantitative relationship between circuit dynamics and patterns of variability is challenging because it requires a formal understanding of how correlations arise mechanistically in recurrently connected networks of neurons. Unfortunately, existing theories provide a somewhat blurry picture, as they often connect some high-level statistics of the circuit connectivity to other summary statistics of the pairwise correlation distribution. More elaborate theories can predict pairwise correlations for any specific pair of neurons, but assume either small firing rate variability or weak correlations, which are unlikely to generally hold in cortical circuits. Here, we developed a novel theoretical framework to obtain the full correlational structure of a stochastic network of nonlinear neurons described by rate variables. Importantly, our theory requires neither the fluctuations nor the pairwise noise correlations to be weak, and works for several biologically motivated, convex single-neuron gain functions. We apply our formalism to visual area MT, for which data on how particular stimuli affect neuronal variability has been published recently (Ponce-Alvarez et al., 2013). We find that a balanced ring model network with a threshold-quadratic nonlinearity captures the stimulus-dependence of both the Fano factor and the noise correlations. Interestingly, this network operates in a regime very different from previous proposals for variability quenching which all relied on multistable attractor dynamics, but closer to the behavior of the inhibition-stabilized balanced networks (Murphy and Miller, 2009; Ahmadian et al. 2013; Hennequin et al. 2014).

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Poster

533. Eye Movements and Perception

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 533.01/GG27

Topic: D.06. Eye Movements

Title: Modeling eye-position gain fields for population coding recovery of stimulus locations

Authors: *A. B. SERENO[1], M. E. SERENO[2], S. R. LEHKY[3]
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Abstract: We created a population of model neurons whose responses were modulated by eye position. This population was used to recover gaze angle, equivalent to recovering the location of a stimulus at fixation. An intrinsic population decoding method was used, namely multidimensional scaling. Each neuron in the population had a different function describing eye-position modulation, or a different gain field. We used quasi-planar gain fields, shaped like a curved sheet with a sigmoidal cross-section, defined by three parameters: *slope* (how steeply the gain field was tilted), *orientation* (direction of tilt), and *shift* (position of sigmoid inflection relative to receptive field center). When the population had broadly dispersed values for all three parameters it was able to do an accurate recovery of stimulus location. When the range of the slope parameter was held constant or restricted to having a small range of values close to some arbitrary value, the population was still able to accurately recover location. However, if the range of the orientation parameter was similarly restricted, the population was able to recover eccentricity of location, but not polar angle. Also, if the range of the shift parameter was similarly restricted, the population was able to recover polar angle of location, but not eccentricity. Using a broad range for all three parameters, it made no difference if the gain field was quasi-planar (sigmoidal) or planar, showing that linear and nonlinear gain fields fare equally well under this model. Populations of completely non-planar gain fields also performed good recovery of location, including gain fields that were parabolic, hyperbolic, or a complex shape that was a linear combination of planar, parabolic, and hyperbolic. An important requirement was that average neural response over the gain field (average response over all eye positions) was not identical for all neurons in the population. Thus, we have characterized how gain field properties can affect recovery of stimulus location and such modeling may be useful in understanding known stimulus mislocalization phenomena. Most importantly, we demonstrate that eye-position gain fields, even without retinal information, are sufficient to accurately recover fixated stimulus locations.

Disclosures: A.B. Sereno: None. **M.E. Sereno:** None. **S.R. Lehky:** None.

Poster

533. Eye Movements and Perception

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Topic: D.06. Eye Movements

Support: NIH R00EY021252

GMU URSP

Title: The contribution of corollary discharge to perceived eye location for different movement orientations and amplitudes

Authors: *S. BANSAL[1], L. C. BRAY[2], M. S. PETERSON[3], W. M. JOINER[2]
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Abstract: Both neural and behavioral studies have suggested that visual stability is mediated by a corollary discharge (CD) signal. This signal is hypothesized to inform the visual areas on the future eye position, but how it encodes impending saccade metrics, particularly for movements of different orientations and amplitudes, is still unclear. To investigate these properties we designed two experiments to determine the effects of amplitude and orientation on the CD-based ability to perceive trans-saccadic target displacements. In the first experiment (N=10) an initial target (T1) randomly appeared at one of three amplitudes (4º, 6º, 8º) and four orientations (upward, downward, leftward, and rightward) from the central fixation point. After a primary saccade to T1 there was a 250 ms blank period to reduce saccade suppression of displacement. T1 then reappeared at a shifted randomized position in-line with the movement (± 0.5º collinear increments) between ± 2.5º and the subject made a manual response to indicate displacement direction. Our results showed that there was a significant effect of amplitude, but for a given amplitude there was no significant effect of orientation ($p<0.01$ and $p=0.19$, 2-way ANOVA) in perceiving displacements when occurring in the direction of the saccade. We also confirmed that the threshold increased systematically with amplitude; mean thresholds were ~10% of movement amplitude. In addition, we found that in spite of the movement variability, subject performance remained greater than chance in perceiving the target displacement accurately (>70%) even when the displacement and saccade error were in conflict. As a consequence of these findings, we reduced our second experiment (N=8) to two amplitudes (4º or 8º), and three orientations (rightward, oblique (45º), or upward) to examine the ability to detect collinear, orthogonal and diagonal T1 shifts. We found that perceptual thresholds for both orthogonal and oblique shifts were lower than collinear displacements ($p\leq0.03$). Based on the different orientations, we also determined and compared the fields of sensitivity for the respective target locations. We find that the sensitivity fields are oriented (broader) along the saccade direction for horizontal and vertical movements, but less so for oblique saccades. However, for all saccade orientations, the

sensitivity field increased in size with movement amplitude while maintaining the original orientation. In summary, the CD-based ability to perceive changes in target displacement decreases with movement amplitude, and it is generally independent of movement orientation, but highly dependent on displacement direction.

Disclosures: S. Bansal: None. **L.C. Bray:** None. **M.S. Peterson:** None. **W.M. Joiner:** None.

Poster

533. Eye Movements and Perception

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Topic: D.06. Eye Movements

Support: NIH Grant EY019266

P30EY001730

ORIP P51OD010425

Research to Prevent Blindness

Title: Gain and timing adaptation for smooth pursuit eye movements

Authors: *S. ONO
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Abstract: Visuomotor adaptation associated with gain and timing control is necessary for precise control of eye movements. It has been shown that smooth pursuit gain adaptation is induced by repeated trials of a step-ramp tracking with two different velocities (double-velocity paradigm) that step-up (10-30 °/s) or step-down (20-5 °/s). The most effective way to induce pursuit adaptation is to change the target speed at the time of pursuit onset (100-120 ms after target onset). Previous studies have shown an adaptive change in initial pursuit gain after repeated double-velocity trials (over 100-200 trials), revealed as overshooting or undershooting eye motion during control testing using single-speed ramp trials. However, it is still uncertain whether a double-velocity paradigm with different timing produces pursuit gain adaptation. Therefore, we use a double-velocity paradigm where target speed changes 400 ms or 800 ms after the target onset during a steady-state phase. After repeated presentation of this paradigm, overshooting eye motion did not occur on control trials using single-speed pursuit. The steady-

state paradigm affected neither initial pursuit gain nor steady-state gain. However, when a brief sinusoidal perturbation of target motion (2.5 Hz, ± 10 °/s) is introduced during a steady-state phase after the adaptation paradigm, a perturbation ocular response showed a significant adaptive change, which was associated with the time when the target speed changed. These results indicate that this gain adaptation occurs at a specific time, which could be due to adaptation mechanisms of visuomotor gain associated with timing control.

Disclosures: S. Ono: None.

Poster

533. Eye Movements and Perception

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Topic: D.05. Visual Sensory-motor Processing

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Taiwan's National Science Council (NSC102-2917-I-002-096 to Y. Y.).

Title: What eye movements tell us about visual saliency in different states of awareness

Authors: *Y.-H. YANG[1,2], S.-L. YEH[2], A. BARTELS[1]
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Abstract: The relation between attention and consciousness is a contentious issue. In the present study we tried to relate attention allocation to stimulus processing as a function of the conscious state of the observer. In particular, we asked whether attention is allocated to visual cues that are globally or locally salient as a function of awareness of the stimulus. We used a paradigm called continuous flash suppression (CFS) to render stimuli invisible to the observer, and measured eye movements to left or right half of the visual field that differed in stimulus content. We assumed that oculomotor responses are a good indicator of attention allocation during conscious as well as unconscious stimulus processing. During CFS, a critical stimulus presented to one eye is

interocularly suppressed by dynamic masks presented to the other eye. We presented the salient stimulus property in either left or right visual field, while the whole stimulus was suppressed by interocular masks. Oculomotor responses were measured online by an eye-tracker. The salient stimulus property was either a single pop-out feature (e.g., a red disk among green disks), or alternating local gradients (e.g., alternative red-green disks), thus forming a global or a local salient stimulus property, respectively. In each trial, we measured the level of awareness using an objective and a subjective criterion: the objective measure was the success rate with which observers judged whether the salient property was in left or right visual fields, and the subjective measure was the confidence rating of their judgment. We found that attention was allocated to the global or local feature depending on the state of awareness: in trials where objective performance was above chance level and subjective rating was high, eye position favored the local over the global stimulus feature. On the other hand, in trials where objective performance was at chance and confidence was low, eye position favored the global over the local stimulus feature. These results suggest that the state of awareness affects the type of visual features that attract attention.

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Poster

533. Eye Movements and Perception

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Topic: D.06. Eye Movements

Support: FRM

James S. McDonnell Foundation

Title: Effect of sensorimotor adaptation of saccades on covert exogenous attention

Authors: O. HABCHI, R. MATHIEU, C. URQUIZAR, A. FARNÈ, *D. PELISSON
INSERM U1028-CNRS UMR5292/CRNL, Lyon-Bron, France

Abstract: Acquisition of visual information relies on a close collaboration between overt movements of the eyes (saccades) and covert shifts of attention. The accuracy of saccades is controlled by sensorimotor adaptation processes based on neural plasticity. Despite the link between saccades and covert attention, the effects of saccadic adaptation on covert shifts of

exogenous attention have never been investigated. In this study we measured the performance of seventy-three healthy volunteers in a speeded visual detection task performed immediately before (pre) and after (post) an exposure phase. Participants were assigned to 8 groups (10 participants per group), each group performing during the exposure phase one combination of three experimental factors: saccade task (adaptation versus mere execution) x saccade type (voluntary versus reactive) x saccade direction (leftward versus rightward). In the saccadic adaptation task, a backward adaptation was induced by the double step target paradigm (McLaughlin 1967), whereas in the saccade execution (control) task, saccades were elicited in a single-step target paradigm. Note that throughout the visual detection task, subjects maintained their eyes on a central fixation point and had to manually press a response-button as soon as they detected the presentation of a visual target in either hemi-field. The results showed that, as expected, saccadic gain significantly decreased in the 4 adaptation groups and only in the "adapted" hemi-field. In contrast, detection reaction time decreased significantly between pre- and post-exposure specifically after adaptation of leftward reactive saccades, thus demonstrating that deployment of purely covert attention can be boosted by saccadic adaptation. Based on this specific effect of adaptation of leftward reactive saccades on covert attention and on the known asymmetrical specialization of cerebral hemispheres in oculomotor and attentional control, we propose a new neurophysiological model where the temporo-parietal junction (TPJ) plays a central role. To test this hypothesis, we currently perform a fMRI-guided Transcranial Magnetic Stimulation (TMS) study of the role of TPJ in adaptation of reactive saccades and in the associated change of covert attention.

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Poster

533. Eye Movements and Perception

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Topic: D.05. Visual Sensory-motor Processing

Support: NIH Grant EY022854

Title: Temporal structure of population activity supplements the instantaneous rate code in gating saccade initiation

Authors: U. K. JAGADISAN[1], *N. J. GANDHI[2]
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Abstract: The superior colliculus (SC) and frontal eye fields (FEF) are critical nodes in the network that facilitates the transformation of sensory input to orienting movements, e.g., saccadic gaze shifts. Crucial to this transformation are neurons in these regions that are activated both by the onset of a visual stimulus (visual burst) as well as prior to a saccade (premotor burst) in their response field. Intriguingly, these so-called visuomotor neurons also have direct projections to the burst generators in the brainstem that are involved in saccade initiation. This raises the question - why does the high frequency visual burst not trigger a saccade, unlike the premotor burst. Here, we record in monkeys performing the delayed saccade task and show that the temporal relationship between neurons in SC and FEF during the visual response is different from that during the premotor response. Specifically, the temporal structure fluctuates in the visual burst while remaining relatively stable in the premotor burst, specifying a code to distinguish between incoming sensory input and motor preparatory output. We propose this temporal inconsistency of the visual response as a putative explanation for why the high frequency visual burst does not initiate a saccade, although it may cross a firing rate threshold at the population level. Such gating of the visual burst is an important functional attribute because owing to the aforementioned direct projections to the brainstem, any increase in the incoming drive from these neurons is poised to trigger a premature (and potentially erroneous) gaze shift. We further show that during large saccades this pattern of temporal inconsistency is reversed in the population recorded from rostral SC, which is active during fixation and bursts for microsaccades. That is, the rostral SC population is temporally consistent during the visual burst occurring in caudal SC and fluctuates at the time of the (large) saccade. We also test the temporal inconsistency hypothesis in a condition where the visual and premotor bursts are not as well separated as in the delayed saccade task. Specifically, the visual burst which triggers express saccades in the gap paradigm had a relatively consistent temporal structure, whereas the visual burst for regular, non-express saccades was inconsistent. In the accompanying abstract, we develop a model of saccade initiation that takes into account temporal structure. We suggest that future studies need to look at the temporal structure of population activity along with measures such as instantaneous firing rate to examine its role in neuronal communication and correlation with behavior.

Disclosures: U.K. Jagadisan: None. **N.J. Gandhi:** None.

Poster

533. Eye Movements and Perception

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National Medical Research Council Singapore (StaR/0004/2008)

Title: Blinks and eye closures - More happening in the brain than meets the eye

Authors: *D. KONG, J. ONG, M. W. CHEE
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Abstract: Blinks and eye closures are two commonly observed oculomotor activities that share similarities yet trigger largely distinct neural activities. Blinks transiently interrupt external visual input, but are rarely noticed, while sustained eye closures shut out external visual environment, altering sensory perception. Here, we tried to delineate functional differences between blinks and eye closure under a variety of visual conditions. 52 participants took part in 4 experiments that involved fMRI and eye-tracking. 1. Voluntary blinks with external visual inputs RSVP sequences of black letters appeared on a white screen. Participants blinked continuously or kept their eyes open for 20s blocks. 2. Blink in darkness or with constant retinal illumination Each 12s blink block was followed by an eyes-open period. Half the runs were conducted in darkness; while in the other half, pulsating light was delivered directly to the retina using a fiber-optic light source placed at the roof of the mouth. This kept retinal illumination constant regardless of blinks. 3. Spontaneous blinks in darkness Participants kept their eyes open in darkness. Spontaneous blinks were identified using eye tracking. 4. Eye closure in darkness or with constant retinal illumination Scans alternated between 12s eyes close and eyes open blocks. Similar to Exp. 2, visual stimulation was either absent or delivered through the fiber-optic system. Blinks, voluntary or spontaneous, consistently activated the parieto-occipital junction regardless of constancy or presence of visual stimulation. During extended eye closure, extensive deactivation was observed in thalamus, insula, frontal eye field, supplementary eye field and cuneus. Interestingly, increased activity was observed in extrastriate visual, somatosensory regions and hippocampus. Beta series connectivity analysis using thalamus as a seed showed that regardless of stimulation condition, thalamus connectivity with the attentional network and visual areas was preserved during blocks of blinking but was lost with extended eye closure. Activation observed during blinks may reflect engagement of compensation for the transient loss of visual input, giving the illusion of perceptual continuity. The co-activation of extrastriate visual, hippocampal and somatosensory areas in the absence of visual stimulation represents sensory activation dissociated from relevant external stimulation as evidenced by reduced thalamocortical functional connectivity. The basis for these latter observations remains unclear.

Disclosures: **D. Kong:** None. **J. Ong:** None. **M.W. Chee:** None.

Poster

533. Eye Movements and Perception

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Topic: D.06. Eye Movements

Support: CELEST (NSF SBE-0354378 and OMA-0835976)

ONR (N00014-11-1-0535)

AFOSR FA9550-12-1-0436

Title: Dissociation of pursuit eye movement and perception of visual motion under binocular and dichoptic condition

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Abstract: Our eyes are constantly moving. The brain uses both visual information and ongoing eye movements for future eye movement planning. However, our knowledge of how the brain integrates these information from two different sources and two eyes is still limited. We developed a presentation system that can show different images to separate eyes with large viewing angles and concurrently track both eyes. We conducted a series of psychophysical experiments, in which healthy human observers pursued a moving target on a moving background. The target and background could be seen by both eyes (binocular condition) or by separate eyes (dichoptic condition), i.e., the target was seen by one eye while the background by the other. We also systematically varied the background and target relative directions. The percept of the target's velocity was affected by the motion of the background in both binocular and dichoptic conditions. For example, when the background moves in the opposite direction of the target, the target appears to move faster subjectively (fast condition), and when the background moves with the same direction as of target's, the target's perceived velocity is slower (slow condition). Counter-intuitively, the eyes always moved slower in the fast condition than the slow condition, even when the target seemed to move faster due to background motion

in the fast condition. This effect remained even when there was no background for the eye seeing the target in the dichoptic condition. Thus, we found that the pursuit eye movement was subject to the background motion, but the pursuit eye movement was not following the perceived velocity of the moving target. Further, the planning of the pursuit seemed to be sent to both eyes, even when the visual information was separated from the two eyes.

Disclosures: B. Cao: None. **E. Eyuboglu:** None. **A. Meckel:** None. **A. Yazdanbakhsh:** None.

Poster

533. Eye Movements and Perception

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Topic: D.05. Visual Sensory-motor Processing

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Title: Target motion predictability determines the predictability of gaze decisions from retinal inputs

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Abstract: In order to stabilize a moving target's retinal image, the brain must make continuous visual estimates of target motion and evaluate the trade-off between smoothly modulating eye movement and issuing a saccade. Pursuit offers the advantage of uninterrupted visual information but is not able to compensate for large retinal errors; saccades, on the other hand, are able to reduce large errors quickly, but they are likely to degrade visual information during the process. If target motion is unpredictable, gaze behavior must be driven by delayed visual estimates. But if the target trajectory can be extrapolated into the future because its motion is predictable, then pursuit and saccades may be coordinated to maximize both visual information and tracking performance. We investigate an existing formulation of the decision rule between pursuit and saccade introduced by Lefevre and colleagues (2002). This quantity, Eye-Crossing

Time (TxE), is defined as the time it would take the eye trajectory to cross the target. We tracked eye movements in human subjects with a Dual Purkinje Image eye tracker (Fourward Technologies) and in monkeys with scleral coils. We used three experimental paradigms: a 1D and 2D double step-ramp experiment, and a single-player version of the video game, Pong. In the double step-ramp paradigm, randomized trial presentation and a large parameter space minimized predictability. In Pong, the target dynamics - a small spot target with constant speed and elastic collisions with the arena walls - were predictable. We extend the existing definition of Eye-Crossing Time (TxE) to two-dimensions and show that there is a decision rule that captures gaze behavior across both experimental paradigms and both species. When conditioned on a saccade 125 ms in the future, TxE distributes equivalently for both the double step-ramp experiment and Pong, and is consistent between humans and non-human primates. Saccades are most likely when TxE is less than zero; pursuit is most likely when TxE is between 0-200 ms. That means that the occurrence of a saccade tells us something about the Eye- Crossing Time in the recent past. But is the converse true? Can we predict gaze behavior from TxE? We find that the likelihood of observing a saccade given the occurrence of an appropriate TxE is peaked at ~130 ms, as expected. But this only held true in the double step-ramp experiment, not in Pong. We find that a TxE based decision rule holds when gaze behavior is driven by feed-forward visual estimates. When motion becomes predictable, gaze behavior is no longer captured by the same decision rule. We apply information theoretic analysis to quantify the interaction between target, gaze, and time.

Disclosures: L.C. Osborne: None. **M.E. Battifarano:** None.

Poster

533. Eye Movements and Perception

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 533.10/GG36

Topic: D.05. Visual Sensory-motor Processing

Title: The relation of perisaccadic perceived location to eye position and time

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Abstract: The features and basis of the extraretinal (exR) signal involved in perisaccadic stability of visual space are far from being fully determined. One procedure for investigating the

exR signal consists of a subject making a saccade in the dark and reporting on the perceived location of a test flash presented around the time of the saccade. A general result coming from this sort of study is that the test flash is mislocalized just before, during and for a short time after the saccade (Matin, 1972, 1976; Honda, 1989, 1990, 1991; Dassonville, Schlag & Schlag-Rey, 1992; Bockisch & Miller, 1999). This mislocalization suggests the existence of an exR signal that begins to change prior to the saccade and continues to change more slowly than the eye movement. However, besides the slow change in exR signal, little is known about the quantitative properties of this signal, and especially in what manner it is influenced by eye position. In view of this, we explored the perceived location of a test flash presented in the dark during and following both normal and reduced amplitude saccades (reduced via an adaptation procedure) to see how, over a broader than usual range of eye positions, perceived location is related to eye position. A main finding of this work is that the perceived location of the flash varies as a linear function of eye position both during and after a saccade, and that the slope of the linear function changes over time. Of interest, the slope of the linear function is the same for both normal and reduced amplitude saccades. Along with these findings, an analysis of the features of the slope-intercept during and after the saccade indicates that besides the change in slope over time, another time varying component, not related to eye position, makes a contribution to perceived location. Together, these results suggest that perceived location is the consequence of a multifaceted exR signal. In traditional terms, this complex signal may come from some amalgam of "outflow signals" (such as "effort of will" and "efference copy") and "inflow signals." Associated neurophysiology may, in part, consist of a mix of receptive field remapping (as found in FEF, V1, V2 and V3) and eye position signals (occurring in LIP, VIP and MT).

Disclosures: **J. Pola:** None. **E. Matin:** None. **L. Matin:** None.

Poster

533. Eye Movements and Perception

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Program#/Poster#: 533.11/HH1

Topic: D.06. Eye Movements

Title: Dynamic network interactions of the human oculomotor system based on intrinsic connectivity

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Abstract: One of the biggest challenges in neuroscience is to understand how various diverging functions (such as perception, action and cognition) arise from the brain's fixed structural connections. Neuroimaging studies of the 'resting' brain consistently show that in timescales of 10-15 minutes brain structures with known high functional synchrony form distinguishable activation networks, such as the default mode network. White matter tractography studies have identified the structural links between the brain regions within these intrinsic connectivity networks (ICNs). However, synchrony between these brain regions over shorter timescales is not stable. This fluctuation in connectivity manifests as changes in community organization of these ICNs. Since the mental states of the subject are unknown in these resting state experiments, little can be learned about the role and significance of these short-scale modulations in these functional brain networks. Here, we studied the dynamics in short-scale modulations in connectivity and global functional organization of ICNs during a visually-guided task designed to activate the oculomotor system. Ten subjects, studied on a 3T Siemens Trio MRI scanner and an eye tracker, preformed a battery of 40s long blocks of alternating visually guided oculomotor tasks (fixation, smooth pursuit, saccades and voluntary cued blink conditions). Human and non-human primate oculomotor research has identified a network of cortical and sub-cortical structures involved in the control and execution of numerous subtypes of eye movements. Here, we asked how these structures within these oculomotor networks interact with other known functional networks. We decomposed our oculomotor task data using independent component analysis to identify and extract the active intrinsic connectivity networks (ICN). Number of structures involved in the control of eye movements, such as the frontal eye fields (FEF), supplemental eye field (SEF) and parietal eye field (PEF) were all part of a single ICN. However, other task activated areas such as lateral frontal eye field (latFEF) and inferior frontal gyrus (IFG) were each identified as separate ICNs. The well-known networks (e.g. the primary vis., higher vis., left executive control network (LECN), RECN, and salience network) were also identified. Utilizing graph analysis, we have studied the modulations of the functional community organization of these ICNs across our different task conditions. Our results showed similar condition-specific modular organizations of the ICNs across subjects. Additionally, most of the ICNs maintained a stable relationship throughout the task block.

Disclosures: **A. Ashourvan:** None. **A. Puce:** None. **N.L. Port:** None.

Poster

533. Eye Movements and Perception

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Topic: D.06. Eye Movements

Support: NSERC DG

Canada Foundation for Innovation

Canada Research Chair

Title: Accurate smooth pursuit eye movements improve hand movements in a manual interception task

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Abstract: Objective: Tracking a moving object with smooth pursuit eye movements enhances our ability to predict the object's trajectory in space (Spering et al., J Neurophysiol 2011) and time (Bennett et al., Exp Brain Res 2010). Intercepting a moving object, such as a flying ball, critically relies on motion prediction. Here we ask if accurately tracking a briefly presented target with smooth pursuit eye movements improves the ability to intercept its trajectory. Methods: We developed a novel paradigm in which human observers, 32 members of the UBC varsity baseball team, were asked to track a small moving dot, back-projected onto a translucent screen, and to intercept it as fast and accurately as possible. Observers were instructed to hit the target with their index finger as soon as it entered a designated "hit zone". During training, the target was shown for the entire trajectory. In experimental trials, the target trajectory was only shown briefly (100, 200 or 300 ms). Thus, observers had to extrapolate the target's trajectory after the dot disappeared from the screen and subsequently intercept the target at its assumed current position anywhere within the hit zone. Stimulus speed (25, 30 or 35 deg/s) and trajectory shape (curved or linear) were also varied. Eye and hand movements were recorded throughout each trial using an Eyelink 1000 and an Ascension TrakSTAR, respectively. We assessed the 2D position error of the finger end position as well as smooth pursuit quality (initial eye acceleration, position error, velocity error, steady-state velocity gain, catch-up saccade characteristics). Results: Eye and hand movement accuracy improved with increasing target presentation duration and were overall better for linear than for curved trajectories. Importantly, interception accuracy increased following more accurate pursuit; 2D finger position error was lower following trials with low eye position and velocity errors and high eye-velocity gain. Interception accuracy was also predicted by saccade characteristics: better interception was observed in trials with smoother pursuit, in which fewer catch-up saccades of smaller amplitude

were made. Conclusions: Smooth pursuit eye movements boost hand movement accuracy, indicating that accurate eye movements lead to a better velocity estimate. Enhanced motion estimation during pursuit may be due to efference copy information. We discuss potential practical implications for improving motor performance in sports and clinical rehabilitation through eye-movement training.

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Poster

533. Eye Movements and Perception

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Topic: D.05. Visual Sensory-motor Processing

Support: Hungarian Brain Research Program - Grant No. KTIA_13_NAP-A-I/18

Title: Neural signatures of perceptual load during natural reading

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Abstract: Letter spacing strongly affects the efficacy of natural reading and recently it has also been shown that increasing letter spacing improves reading in dyslexia. However, the neural basis of the effect of letter spacing on natural reading is unknown. Here we used concurrent eye-tracking (ET) and EEG recordings to investigate the neural correlates of letters spacing during natural reading. Twenty-four young adults were instructed to read text paragraphs at their own pace, while the text was presented line-by-line and the perceptual load was controlled by manipulating the letter spacing. Our novel methodological approach involved simultaneous recording of ET and EEG, adaptive analysis of ET data, artifact elimination using independent component analysis of EEG recordings with incorporated ET information, scalp current density transformation, time-frequency decomposition based spectral dynamics analysis, extraction of fixation-related potentials (FRPs) and fixation-related spectral perturbations (FRSPs), hierarchical single-trial analysis of FRPs and FRSPs. The obtained results revealed significant effects of letter spacing on FRP and FRSP features during natural reading while controlling for

confounding ET variables such as saccade amplitude and fixation duration. FRP amplitudes decreased in the 170-220 ms time interval with increase of letter spacing. Most significant effects were found in the right temporo-occipital and parietal brain regions. FRSP results showed a very similar trend for theta frequency band power, but in a broader spatio-temporal region. The power of theta oscillations exhibited a significant decrease with the increase of letter spacing with the strongest effect around 190 ms in the right temporo-occipital region. The same trend also appeared for beta band power, however, most significant effects were found around 100 ms in the right occipital and temporo-occipital regions in this case. Our results revealed the EEG signatures of the effect of letter spacing during natural reading and thus pave the way for future research aiming at understanding why decrease of letter spacing impairs reading in dyslexia.

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Poster

533. Eye Movements and Perception

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Topic: D.05. Visual Sensory-motor Processing

Support: NIH Grant EY022854

Title: A recurrent excitation-inhibition model that captures population temporal structure explains saccade initiation

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Abstract: It is difficult to reconcile popular models of movement initiation such as threshold-based gating and the optimal subspace hypothesis with the observed activity pattern of visuomotor neurons in the superior colliculus (SC) and frontal eye fields (FEF). We recently proposed an alternative hypothesis that uses the temporal structure of population activity to explain why downstream structures process the visual burst differently from the premotor burst in that the former does not trigger a saccade while the latter does (see accompanying abstract). Here, we develop rate-based models that incorporate the effects of temporal structure to explain saccade initiation. We explore various aspects of model design that enable the exploitation of population activity structure. We first demonstrate that an accumulator network with synaptic facilitation can distinguish between consistent and fluctuating population inputs. Heuristically,

this “decoder” keeps track of the short-term history of population activity, uses this memory to evaluate consistency of the inputs, and responds selectively when the activity pattern is deemed consistent over some time. This is achieved by using short-term facilitatory connections from the input population to a leaky accumulator, with a Hebb-like learning rule and weight normalization. We also look at whether a network with static synapses can use its leak time constants to achieve optimal temporal integration of consistent inputs over fluctuating inputs. In addition to these putative neural mechanisms for interpreting temporal structure, we also propose a mechanism for why the inconsistency arises in the visual burst and not the premotor burst. Independent, noisy inhibition on the recurrent networks in SC and FEF causes the activity bursts in single neurons in response to an incoming visual input to be relatively uncorrelated. Following the GO cue, reduced levels of inhibition coupled with correlated low-level activity allows the recurrent excitation to take over the dynamics producing correlated bursts that are temporally consistent. We examine the role of the balance between excitation and inhibition in creating experimentally observed attributes of population activity, including stable persistent activity during the delay period in the delayed saccade task.

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Poster

533. Eye Movements and Perception

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Topic: D.06. Eye Movements

Support: EY19281

Title: Illusory objects are altered by saccadic eye movement preparation

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Abstract: The human visual system continuously interpolates fragmented image features across space, time and eye movements to construct a coherent percept of rigid objects. We investigate how high-level brain areas that control the planning and execution of eye movements interact with other brain areas that integrate spatial information and encode structures. We employed Kanizsa figures, in which illusory contours are interpolated from isolated “pacmen” inducers to

form an apparent occluding shape that can appear lighter or darker than its background. Observers judged the apparent lightness of real and illusory Kanisza squares relative to a grey background. The pacmen inducers were dark (<1 cd/m2) and the physical luminance of the target square was higher than, lower than, or equal to the luminance of the background (15 cd/m2), according to a method of constant stimuli. The target was presented at 11° eccentricity for 80 ms while an observer fixated the center of the display, or within 200 ms of the onset of a saccade toward the center of the upcoming target. Perceived lightness was inferred from the 50% point of a cumulative Gaussian fit to the proportion of trials in which the observer reported that the target appeared lighter than the background as a function of the physical target luminance. For the no-eye-movement condition, the point of subjective equality (PSE) was biased toward darker targets: a square that was physically 3% darker than its background was judged to be equally light, suggesting a perceptual lightening of the target. When targets were presented immediately before a saccade, this perceptual bias doubled in magnitude: the PSE occurred when the target was 6% darker than the background. Therefore, the perceived lightness of the Kanisza figure was greater immediately before a saccade than when no eye movement was planned. Lightness discrimination sensitivity (the slope of the psychometric function) did not vary across conditions, ruling out the possibility that changes in perceived lightness were a corollary of previously reported changes in perceived contrast of features at the goal of a saccade. Our data thus reveal three important novel findings: 1) the systems involved in generating eye movements influence the computations involved in visual interpolation; 2) visual objects, not just their component features, are altered by saccadic preparation, and 3) the perceptual processes associated with saccadic preparation enhance illusory rather than veridical percepts. These observations identify an interaction between eye movement control and feature integration that promotes the representation of stable objects across the visual field.

Disclosures: **W.J. Harrison:** None. **G. Maiello:** None. **P.J. Bex:** None.

Poster

533. Eye Movements and Perception

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National Alliance for Research on Schizophrenia and Depression

Title: Perisaccadic perception of visual space in people with schizophrenia

Authors: *A. RICHARD, J. CHURAN, V. WHITFORD, G. O'DRISCOLL, D. TITONE, C. PACK
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Abstract: Background Corollary discharge signals are found in the nervous systems of many animals, where they serve a large variety of functions related to the integration of sensory and motor signals. In particular, this signal is thought to serve a number of purposes related to the maintenance of accurate visual perception. In humans, an important corollary discharge signal is generated by oculomotor structures and communicated to sensory systems in concert with the execution of each saccade. The properties of the oculomotor corollary discharge can be probed by asking subjects to localize stimuli that are flashed briefly around the time of a saccade, where the results of such experiments typically reveal large errors in localization. Here, we have exploited these well-known psychophysical effects to assess the potential dysfunction of corollary discharge signals in people with schizophrenia. **Methods** Using a standard paradigm (e.g. Lappe et al., 2000), we studied mislocalization in schizophrenic subjects and controls making horizontal 20° saccades. Subjects (8 patients and 6 controls) were instructed to report the perceived position a briefly (12 ms) flashed vertical bar presented over a range of horizontal positions in a time-window ± 200 ms around saccade onset. **Results** We found that, compared with controls, patients with schizophrenia exhibited larger errors in localizing visual stimuli. The pattern of errors could be modeled as an over-damped corollary discharge signal that encodes instantaneous eye position. **Conclusions** The dynamics of this signal predicted symptom severity among patients, suggesting a possible mechanistic basis for widely observed behavioral manifestations of schizophrenia.

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Poster

533. Eye Movements and Perception

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Program#/Poster#: 533.17/HH7

Topic: D.06. Eye Movements

Title: A meta-analysis of functional imaging studies of smooth pursuit eye movement abnormalities in patients with schizophrenia

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Abstract: Abnormal smooth-pursuit eye movements (SPEM) are well documented in patients with schizophrenia (SZ). Studies show SPEM abnormalities in up to 80% of patients and 50% of their unaffected first degree relatives (Holzman et al., 1974; Chen et al., 2004). However, no conclusive evidence exists that can delineate the neural basis of dysfunctional SPEMs in patients with SZ. In the present study, we utilized activation-likelihood estimation (ALE) meta-analytic techniques to synthesize the results of many neuroimaging studies in order to identify common patterns of activation among them. Particularly, we focused on studies which compared the neural activation patterns of patients with SZ to healthy controls during a smooth-pursuit based task. We performed a literature search of the online databases PubMed and Google Scholar, using the keywords "smooth pursuit, schizophrenia, eye movement" to identify relevant studies. Any paper that directly compared patients to controls on a smooth-pursuit task, utilized whole brain analyses, and reported the foci of significantly different activation in a standard stereotactic space was included in ALE meta-analysis. ALE finds convergence of location between different experiments, and does not depend on author assigned labels (Turkeltaub et al., 2002). Instead, they utilize foci reported in a standard stereotactic space as raw data. All six studies we reviewed (O'driscoll et al., 1999; Tregellas et al., 2004; Hong et al., 2005; Lencer et al., 2005; Keedy et al., 2006; Nagel et al., 2007), used a horizontal smooth-pursuit task, and reported significantly different activation localized in the frontal eye fields and/or the V5 complex. However, our meta-analysis revealed a small cluster of significant ALE scores only in the right medial thalamus. These results are consistent with the finding of Cui et al. (2003) who suggested that the thalamus is involved in an efferent feedback loop of extra-retinal motion signals in primates, as well as a paper by Cronenwett and Csernansky (2010) that detailed thalamic pathology in patients with schizophrenia. Our finding of abnormal activity patterns in the right medial thalamus support the need for the use of whole brain imaging and analysis, instead of region of interest based analyses, in all future neuroimaging studies of patients with SZ. We believe that the differences between the tasks used in the experiments included in our meta-analysis confounded our results. Therefore, any future research of smooth-pursuit dysfunction in patients with SZ would benefit from the adoption of a standardized smooth-pursuit task, similar to the standardized anti-saccade task reported in Leigh et al. (2013)

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Poster

533. Eye Movements and Perception

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Philanthropic Educational Organization National Scholar Award

Title: Oculomotor executive function abnormalities with increased tic severity in Tourette Syndrome

Authors: *C. B. JETER[1], S. S. PATEL[2], J. S. MORRIS[3], A. Z. CHUANG[4], I. J. BUTLER[5], A. B. SERENO[6]
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Abstract: Background: Reports conflict as to whether Tourette Syndrome (TS) confers deficits in executive function. This study's aim was to evaluate executive function in youths with TS using oculomotor tasks while controlling for confounds of tic severity, age, medication and severity of comorbid disorders. **Method:** Four saccade tasks requiring the executive functions of response generation, response inhibition, and working memory (prosaccade, antisaccade, 0-back and 1-back) were administered. Twenty youths with TS and low tic severity (TS-low), nineteen with TS and moderate tic severity (TS-moderate), and twenty-nine typically developing control subjects (Controls) completed the oculomotor tasks. **Results:** There were small differences across groups in the prosaccade task. Controlling for any small sensorimotor differences, TS-moderate subjects had significantly higher error rates than Controls and TS-low subjects in the 0-back and 1-back tasks. In the 1-back task, these patients also took longer to respond than Controls or TS-low subjects. **Conclusions:** In a highly controlled design, the findings demonstrate for the first time that increased tic severity in TS is associated with impaired response inhibition and impaired working memory and that these executive function deficits cannot be accounted for by differences in age, medication or comorbid symptom severity.

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Poster

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Topic: D.05. Visual Sensory-motor Processing

Support: USAF School of Aerospace Medicine (Oculometric Motion Capability Assessment Tool)

NSF Program in Perception, Action, and Cognition (#0924841)

Title: Oculometric assessment of dynamic visual processing

Authors: *D. B. LISTON[1,2], L. STONE[1]
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Abstract: Eye movements are the most frequent (~3 per second), shortest-latency (~200 ms), and biomechanically simplest (1 joint, no inertial complexity) voluntary motor behavior in primates, providing a model system to assess sensorimotor disturbances arising from trauma, fatigue, aging, or disease (e.g., Diefendorf & Dodge, 1908). We developed a 15-minute test that derives ten metrics from the oculomotor response to visual motion, including pursuit initiation and steady-state tracking, as well as direction and speed tuning metrics that can be directly converted into psychometric thresholds (e.g., Stone et al., 2009). Methods. Subjects (N=41) fixated a central spot for a randomized duration. When the fixation spot was extinguished, a small peripheral target spot simultaneously appeared at 3.2 to 4.8 deg eccentricity and immediately began moving for 700-1000 ms back toward and past the initial fixation point (Rashbass, 1961). On each of the 180 trials, the velocity of the target spot was randomly drawn from a distribution of five speeds (16, 18, 20, 22, 24 deg/s) and of 180 possible directions sampled every 2 deg. The large temporal and spatial uncertainty minimized the utility of prior expectations and required a broad distribution of attention across space, time, and direction. Results. We observed a median pursuit latency of 180 ms, initial acceleration of 124 deg/s^2, and median steady-state gain of 0.82. We found median oculometric direction-discrimination thresholds of 2.7 deg (cardinal) and 6.0 deg (oblique), and speed-discrimination Weber fraction of 34%, consistent with psychophysical values for unpracticed observers under high-uncertainty

conditions (e.g., Krukowski and Stone, 2005). Although some of our metrics were correlated, some were not (e.g., the two oblique-effect metrics were uncorrelated with the others, r2 < 0.2) and all were uncorrelated with subject age and foveal acuity (r2 < 0.10. Conclusion. Our 15-minute clinical test delivers ten metrics that quantify multiple aspects of dynamic visual function that would require many hours or even days of psychophysical testing to measure using standard methods. Our test could serve as an efficient screening tool for neural disorders affecting visuomotor processing.

Disclosures: D.B. Liston: None. **L. Stone:** None.

Poster

533. Eye Movements and Perception

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 533.20/HH10

Topic: D.05. Visual Sensory-motor Processing

Title: Electrodermal activity and eye movements inform the usability of passwords

Authors: *J. C. ROMANO BERGSTROM[1], K. GREENE[2], D. HAWKINS[1], C. GONZALEZ[1]
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Abstract: While measuring physiological responses is a common practice in the field of neuroscience, it is rare in the usability arena and in password usability studies, in particular. This is unfortunate, as the use of such implicit measures could complement more traditional, explicit metrics of performance like password entry times and errors. Capturing participants' electrodermal activity and eye-movement patterns during password entry can provide unique insights about users' intentions, perceptions of difficulty, and emotional state that would otherwise be impossible to gain via behavioral and self-report measures. Here, we build on prior work examining the usability of a set of complex, system-assigned passwords across input devices (Greene et al., 2014) and adherence to English grammatical rules (Romano Bergstrom et al., 2014). We maintain similar stimuli and methodology but expand upon previous research by additionally recording electrodermal activity (i.e., skin conductance response [SCR]) and eye movements, using fingertip electrodes and a desktop eye tracker. We expect SCR for more difficult passwords to be of greater amplitude, greater frequency, and shorter latency than easier passwords, where password difficulty is a function of multiple factors such as length, frequency and placement of special symbols, and linguistic and phonological difficulty. Additionally, more difficult passwords should result in more repeat fixations, fixations that are more frequent and of

longer duration than for easier passwords. Physiological measures may improve password difficulty classification accuracy of a naïve Bayesian classifier over and above behavioral measures of error rates and completion times. The potential to differentiate difficult from easy passwords based on SCR and fixation frequency and duration implies that we have indeed found a promising use for implicit indicators of stress, encoding, and retrieval processes associated with password learning and entry across devices. The current exploratory work demonstrates the potential practical value of combining physiological and behavioral measures in password usability research. This should be of interest to the usability, security, and neuroscience research communities, as it provides a novel measurement approach for the former and an interesting applied research problem for the latter.

Disclosures: J.C. Romano Bergstrom: None. **K. Greene:** None. **D. Hawkins:** None. **C. Gonzalez:** None.

Poster

533. Eye Movements and Perception

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 533.21/HH11

Topic: D.04. Vision

Title: The cognitive control of gaze patterns when decoding the affordances of complex tool use

Authors: *N. NATRAJ[1], Y. M. PELLA[1], A. M. BORGHI[2], L. WHEATON[1]
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Abstract: Tools are a unique class of objects as they automatically potentiate action knowledge (graspability, usability) during passive viewing, a property called affordances. It is thought that such affordances are decoded by parietofrontal regions that store motoric properties of tools and tool-use. However, overlapping parietofrontal networks also control eye movements; it is unclear if a relationship exists between exploratory oculomotor control and action understanding when viewing static images of complex tool-use, the predominant stimuli in affordance studies. To address this issue, we recorded eye movements in healthy human adults as they passively evaluated static tool-use images across a combination of three tool-use contexts and four types of tool-grasps, giving rise to 12 experimental conditions with varying affordances. We hypothesized that the control of exploratory eye movements (saccade initiation times, gaze patterns and scene feature biases) over distinct features of tool-use scenes across conditions to

either be maximally unpredictable/random or alternatively, always follow a predictable pattern independent of affordances. This would imply that parietofrontal responses to tool-use images represent action understanding alone rather than an additional control of fixations and saccades. Given the high dimensionality of the dataset and the multivariate time varying nature of eye movements, a novel statistical framework based on pattern recognition theories was developed to evaluate the hypothesis. While saccade initiation times were independent of affordances, contrary to our hypothesis participants exhibited differential gaze patterns and scene feature biases that were driven primarily by the intent of the tool-grasp and subsequently by the context of tool-use action with unique interaction effects. Additionally, a discrete Hidden Markov Model was developed that predicts a hierarchical control scheme of eye movements in each visual hemifield modulated by affordances. Results here show that gaze behavior is sensitive to affording properties in tool-use scenes, suggestive of a coupling between oculomotor control and action understanding over parietofrontal networks. This has potential implications to affordance studies evaluating neural and/or behavioral responses to tool-use understanding.

Disclosures: **N. Natraj:** None. **L. Wheaton:** None. **A.M. Borghi:** None. **Y.M. Pella:** None.

Poster

534. Nociceptors: Molecular and Pharmacological Studies

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 534.01/HH12

Topic: D.08. Pain

Support: National Research Foundation of Korea (NRF) Grant 2012R1A3A2048834

Title: Acute inflammation reveals potential nociceptive role of peripheral GABA A receptors

Authors: ***I. JANG**[1], Y. KIM[1], S. JUNG[2], H. FURUE[3], S. OH[1]
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Abstract: Gamma-aminobutyric acid (GABA) depolarizes dorsal root ganglia (DRG) neurons through GABA A receptor activation. In the present study, we tested whether GABA A receptors are involved in nociceptive signaling and pain behavior during formalin-induced acute inflammatory pain in adult mice. We found that peripheral administration of the GABA A receptor agonist, muscimol, restored spontaneous licking behavior after subsidence of formalin-induced pain behaviour. We performed extracellular single-unit recordings from spinal cord wide

dynamic range neurons *in vivo* and showed that spike frequency was increased by muscmiol injection into hind paw after, but not before, formalin treatment. Using Ca2+ imaging *in vitro* we show that formalin, as well as the major inflammatory mediator prostaglandin E2, can potentiate GABA-induced Ca2+ transients in cultured DRG neurons, an effect that is blocked by the prostaglandin EP4 receptor antagonist AH23848. Taken together, these results demonstrated that GABA A receptors may contribute to excitation of peripheral sensory neurons in inflammation through PGE2-EP4 signaling.

Disclosures: I. Jang: None. **Y. Kim:** None. **S. Jung:** None. **H. Furue:** None. **S. Oh:** None.

Poster

534. Nociceptors: Molecular and Pharmacological Studies

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 534.02/HH13

Topic: D.08. Pain

Title: Investigating the role of TRESK in sensory afferent function using a selective opener

Authors: *L. CAO[1], A. LOUCIF[1], P.-P. SAINTOT[1], M. K. PATEL[2], C. ADAMS[1], K. KUAN[1], R. FISH[1], M. RIGBY[1], B. ANTONIO[3], K. OMOTO[1], D. PRYDE[1], E. B. STEVENS[1]
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Abstract: Two-pore-domain background potassium (K2P) channels are a unique structural group of ion channels with each subunit containing four transmembrane domains and two pore domains which assemble as functional dimers. K2P channels are important for the generation and modulation of the resting membrane potential and play an important role in regulating neuronal excitability. The calcineurin-regulated K2P channel, TRESK has been suggested as potential therapeutic target for pain due to a genetic association between a TRESK frameshift mutation and migraine with aura in a large pedigree, along with its unique expression pattern largely limited to sensory neurones. In this study we have used a highly selective TRESK opener alongside an immunohistochemical approach, using a TRESK antibody, to investigate the role of TRESK in sensory neuron excitability and neurotransmitter release. The TRESK opener was identified following an HTS campaign using a thallium flux assay. Using manual patch clamp technique, compound A caused a maximum enhancement of 226 ± 43 % and an EC_{50} of 0.6 μM for human TRESK (n=3) and a maximum enhancement of 394 ± 21 % and an EC_{50} of 0.9 μM for rat TRESK (n=3). No clear effect was observed against other potassium channels (TREK-1,

TASK-3, KCNQ1, BK and hERG) at 10 μM. Using current clamp recording, the firing frequency of rat dorsal root ganglion (DRG) neurones was reduced by 41.5 ± 17 % (n=7) when compound A was applied at 3 μM. A multi-unit *ex-vivo* rat skin-nerve preparation was used to test the effect of compound A on heat evoked (36-52^{o}C ramp) tibial nerve firing. Compound A had no significant inhibitory effect (11 ± 2.0 %, n=7) on the heat-evoked firing frequency, while the KCNQ2/3 opener, retigabine, significantly inhibited firing by 38.4 ± 2.0% (n=7), compared to their respective vehicle controls. In contrast, compound A at both 3 uM and 10 uM significantly reduced CGRP release from (lumbar L4-L6) spinal synaptosomes evoked by 10 μM veratridine. Using immunohistochemistry, expression of TRESK in peripheral and central compartments (rat skin, DRG and dorsal horn) was compared to the neuronal marker PGP9.5. TRESK antibody staining was detected in DRG neurons and dorsal horn, but was absent from peripheral nerve. In conclusion, using both a selective TRESK opener and immunohistochemistry, we have demonstrated that TRESK has a key role in regulating neurotransmitter release in the dorsal horn, but plays little or no role in regulating peripheral excitability.

Disclosures: **L. Cao:** A. Employment/Salary (full or part-time):; Pfizer. **A. Loucif:** A. Employment/Salary (full or part-time):; Pfizer. **P. Saintot:** A. Employment/Salary (full or part-time):; Pfizer. **C. Adams:** A. Employment/Salary (full or part-time):; Pfizer. **K. Kuan:** A. Employment/Salary (full or part-time):; Pfizer. **D. Pryde:** A. Employment/Salary (full or part-time):; Pfizer. **K. Omoto:** A. Employment/Salary (full or part-time):; Pfizer. **B. Antonio:** A. Employment/Salary (full or part-time):; Pfizer. **E.B. Stevens:** A. Employment/Salary (full or part-time):; Pfizer. **M.K. Patel:** None. **R. Fish:** A. Employment/Salary (full or part-time):; Pfizer. **M. Rigby:** A. Employment/Salary (full or part-time):; Pfizer.

Poster

534. Nociceptors: Molecular and Pharmacological Studies

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Topic: D.08. Pain

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Eckardt Scholars Program

College of Arts and Sciences, Lehigh University

Department of Biological Sciences, Lehigh University

HHMI BDSI Program

Title: Effects of pharmacological agents on pain tolerance in lynx1 knockout mice through the manipulation of nicotinic acetylcholine receptors

Authors: *K. R. ANDERSON, C. M. GARRISON, K. L. ACKERMAN, J. M. MIWA
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Abstract: Pain management is a crucial yet challenging field that causes over 80 billion dollars in hospital costs annually. A subset of patients are resistant to the current treatment options, which are also often accompanied by adverse side effects. A search for alternative therapeutic avenues, therefore, is warranted. A potential alternative pain therapy is based upon nicotine receptors, which have a role in pain alleviation (antinociception). The binding of certain agonists, such as the exogenous drug of abuse nicotine, to nicotinic acetylcholine receptors (nAChRs) have been shown to produce analgesic effects. nAChRs are part of the cholinergic system which mediates a host of CNS functions, including pain tolerance. The cholinergic system exists on a gradient of activation, which can be influenced by several regulatory factors, one being a class of protein modulators, lynx proteins. The lynx1 gene codes for an allosteric modulatory protein which binds to nAChRs and inhibits its function, in order to balance neuronal activity and survival in the CNS. lynx1 acts as a brake on the cholinergic system and shifts the cholinergic gradient towards underactivation. lynx1 was not previously known to function in pain pathways but its relationship with the cholinergic system suggests a direct upstream function in pain thresholds and pain tolerance. In order to test the relationship between nicotine, lynx1, and pain tolerance lynx1 knockout and wildtype mice are given saline or nicotine and tested for pain thresholds on a thermal sensitivity test. It is predicted that in the presence of lynx1, the analgesic effects of nicotine will be reduced but in the absence of the lynx1 gene, cholinergic tone will be raised, thus increasing pain tolerance. This hypothesis was supported as the loss of the lynx1 gene in lynx1 knockout mice, resulted in an increase in pain tolerance which is further amplified by nicotine. Several inhibitors were given in conjugation in order to test which specific subtypes of nicotinic receptors are involved. Our data suggest a selective release on α4* receptor inhibition in lynx1 knockout mice, whereas an α4* component is not expressed in wild-type mice. This data suggests the lynx1 can act as a specific receptor modulator to regulate pain thresholds.

Disclosures: K.R. Anderson: None. **C.M. Garrison:** None. **K.L. Ackerman:** None. **J.M. Miwa:** None.

Poster

534. Nociceptors: Molecular and Pharmacological Studies

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 534.04/HH15

Topic: D.08. Pain

Support: MINECO SAF2010-14990

Title: Role of TASK-3 leak potassium channels in peripheral cold thermosensation

Authors: C. MORENILLA-PALAO[1], E. LUIS[1], C. FERNÁNDEZ-PEÑA[1], E. QUINTERO[1], J. L. WEAVER[2], D. A. BAYLISS[2], *F. VIANA[3]
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Abstract: Animals sense cold ambient temperatures through the activation of peripheral thermoreceptors that express TRPM8, a cold- and menthol-activated transient receptor potential ion channel. These cold thermoreceptors can discriminate a very wide range of temperatures, from innocuous to noxious. The molecular mechanism responsible for the variable sensitivity of individual cold receptors to temperature is still unclear. Previous studies have suggested an important role of several potassium channels in cold thermotransduction, by acting as excitability brakes. However, the molecular and functional characterization of these potassium channels in thermoreceptors is still incomplete. To address this question, we performed a detailed ion channel expression analysis of cold sensitive neurons, combining BAC transgenesis with a molecular profiling approach in FACS purified TRPM8 neurons from mouse DRG. We found that TASK-3 (Kcnj9) leak potassium channels are highly enriched in TRPM8-positive neurons and expressed in a fraction of these thermoreceptors. Using whole-cell patch-clamp recordings we characterized leak potassium currents sensitive to low external pH (a signature for TASK-1 and TASK-3 channels) in TRPM8-expressing neurons. The density of pH-sensitive current was reduced in TRPM8-positive neurons of TASK-3 KO mice. Moreover, the thermal threshold of the population of TRPM8 cold neurons is decreased in mice lacking TASK-3. Behavioral studies showed that these mice display hypersensitivity to cold. Our results demonstrate a novel role of TASK-3 channels in cold thermosensation, and suggest that a channel-based combinatorial expression within TRPM8 cold thermoreceptors leads to their molecular specialization and functional diversity.

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Poster

534. Nociceptors: Molecular and Pharmacological Studies

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

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Topic: D.08. Pain

Support: NIH RO1 NS NS 073939

NIH RO1 NS NS 074999

UT System STAR Award

Title: Phosphorylation of Epac1 by GRK2 inhibits Epac1-to-Rap1 signaling and prevents chronic pain

Authors: *A. KAVELAARS[1], P. SINGHMAR[1], X. HUO[1], N. EIJKELKAMP[2], S. R. BERCIANO[3], F. MAYOR, Jr.[3], Y. ZHOU[4], F. C. MEI[4], C. MURGA[3], X. CHENG[4], C. J. HEIJNEN[1]
[1]Neuroimmunology Lab., Univ. of Texas MD Anderson Cancer Ctr., Houston, TX; [2]NIDOD laboratory, Univ. Med. Ctr. Utrecht, Utrecht, Netherlands; [3]Unisversidad Autonoma de Madrid, Madrid, Spain; [4]Dept Integrative Biol. and Pharmacol., Univ. of Texas Hlth. Sci. Ctr., Houston, TX

Abstract: Chronic pain is a major clinical problem, yet the mechanisms underlying the transition from acute to chronic pain remain poorly understood. We show that reduced expression of nociceptor G protein-coupled receptor kinase 2 (GRK2) promotes cAMP signaling to the guanine nucleotide exchange factor EPAC1 and prolongs the PGE_2-induced increase in pain sensitivity (hyperalgesia). Using a model of transition from acute to chronic inflammatory pain, we show that reduced GRK2 and/or increased EPAC1 in dorsal root ganglion (DRG) neurons promotes chronic pain. In addition, when we either increased GRK2 *in vivo* by viral-based gene-transfer or decreased EPAC1 *in vivo*, as was the case for mice heterozygous for Epac1 or mice treated with Epac1 antisense-oligodeoxynucleotides, the transition to chronic pain was prevented. In search for the underlying mechanism that promotes Epac1 signaling when GRK2 is low, we identified GRK2 as the first kinase known to phosphorylate Epac1. We identified the residue in Epac1 that is phosphorylated by GRK2. In addition, we demonstrate that phosphorylation of this residue in Epac1 by GRK2 inhibits Epac-mediated activation of the small G protein Rap1 and agonist-induced membrane association of Epac1. These findings add Epac1

to a growing list of non-G protein coupled receptor GRK2 substrate and further establish the key role of GRK2 in pain sensing neurons in protecting against chronic pain. Our data suggest that therapies targeted at balancing nociceptor GRK2 and EPAC1 levels have promise for the prevention and treatment of chronic pain.

Disclosures: **A. Kavelaars:** None. **P. Singhmar:** None. **X. Huo:** None. **N. Eijkelkamp:** None. **S.R. Berciano:** None. **F. Mayor:** None. **Y. Zhou:** None. **F.C. Mei:** None. **C. Murga:** None. **X. Cheng:** None. **C.J. Heijnen:** None.

Poster

534. Nociceptors: Molecular and Pharmacological Studies

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Topic: D.08. Pain

Support: NIH Grant 1-R01-DA-033059

VA Merit Review I01-RX000378

Title: NMDA receptor-induced substance P release from primary afferent terminals is inhibited by μ and κ, but not δ, opioid receptors

Authors: ***W.-L. CHEN**[1], H. KIM[1], H. S. ENNES[1], W. WALWYN[1], J. A. MCROBERTS[1,2], J. G. MARVIZON[1,2]
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Abstract: Recently, we showed that BDNF acting on trkB receptors induces a gain of function of NMDA receptors in primary afferent terminals that occurs after the onset of neuropathic pain (Chen et al., Eur. J. Neurosci., 2014). Here we present evidence that μ opioid receptors (MORs) and κ-opioid receptors (KORs) induce a counterbalancing loss of function of these NMDA receptors. NMDA receptor function was assessed as the ability of NMDA + D-Ser (10 μM for 2 min) to induce substance P release in rat spinal cord slices. Substance P release was measured as neurokinin 1 receptor (NK1R) internalization in lamina 1 neurons. NMDA-induced NK1R internalization in spinal cord slices was abolished by 1 hr preincubation with the MOR agonists morphine (1 μM), DAMGO (0.1 μM) and endomorphin-2 (1 μM). DAMGO had no effect when applied together with NMDA and produced a maximum effect with preincubations of 15 min or

longer. A concentration-response for DAMGO yielded an IC50 of 14 nM and was right-shifted by the MOR antagonist CTAP with a KB of 215 nM (Gaddum-Schild analysis). The KOR agonist U50,488 (1 μM) inhibited NMDA-induced NK1R internalization by about 50%, and this inhibition was reversed by the KOR antagonist nor-binaltorphimine (1 μM) but not by the MOR antagonist CTAP (10 μM). The δ-opioid receptor (DOR) agonists DPDPE (1 μM), [D-Ala2]-deltorphin II (1 μM) and SNC-80 (1 μM) produced different levels of inhibition; however, their effect was eliminated by the MOR antagonist CTAP (10 μM) but not by the DOR antagonist naltrindole (10 μM), indicating that it was mediated by MORs. The GABAB agonist baclofen (30 μM) did not affect NMDA-induced NK1R internalization. DAMGO inhibited NMDA-induced NK1R internalization in slices preincubated with BDNF (20 ng/ml), but not in slices preincubated with the protein tyrosine phosphatase inhibitor BVT948 (10 μM), suggesting that MORs inhibit the NMDA receptors by increasing the dephosphorylation of the NR2B subunit by a protein tyrosine phosphatase. To determine whether MORs inhibit NMDA receptor-induced substance P release *in vivo*, rats were given intrathecal BDNF (0.3 μg) to increase NMDA receptor function, then intrathecal DAMGO (3 nmol) or saline 3 hr after BDNF and finally intrathecal NMDA + D-Ser (10 nmol each) 1 hr later. NMDA induced NK1R internalization after BDNF, and this effect was eliminated by DAMGO. Alternatively, NMDA receptor function was increased by chronic constriction injury of the sciatic nerve; 6 hr later, intrathecal DAMGO (3 nmol) also inhibited NMDA-induced NK1R internalization. These results show that MORs and KORs, but not DORs or GABAB receptors, decrease NMDA receptor function in primary afferent terminals.

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Poster

534. Nociceptors: Molecular and Pharmacological Studies

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Program#/Poster#: 534.07/HH18

Topic: D.08. Pain

Support: USPHS NS078173

Title: The TrkA receptor and not the p75NTR mediates thermal hyperalgesia from NGF in the rat hind paw

Authors: *A. KHODOROVA[1], G. NICOL[2], G. STRICHARTZ[1]
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Abstract: Recently we have shown that p75 neurotrophin receptor ($p75^{NTR}$) and its activation of the sphingomyelin signaling cascade are essential for mechanical hypersensitivity resulting from NGF (Neuroscience, 2013). Here we evaluate the role of the same effectors in thermal hypersensitivity induced by NGF. Sensitivity of rats' plantar skin to thermal stimulation after local subcutaneous (s.c.) injection of NGF was measured using the Hargreaves method. Intraplantar injection of NGF (500ng/paw) resulted in ~40% reduction of paw withdrawal latency (PWL) that was measured through 22-24h and had recovered by 48h. Pre-injection of the paw with the $p75^{NTR}$ blocking anti-body (gift of L. Reichardt, UCSF) 4h before NGF did not affect the degree of acute thermal hyperalgesia over 0.5-3.5h, but shortened its duration such that the PWL fully recovered by 22h. In controls, pre-injections of IgG were without effect. Thus, whereas mechano-sensitizing effects of both Pro-NGF and NGF were prevented by pre-injection of the paw with the $p75^{NTR}$ blocking anti-body, that antibody failed to prevent the acute thermal hypersensitivity from NGF. In addition, GW4869 (2mM), an inhibitor of the nSMase enzyme on the $p75^{NTR}$ signaling pathway, that inhibited mechanical hyperalgesia from NGF, failed to prevent thermal hyperalgesia. Paw injection of a non-selective inhibitor of nSMase activation, glutathione, pre- and co-injected with NGF (1.8µmoles/paw total dose, at a 15min interval) prevented significant acute thermal- sensitization at 22-48min, but was without effect at later time points, or at lower doses. A wide spectrum inhibitor of the TrkA receptor, K252a, at 2mM fully prevented thermal hyperalgesia from NGF, at 200µM its development was delayed, and at 20µM it was unaffected. TrkA blocking anti-body (gift of L. Reichardt, UCSF) prevented the reduction of PWL from subsequently injected NGF. These findings suggest that TrkA receptor has a predominant role in the development of thermal hypersensitivity induced by NGF, while $p75^{NTR}$ may be engaged in its maintenance.

Disclosures: A. Khodorova: None. **G. Nicol:** None. **G. Strichartz:** None.

Poster

534. Nociceptors: Molecular and Pharmacological Studies

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 534.08/HH19

Topic: D.08. Pain

Title: Involvement of P2X3 in peripheral sensitization of UVB-induced inflammatory pain

Authors: E. KASAI, S. OMACHI, T. ASAKI, G. SAKAGUCHI, *S. SHINOHARA
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Abstract: In the field of pain research, the importance of translational pain models become increasing. Among them, ultraviolet B (UVB)-induced skin inflammation model is one of the best characterized experimental pain models in the clinical. UVB-irradiation induces mechanical hyperalgesia at the site of irradiation (primary hyperalgesia), is thought to be elicited predominantly via peripheral sensitization. In this study, we investigated physiological changes and sensitivity to analgesics in UVB-irradiated rats. We also examined whether P2X3 receptors are involved in peripheral sensitization in UVB model.UVB-irradiation to the rat hind paws produced a significant dose-dependent reduction in mechanical paw withdrawal thresholds as reported in human. Slight erythema, mild edema, increase in blood flow and up-regulation of inflammatory factors are also observed in the rats. The sensitivity profile was evaluated using several analgesics such as ibuprofen, morphine and pregabalin. Ibuprofen (30 mg/kg, p.o.) and morphine (1 mg/kg, s.c.) significantly reduced mechanical hyperalgesia but pregabalin (10 mg/kg, p.o.) had little effect in the UVB-irradiated rats. These pharmacological findings are similar to those in human model. Injection of adenosine 5'-triphosphate (ATP) to rat hind paws produced mechanical hyperalgesia and it was potentiated in the UVB-irradiated rats. ATP-evoked hyperalgesia in UVB-irradiated rat was dose dependently attenuated by pre-administration of P2X3 antagonist (A317491, 1-100 mg/kg, s.c). Minimal effective dose of A317491 was lower in the UVB-irradiated rats than non-irradiated. Immunohistochemical analysis revealed that injection of ATP into UVB-irradiated skin induced phosphorylation of extracellular signal-regulated kinases 1/2 (ERK1/2), a potent marker of activated neurons, in L4/L5 dorsal root ganglion (DRG) neurons expressing P2X3. These results suggested that P2X3 receptors have critical contribution to ATP-evoked hyperalgesia and also contribute to peripheral sensitization in UVB model. In conclusion, rat UVB model shows similar pathophysiological and harmacological properties to those in human UVB model. The rat UVB model might be a good translational model for investigation of peripheral sensitization in pathological pain.

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Poster

534. Nociceptors: Molecular and Pharmacological Studies

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Topic: D.08. Pain

Support: NSF IOS-1051734 BDB

Title: Endovanilloids modulate synaptic transmission by three distinct mechanisms

Authors: *Y. WANG, B. D. BURRELL
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Abstract: Endovanilloids are endogenous ligands that act on TRPV channels in the central nervous system and play an essential role in cardiovascular and respiratory functions, emesis, locomotion, anxiety and pain modulation. Anandamide (AEA) and 2-arachidonoyl glycerol (2-AG) are examples of endogenous vanilloid neurotransmitters that act on both TRPV and cannabinoid receptors (Zygmunt et al. (2014), PLoS ONE 8: e61618). Using the well-characterized nervous system of the medicinal leech, it is possible to isolate the TRPV-mediated effects of 2-AG and AEA since invertebrates lack of CB receptors. In this project, we examined the role of Cl- homeostasis in determining the sign of endovanilloids-mediated synaptic modulation. Endovanilloids were observed to elicit three distinct forms of persistent (≈1hr) synaptic plasticity. In synapses made by polymodal nociceptive afferents, AEA, 2-AG and the TRPV1 agonist, capsaicin (CAP), depressed synaptic transmission via direct activation of a TRPV-like receptor as previously described (Yuan & Burrell (2010) J Neurophysiol 104: 2766). Pretreatment with the TRPV1 antagonist, SB 366791 (SB), prevented this endovanilloid-induced form of long-term depression (LTD). In synapses made by the non-nociceptive, pressure-sensitive afferents, AEA, 2-AG and CAP potentiated synaptic transmission. This endovanilloid-induced long-term potentiation (LTP) was due to a decrease in inhibitory GABAergic signaling (disinhibition) that could be blocked by pretreatment with either bicuculline (GABA receptor antagonist) or VU 0240551 (an inhibitor of the Cl- exporter KCC2). The polymodal nociceptive synapses that are directly depressed by endovanilloids are protected from this disinhibition due to an elevated intracellular Cl- concentration (they are excited, not inhibited, by GABA). Finally, in synapses made by mechanical nociceptive afferents, AEA, 2-AG and CAP also depressed synaptic transmission, but not due to the direct activation of the TRPV-like receptors, which these afferents lack. As in the case of polymodal nociceptors, mechanical nociceptive afferents are also excited by GABA due to an elevated intracellular Cl- concentration. In these synapses, endovanilloid-mediated LTD was found to be due to a decrease in this excitatory GABAergic input (disexcitation) given that it could be blocked by pretreatment with either bicuculline or bumetanide (an inhibitor of Cl- importer NKCC1). These results indicate that endovanilloids can modulate synapses by three distinct mechanisms and that Cl- homeostasis plays a critical role in determining the sign of this synaptic plasticity.

Disclosures: Y. Wang: None. **B.D. Burrell:** None.

Poster

534. Nociceptors: Molecular and Pharmacological Studies

Location: Halls A-C

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Topic: D.08. Pain

Support: GM102575

NS065926

Title: AMPK-mediated control of P bodies as a novel mechanism of gene expression control in peripheral sensory neurons

Authors: G. L. MEJIA[1], O. K. MELEMEDJIAN[3], G. DUSSOR[4], *T. J. PRICE[2]
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Abstract: Changes in gene expression have long been recognized as a central mechanism for altered sensitivity and excitability of nociceptors. We, and others, have focused on translation control, in particular local, activity-dependent translation control as a novel means to modulate gene expression in response to injury. In this context, an increase in local translation, downstream of extracellular signal regulated kinase (ERK) and/or mechanistic target of rapamycin complex 1 (mTORC1) activation leads to an enhancement of pain sensitivity and an increase in measures of excitability. A possible mechanism to mitigate these effects is activation of adenosine monophosphate activated protein kinase (AMPK) because signaling via this kinase leads to inhibition of ERK and mTORC1 signaling to translation machinery. In addition to these effects, inhibition of translation via AMPK may also lead to changes in mRNA turnover. We have tested that hypothesis here examining major sites of mRNA repression and decay in cells, called P bodies, upon AMPK activation in trigeminal (TG) and dorsal root ganglion (DRG) neurons. We find that translation (using the sunset technique) and P body formation are reciprocally regulated upon pharmacological activation of AMPK in TG and DRG neurons. While AMPK activation leads to a decrease in puromycin incorporation into nascently synthesized peptides, it also causes a robust increase in P bodies (as revealed by rck/p54-positive puncta) suggesting mRNA sequestration from translation machinery and potentially mRNA degradation because P bodies are major sites for mRNA decapping in cells. Moreover, AMPK activation *in vivo* with metformin leads to enhanced P body formation in DRG neurons. Our

findings enhance our understanding of gene expression regulation in the peripheral nervous system and suggest a potential role for P bodies in pain plasticity.

Disclosures: G.L. Mejia: None. **O.K. Melemedjian:** None. **G. Dussor:** None. **T.J. Price:** None.

Poster

534. Nociceptors: Molecular and Pharmacological Studies

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Topic: D.08. Pain

Support: NIH grant NS065926

NIH grant GM102575

Title: An essential role for eIF4E phosphorylation in peripherally-mediated pain plasticity

Authors: *J. K. MOY[1,2], M. N. ASIEDU[3,2], A. KHOUTORSKY[4], D. V. TILLU[3], O. K. MELEMEDJIAN[2], G. L. MEJIA[3,2], J.-Y. KIM[2], N. SONENBERG[4], G. DUSSOR[3,2], T. J. PRICE[3,2]
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Abstract: An essential role for eIF4E phosphorylation in peripherally-mediated pain plasticity Jamie K Moy[1,2], Marina NK Asiedu[1,2], Arkady Khoutorsky[3], Dipti V Tillu[2], Ohannes K Melemedjian[1], Galo L Mejia[1,2], Ji-Young Kim[1], Nahum Sonenberg[3] and Gregory Dussor[1,2], Theodore J Price[1,2] [1]University of Arizona, Department of Pharmacology [2]University of Texas at Dallas, School of Behavioral and Brain Sciences [3]McGill University, Department of Biochemistry Translational control of gene expression is a key process for the regulation of plasticity in the nervous system. Multiple lines of evidence indicate that translation control plays a critical role in peripheral pain plasticity but precise mechanisms underlying this effect have not been elucidated. Here we have tested the hypothesis that eIF4E, which binds the 5'CAP of mRNAs, plays an important role in pain plasticity via upstream phosphorylation through the MAPK pathway, specifically ERK and MNK1/2. We used transgenic mice harboring a Ser209Ala mutation in eIF4E to assess the role of eIF4E phosphorylation in pain plasticity.

Behavioral studies were done on eIF4E^{S209A} mice and their wild-type (WT) littermates. We used biochemical techniques to assess changes in brain-derived neurotrophic factor expression in these mice. Finally, we used patch clamp electrophysiology to assess changes in excitability in nociceptors lacking eIF4E phosphorylation. eIF4E^{S209A} mice showed clear deficits in behavioral hypersensitivity induced by nerve growth factor (NGF) and protease activated receptor type 2 (PAR2) agonists. Although overall protein synthesis rates (measured with sunset assay) were not lower in these mice, BDNF protein expression was markedly reduced indicating that eIF4E phosphorylation selectively targets certain mRNAs in DRG neurons. Electrophysiological characterization of DRG neurons from eIF4E^{S209A} revealed no differences in baseline properties, however, hyperexcitability induced by NGF was abrogated in transgenic mice. eIF4E phosphorylation plays a crucial role in sensitization of the peripheral nervous system following injury. Therefore, targeting eIF4E phosphorylation via the ERK/MNK1/2 pathway is a novel target for the manipulation of pain plasticity. **Acknowledgements** Supported by NIH grants NS065926 and GM102575

Disclosures: **J.K. Moy:** None. **M.N. Asiedu:** None. **A. Khoutorsky:** None. **D.V. Tillu:** None. **O.K. Melemedjian:** None. **G.L. Mejia:** None. **J. Kim:** None. **N. Sonenberg:** None. **G. Dussor:** None. **T.J. Price:** None.

Poster

534. Nociceptors: Molecular and Pharmacological Studies

Location: Halls A-C

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Topic: D.08. Pain

Support: Z01DE000664-16

Title: Cdk5 is an important modulator of pain signaling

Authors: M. PROCHAZKOVA[1], E. UTRERAS[2], A. TERSE[1], B. HALL[1], *A. B. KULKARNI[1]
[1]Functional Genomics Section, LCDB, NIDCR, NIH, BETHESDA, MD; [2]Dept. of Biol., University of Chile, Chile

Abstract: Cyclin-dependent kinase 5 (Cdk5), a unique member of the serine/threonine kinase family, plays an important role in brain development and functions. Deregulation of its activity is implicated in neurodegenerative and neuropsychiatric disorders. Considering this link between

Cdk5 and brain functions, we explored its role in the regulation of nociceptive signaling. Our initial studies revealed that the expression of Cdk5 and its activator, p35, is upregulated in nociceptive neurons during peripheral inflammation. Moreover, we observed in genetically altered Cdk5 mouse models that perturbation in Cdk5 activity in DRG correlated with altered peripheral thermal pain sensation. Cdk5 regulation of inflammatory pain involved crosstalk with different signaling pathways. Our current studies based on operant behavioral analysis link Cdk5 with orofacial mechanical, thermal, and chemical pain sensation. We found that this link is partly based on the ability of Cdk5 to phosphorylate some of the receptors of pain. All of these findings demonstrate that Cdk5 is an important modulator of pain signaling.

Disclosures: M. Prochazkova: None. **A.B. Kulkarni:** None. **A. Terse:** None. **B. Hall:** None. **E. Utreras:** None.

Poster

534. Nociceptors: Molecular and Pharmacological Studies

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Support: Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan (No 24593047)

Title: Nociceptive sensitization by activation of Protease-Activated Receptor 2 (PAR-2) in rats

Authors: *K. KIDO, E. MASAKI
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Abstract: Background- It was reported that mast cell degranulation after incision induced hyperalgesia in a postoperative pain model. Proteinase-activated receptor 2 (PAR-2) is a G protein-coupled receptor, seven trans-membrane domain receptor family, which is expressed in the peripheral sensory neurons and may play an important role in development of inflammatory and injured pain by binding with tryptase from mast cells. In this study, we examined if activation of peripheral PAR-2 could modulate nociception, using behavioral testing and *in vitro* skin-nerve preparation in rats. Method- The PAR-2-activating peptide SLIGRL-NH2 was administered by the intraplantar route in the rat. Guarding behavior (biting and licking) was observed after administration of SLIGRL-NH2 for 30 minutes. Mechanical thresholds were assessed using von Frey filaments. Withdrawal latencies to heat were assessed applying a

focused radiant heat on the injected area of plantar skin. Using the rat glabrous *in vitro* skin-tibial nerve preparation, afferent activities from single mechanosensitive nociceptors were recorded. Spontaneous activities, and responses to mechanical stimuli were recorded before and after SLIGRL-NH2 application or phosphate buffered saline (PBS) control. Responses to heat and cold stimuli were recorded once after exposure to either with SLIGRL-NH2 application or PBS control. Results- The guarding pain increased after SLIGRL-NH2 injection within 30min. Mechanical and thermal hyperalgesia were also produced after administration. *In vitro* skin-nerve preparation, 100μM SLIGRL-NH2 increased spontaneous activities in 42.5 % C-fibers within 5 minutes. 100μM SLIGRL-NH2 application did not change the responses to mechanical stimuli in C- fibers but 1mM increased the responses. C-fibers were sensitized to heat and cold stimuli. Conclusion- PAR-2-mediated excitation and sensitization of peripheral primary nociceptors may contribute to PAR-2-mediated pain via TRPV1, TRPA1 and TRPV4.

Disclosures: K. Kido: None. **E. Masaki:** None.

Poster

534. Nociceptors: Molecular and Pharmacological Studies

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Topic: D.08. Pain

Support: NIH Grant R01 DA021332

Title: Epigenetic regulation of spinal cord gene expression controls opioid-induced hyperalgesia and tolerance

Authors: *D.-Y. LIANG[1], Y. SUN[2], P. SAHBAIE[2], X. SHI[2], W.-W. LI[2], D. J. CLARK[2]
[1]Anesthesiol., [2]Stanford Univ., Palo Alto, CA

Abstract: The long term use of opioids for the treatment of pain leads to a group of maladaptations which includes opioid-induced hyperalgesia (OIH). Previous studies show morphine-induced epigenetic alteration in spinal cord (SC) tissue regulates OIH. Specifically, OIH typically resolved within few days after cessation of morphine treatment but was prolonged for weeks following manipulation of histone deacetylase activity during treatment. The present work searches for gene targets of these epigenetic effects responsible for OIH prolongation. To investigate involvement of histone acetylation on OIH, selective HDAC inhibitor suberoylanilide hydroxamic acid (SAHA) was administered daily to groups of animals undergoing repeated

morphine exposure. After examining expression changes in implicated SC genes by qPCR, chromatin immuoprecipation (ChIP) of candidate genes were carried out, and complementary studies involved mechanical threshold determination, pharmacological assessments, ELISA and immunohistochemistry. Dose-response relationships and tolerance were assessed using the tail flick assay. The data show:1) HADC inhibitor and chronic morphine treatment enhanced OIH with significant SC up-regulation of BDNF and PDYN. Promoter regions of PDYN and BDNF exon-IV genes were strongly associated with aceH3K9 after morphine and SAHA treatment; 2) Selective TrkB receptor antagonist (ANA-12) treatment reduced mechanical hypersensitivity given one or seven days after cessation of morphine with/out SAHA treatment; 3)Treatment with single dose of selective KOR receptor (nor-BNI) antagonist was not sufficient to reverse sensitization due to prior morphine plus SAHA treatment, though it could reverse sensitization in mice treated with morphine; 4)The co-administration of either receptor antagonist agent daily with morphine or morphine plus SAHA resulted in attenuation of hyperalgesia one day after cessation of treatment. Seven days later ANA-12 or nor-BNI treated animals that had received morphine continued to exhibit less mechanical hypersensitivity. Only co-administered ANA-12 had lasting effects on sensitization after morphine plus SAHA treatment; 5) TrkB and KOR receptor antagonists have a different effects on morphine analgesic tolerance The present study identified two genes whose expression is regulated by epigenetic mechanisms during morphine exposure. Epigenetic processes may be responsible for integrating the effects of environmental factors (drug administration) with intrinsic processes like pain signaling pathways to cause alterations in neuronal function.

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Poster

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Title: The role of mechanosensitive ion channels in osteoarthritis pain

Authors: ***H. HE**[1], A. DAVIDOVA[2], R. SHARIF NAEINI[2]
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Abstract: Osteoarthritis (OA) is a disabling and prevalent condition affecting 27 million US citizens and 5 million Canadian citizens. Pain is the primary symptom of the disease and is largely manifested as mechanical hypersensitivity to joint palpation/movement. However, the underlying mechanisms are poorly understood. The enhanced mechanosensitivity can be due to changes in excitability of the pain sensing neurons (nociceptors), or to a sensitization of the mechanosensing apparatus. The central component of this apparatus is a mechanosensitive ion channel that converts mechanical inputs into electrical signals. This project aims at examining the changes that occur in the function of MSCs in knee joint nociceptors in naïve and osteoarthritic animals. Using a mouse model of OA (monoiodoacetate (MIA)), we will assess if the sensitivity of MSCs in joint-innervating nociceptors is altered in the setting of OA pain. We hypothesize that MSCs are sensitized to mechanical stimulation in OA and blocking their activity will not only reduce this hypersensitivity at the cellular level, but also behaviorally. Our initial behavioral observations from knee-injected mice validate our MIA model as mice become hypersensitive to knee flexion and extension. Electrophysiology recordings from acutely dissociated knee nociceptors indicate that there are no changes in the resting membrane potential. Furthermore, our preliminary data indicates that the activation threshold of MSCs in these nociceptors of OA mice is significantly lower than that of nociceptors from naïve mice. This suggests that the sensitivity of MSCs may contribute to the mechanical hypersensitivity observed in behavioral tests. In conclusion, our preliminary results suggest that MSCs in nociceptors may be a novel therapeutic strategy in the treatment of OA pain.

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Poster

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Topic: D.08. Pain

Support: NSF Grant 1051734

Title: Endocannabinoids/endovanilloids attenuate injury-induced hyperalgesia but not mechanical allodynia

Authors: *T. L. SUMMERS, B. HANTEN, W. PETERSON, B. D. BURRELL
Basic Biomed. Sci., Univ. of South Dakota, Vermillion, SD

Abstract: The endocannabinoid/endovanilloid system is thought to play an important role in modulating nociceptive signaling at both the central and peripheral levels, making it a potential therapeutic target for treating chronic pain. The central analgesic effects of these neurotransmitters are thought to be due, in part, to depression of excitatory synaptic transmission within nociceptive pathways. However, results from both clinical and laboratory-based studies have found that endocannabinoid/endovanilloid can also contribute to nociceptive sensitization (e.g. mechanical hyperalgesia or allodynia) due to a depression of inhibitory synapses resulting in disinhibition of pain circuits (Christie & Mallet, 2009; Pernia-Andrade et al., 2009). Using the medicinal leech as a model system, our lab has observed that while endocannabinoid/endovanilloid depress nociceptive synapses, they actually enhance non-nociceptive synaptic transmission (Yuan & Burrell, 2010; Higgins et al. 2013). Here, we examined the functional relevance of these synaptic effects by testing whether endocannabinoid/endovanilloid have similar bidirectional effects on behavioral responses to nociceptive vs. non-nociceptive stimuli. First, uninjured leeches were injected with either 2-arachidonoylglycerol (2-AG; 75μM) or anandamide (AEA; 100μM) and then tested for behavioral responses to nociceptive and non-nociceptive stimuli. Both AEA and 2-AG sensitized responses to non-nociceptive stimuli while simultaneously decreasing responses to nociceptive stimuli. Both the pro- and anti-nociceptive effects of AEA and 2-AG were blocked by co-injection of SB366791, an inhibitor of the transient receptor potential vanilloid (TRPV) channel (invertebrates lack CB1 & CB2 receptors). Next, experiments were conducted to determine the effect of 2-AG and AEA on non-nociceptive and nociceptive stimuli responses in an injury-induced sensitized animal. Animals received a crush injury to their posterior sucker which resulted in a significant increase in responses to non-nociceptive (allodynia) and nociceptive (hyperalgesia) stimuli. In a subgroup of these sensitized animals, injection of 2-AG (75μM) on the third day after injury restored nociceptive stimuli responses to pre-injury levels. However, 2-AG treatment did not attenuate the sensitized response to non-nociceptive stimuli. These results indicate that endocannabinoid/endovanilloid treatment may be effective in treating hyperalgesia, but is ineffective at treating or may even worsen mechanical allodynia.

Disclosures: T.L. Summers: None. **B. Hanten:** None. **W. Peterson:** None. **B.D. Burrell:** None.

Poster

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COSTAR 2T32DE014318-12A1

Title: Allosteric interactions within delta opioid receptor - kappa opioid receptor (DOR-KOR) heteromers in peripheral sensory neurons

Authors: *B. A. MCGUIRE, T. A. CHAVERA, W. P. CLARKE, K. A. BERG
UT Hlth. Sci. Ctr., San Antonio, TX

Abstract: G-protein coupled receptor (GPCR) heteromers may have unique pharmacological properties, signaling characteristics and anatomical specificity. Thus, GPCR heteromers may provide a unique pharmacological target for the treatment of a variety of disease states. Due to methodological limitations, most of the work characterizing GPCR heteromers has been performed in heterologous expression systems, with few experiments in physiologically relevant systems. We have found that DOR-KOR heteromers form in peripheral nociceptors (i.e., pain-sensing neurons) and, when activated, produce robust antinociceptive responses (Berg et al., 2012, *Mol Pharmacol* 81:264-272). Further, selective KOR antagonists differentially alter the potency and efficacy of DOR agonists in both a rat behavioral model of thermal nociception and in primary cultures of peripheral nociceptors (i.e., *ex vivo* model). Here we sought to further demonstrate that DOR-KOR heteromers are functional in nociceptors by examining bidirectional allosteric interactions between protomers of DOR-KOR heteromers. We determined if the selective DOR antagonist naltrindole (NTI) altered responses to the KOR agonist ICI 199441 *ex vivo* and *in vivo*. In primary cultures of rat peripheral sensory neurons, ICI 199441- mediated inhibition of PGE_2-stimulated cAMP accumulation was determined in the presence and absence of NTI (2nM, 100 x Ki). Neither basal nor PGE_2-stimulated cAMP levels were altered by NTI, however, the concentration response curve to ICI 199441 was shifted to the left by more than 100-fold; the EC50 for ICI 199441 was 15 nM versus 0.12 nM in the absence or presence of NTI, respectively (n=4). Similarly, NTI shifted the dose response curve for ICI 199441-mediated inhibition of PGE_2-induced thermal nociception to the left by 10-fold. Rats were injected with NTI (40µg) or vehicle 15 min before intraplantar coinjection of varying doses (0.1-10 µg) of ICI 199441 along with PGE_2 (0.3 µg). Paw withdrawal latencies to thermal stimulation were measured for 20 min. The ICI 199441 ED_{50} for reduction of PGE_2-induced thermal allodynia was 2.7ug versus 0.24ug in the absence or presence of NTI, respectively (n=6 animals

per group). This demonstration of bidirectional allosteric interactions between the protomers of DOR-KOR heteromers provides strong evidence for functional DOR-KOR heteromers in peripheral nociceptors. We propose that the DOR-KOR heteromer may be a suitable target for pain pharmacotherapy.

Disclosures: B.A. McGuire: None. **T.A. Chavera:** None. **W.P. Clarke:** None. **K.A. Berg:** None.

Poster

534. Nociceptors: Molecular and Pharmacological Studies

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Support: NHMRC

Title: Cathepsin S induces peripheral and central activation of pain via biased agonism of Protease-Activated Receptor 2

Authors: *T. LIEU, P. ZHAO, N. BARLOW, D. P. POOLE, B. SEBASTIAN, N. W. BUNNETT
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Abstract: Proteases such as mast cell tryptase cleave protease-activated receptor-2 (PAR2) at R36/S37 and reveal a tethered ligand that binds to and activates the cleaved receptor. PAR2 can activate transient receptor potential (TRP) ion channels and thereby excite nociceptors to induce neurogenic inflammation and pain. Whether proteases that cleave PAR2 at distinct sites are biased agonists that also activate TRP channels to induce neurogenic inflammation and pain is unexplored. Cathepsin S (Cat-S) is a lysosomal protease secreted by antigen presenting cells such as macrophages and spinal microglial cells during inflammation. We used mass spectrometry to identify the Cat-S cleavage site, bioluminescence resonance energy transfer (BRET) to examine PAR2 and G protein coupling, in-vitro cell-based assays to characterize PAR2 signaling by Cat-S, *in vivo* assessment of Cat-S-induced inflammatory and pain mechanisms, and RT-PCR and immunofluorescence to localize PAR2 expression. We observed that Cat-S cleaves N-terminal fragments of PAR2 at E56/T57, down-stream from the canonical trypsin site, as determined by mass spectrometry. In contrast to trypsin, which cleaves PAR2 at the cell-surface and causes receptor internalization, PAR2 cleaved by Cat-S remained at the

plasma membrane. Cat-S and a decapeptide corresponding to the Cat-S-revealed tethered ligand stimulated PAR2 coupling to Gαs but not Gαq determined by BRET to examine the conformational change and interactions between PAR2 and G proteins. Cat-S stimulated PAR2-dependent formation of cAMP, but Cat-S was unable to activate mutant PAR2 lacking the Cat-S cleavage site. Unlike trypsin, Cat-S did not mobilize intracellular Ca2+, activate ERK1/2, recruit β-arrestins nor induce PAR2 endocytosis. This is evidence for agonist-dependent signaling bias for Cat-S activity for PAR2. Intraplantar injection of Cat-S caused inflammation and mechanical hyperalgesia in mice that was attenuated by deletion of PAR2 and TRPV4. The Cat-S inhibitor MV026031 and the PAR2 antagonist GB88 prevented inflammation and pain induced by intraplantar injection of formalin. Central mechanisms of Cat-S activation of spinal neurons are unexplored. We found that PAR2 is expressed throughout the murine spinal cord. Immunofluorescence revealed that PAR2-immunoreactivity is localized to lamina I of the dorsal horn and co-localizes with CGRP positive neurons. Intrathecal injection of Cat-S into the lumbar section of the spinal cord caused sustained mechanical hyperalgesia in wt-mice for up to 4 hours ($p<0.001$, $n=4$). Determination of Cat-S-dependent activation of PAR2 on dorsal horn neurons remains to be investigated.

Disclosures: **T. Lieu:** None. **P. Zhao:** None. **N. Barlow:** None. **D.P. Poole:** None. **B. Sebastian:** None. **N.W. Bunnett:** None.

Poster

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Support: NIH Grant NS014624

Title: CXCL10 directly sensitizes a subset of cutaneous nociceptors through CXCR3 and contributes to itch in a murine model of allergic contact dermatitis

Authors: L. QU[1], K. FU[1,2], S. G. SHIMADA[1], *R. H. LAMOTTE[1]
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Abstract: The role of the chemokine, CXCL10, and its receptor CXCR3, has been implicated in the pathophysiology of allergic contact dermatitis, but unexplored for the itch and pain that can accompany this disorder. We examined whether CXCL10 directly activated acutely dissociated,

small-diameter, cutaneous, sensory neurons through CXCR3, and contributed to itch- and pain-like behaviors in a murine model of allergic contact dermatitis (contact hypersensitivity or CHS) induced by a hapten, squaric acid dibutylester (SADBE). Calcium imaging revealed that CXCL10 directly evoked a Ca2+ increase in a subset of small-diameter, dorsal root ganglion (DRG) neurons, fluorescently tagged as cutaneous, via the activation of CXCR3. The larger proportion of neurons responsive to CXCL10 in CHS vs. control mice was suggestive of an increased number of cutaneous sensory neurons expressing CXCR3 after the development of CHS. The neurons from CHS mice exhibited a depolarized membrane potential accompanied by action potentials and a reduction in input resistance upon CXCL10 application. Pretreatment with a CXCR3 antagonist significantly attenuated the direct excitatory effects of CXCL10. CXCL10 activated a chloride channel in cutaneous sensory neurons from CHS mice, which may account for the depolarization induced by CXCL10. In behavioral tests, subcutaneous injection of a CXCR3 antagonist significantly attenuated the spontaneous itch- but not pain-like behaviors directed to the site of CHS. CXCL10 elicited site-directed itch-like but not pain-like behaviors when injected into a site of CHS but neither type of behavior in control mice, which is likely due to the paucity of functional CXCR3 expressed in cutaneous sensory neurons under normal conditions. The CXCL10-evoked itch-like behavior in CHS mice was attenuated by a CXCR3 antagonist or a chloride channel blocker. These results suggest that CXCL10/CXCR3 signaling directly activates a subset of cutaneous primary sensory neurons through chloride channels and may contribute to the itch accompanying allergic contact dermatitis.

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Poster

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VA Merit Review I01-RX000378

Title: NMDA receptor-induced substance P release from primary afferent terminals is inhibited by α2A adrenergic receptors, lack of synergism with μ-opioid receptors

Authors: *H. KIM[1], W. CHEN[1], H. S. ENNES[1], W. WALWYN[1], J. A. MCROBERTS[1,2], J. G. MARVIZON[1,2]
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Abstract: Recently, we showed that BDNF acting on trkB receptors induces a gain of function of NMDA receptors in primary afferent terminals, which occurs right after the onset of neuropathic pain (Chen et al., Eur. J. Neurosci., 2014). Here we present evidence that α2A adrenergic receptors induce a counterbalancing loss of function of these NMDA receptors. NMDA receptor function was assessed as the ability of NMDA + D-Ser (10 μM for 2 min) to induce substance P release in rat spinal cord slices. Substance P release was measured as neurokinin 1 receptor (NK1R) internalization in lamina 1 neurons. NMDA-induced NK1R internalization in spinal cord slices was inhibited by 1 hr preincubation with the α2A adrenergic receptor agonists clonidine (1 μM), guanfacine (10 nM), tizanidine (100 nM) and medetomidine (1 μM). A concentration-response for guanfacine yielded an IC50 of 0.22 nM and was right-shifted by the selective α2A receptor antagonist BRL44408 with a KB of 16 nM (Gaddum-Schild analysis). To determine whether α2A receptors inhibit NMDA receptor-induced substance P release *in vivo*, rats were given intrathecal BDNF (0.3 μg) to increase NMDA receptor function, then intrathecal guanfacine (0.3 nmol) or saline 3 hr after BDNF and finally intrathecal NMDA + D-Ser (10 nmol each) 1 hr later. NMDA induced NK1R internalization after BDNF, and this effect was eliminated by guanfacine. These results show that α2A receptors decrease NMDA receptor function in primary afferent terminals. We also investigated whether the inhibition of substance P release by α2A receptors was synergistic with its inhibition by μ-opioid receptors. Spinal cord slices were preincubated for 1 hr with different concentrations of guanfacine, DAMGO (a μ-opioid receptor agonist), or guanfacine and DAMGO combined at a ratio of 1:10. Slices were then stimulated for 2 min with NMDA and D-Ser. NMDA-induced NK1R internalization data obtained with each agonist alone or the two agonists combined were analyzed simultaneously by fitting two dose-response curves to the data. Akaike's Information Criterion was used to determine the probability that the IC50 was the same for each agonist alone compared to the two agonists combined. The probability that the IC50 was the same for the concentration-responses of guanfacine and guanfacine + DAMGO was 76%. The probability that the IC50 was the same for the concentration-responses of DAMGO and DAMGO + guanfacine was 48%. Therefore, there was no detectable synergy between α2A adrenergic receptors and μ-opioid receptors to inhibit substance P release in the spinal cord.

Disclosures: H. Kim: None. **W. Chen:** None. **H.S. Ennes:** None. **W. Walwyn:** None. **J.A. McRoberts:** None. **J.G. Marvizon:** None.

Poster

534. Nociceptors: Molecular and Pharmacological Studies

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 534.21/HH32

Topic: D.08. Pain

Support: NIHR01HL62296

Title: Pharmacological evidence for a dominant role of NaV 1.7 in action potential regulation in guinea pig and human vagal nociceptors

Authors: A. HERBSTOMER[1], M. KOLLARIK[1], S. MEEKER[1], J. KRAJEWSKI[2], B. LI[2], K. JOHNSON[2], E. NISENBAUM[2], *B. J. UNDEM[1]
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Abstract: Pharmacological evidence for a dominant role of NaV 1.7 in action potential regulation in guinea pig and human vagal afferent nociceptors. R. Adam Herbstsomer, Marian Kollarik, Sonya Meeker, Jeffrey Krajewski, Baolin Li, Kirk Johnson, Eric Nisenbaum, Bradley J. Undem We used an *ex vivo* guinea pig (GP) trachea-vagus nerve preparation to evaluate the effect of NaV1 blockers on the action potential (AP) response single tracheal afferent terminals with cell bodies in nodose and jugular ganglia. One nerve was studied per animal. In addition we evaluated the C-wave of the compound action potential (CAP) in human peribronchial vagus nerves. In 70% of the nodose and jugular afferent nerves, tetrodotoxin (TTX) potently (EC50 30-100nM) and effectively increased voltage threshold for AP formation. It abolished the action potential discharge in response to citric acid (1ml of a 1 mM solution applied directly to the receptive field) in all nodose Aδ fibers (n=19) and inhibited the acid response in jugular C-and Aδ afferent nerves by 80% (n=16). A selective NaV 1.7 blocker (Lilly's compound A) potently inhibited GP cloned NaV 1.7 current, and inhibited by 80% the TTX-sensitive current in GP isolated nodose neurons. It was nearly equipotent as TTX in increasing voltage thresholds in nodose (n=13) and jugular fiber terminals (n=20) in the trachea. It was slightly less effective than TTX in blocking citric acid responses, maximally (1 μM) blocking discharge in nodose and jugular fibers by 70% and 60%, respectively. ICA-121431 (10 μM), a selective blocker of human NaV1.1, 1.2 and 1.3 (*McCormack et al PNAS, 2013*) had no effect on the GP cloned NaV1.7 current, and had no effect on voltage threshold or citric acid response in nodose or jugular tracheal fibers. Likewise, A803467 (30 μM, a concentration that blocked the TTX-insensitive NaV 1.8 current in GP nodose neurons by 80%) was without effect on GP nodose and jugular terminals. TTX (0.1 μM) and Compound A (1μM) each reversibly blocked (~90% inhibition) the C-wave of the CAP in human peribronchial vagi, whereas ICA-121431 (10 μM) had little effect.

These data add pharmacological support to our previous work with NaV1.7 shRNA *(Muroi et al. J. Physiol 2011)* that NaV 1.7 is a dominant NaV1 involved in action potential formation in vagal afferent Aδ and C-fibers.

Disclosures: A. Herbstomer: None. **M. Kollarik:** None. **S. Meeker:** None. **J. Krajewski:** None. **B. Li:** None. **K. Johnson:** None. **E. Nisenbaum:** None. **B.J. Undem:** None.

Poster

534. Nociceptors: Molecular and Pharmacological Studies

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 534.22/II1

Topic: D.08. Pain

Support: Grants-in-Aid for Scientific Research

Title: Phosphorylation of mTOR maintains pain hypersensitivity after the tissue injury

Authors: Y. IZUMI[1], M. SASAKI[2], *F. AMAYA[2]
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Abstract: (Background) We previously showed that insulin-like growth factor 1 (IGF1) in injured tissue contributes to the maintenance of peripheral sensitization after a plantar incision. Mammalian target of rapamycin (mTOR) is activated by IGF1 and controls protein synthesis in the nervous system. Here, we investigated the role of IGF1/mTOR signaling in the dorsal root ganglion (DRG) for the development of pain hypersensitivity following plantar incision. (Method) Male SD rats were used for the experiments. Rats were treated with plantar incision procedure or intraplantar injection of IGF1 under deep isoflurane anesthesia. Behavioral analysis was performed to determine thermal and mechanical noxious threshold. L4 and L5 DRG were taken and processed for the immunohistochemistry. Phosphorylated mTOR (p-mTOR) and Vesicular glutamate transporter 2 (VGLUT2) expression were visualized by indirect immunofluorescence method. (Result) The number of p-mTOR-positive neurons was increased after the incision. Systemic Rapamycin treatment inhibited p-mTOR expression in the DRG and thermal hypersensitivity after the incision. Local injection of IGF1 increased p-mTOR expression in the DRG, and treatment with the IGF1 receptor inhibitor picropodophyllin alleviated incision-induced p-mTOR induction. VGLUT2 expression was increased after the plantar incision or local IGF1 treatment increased VGLUT2 expression in the DRG neurons. The

incision-induced increase in VGLUT2 expression was inhibited by rapamycin. (Conclusion) These results demonstrated that tissue injury induces phosphorylation of mTOR in DRG neurons via IGF1 dependent manner. Increased expression of VGLUT2 by the mTOR activation contributes to the maintenance of thermal hyperalgesia after tissue injury.

Disclosures: Y. Izumi: None. **M. Sasaki:** None. **F. Amaya:** None.

Poster

534. Nociceptors: Molecular and Pharmacological Studies

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 534.23/II2

Topic: D.08. Pain

Title: Different effects of the cytokine Interferon-γ on slowly or fast conducting nociceptive nerve fibers in rat *in vivo*

Authors: *F. RICHTER, S. WANDT, J. FINK, M. RAUSCHELBACH, H. WITZENHAUSEN, H.-G. SCHAIBLE
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Abstract: Whether the cytokine Interferon-γ (IFN-γ) has proinflammatory or anti-inflammatory effects is still disputed. In this study we investigated whether IFN-γ has the potency to induce mechanical hyperalgesia in normal or in acutely inflamed (kaolin/carrageenan) knee joints and whether such effects are specific to an action on the IFN-γ receptor. Healthy adult WISTAR rats were anesthetized with sodium thiopentone (100 mg/kg, i.p.). The knee joint was mechanically stimulated by innocuous (20 mNm) or noxious (40 mNm) rotations of the lower leg against the fastened femoral bone for 15 sec each. Action potentials were recorded from nerve fibers that were classified as C- or as Aδ-fibers by their conduction velocity (<1.4 m/s or <10 m/s, respectively). Compounds were injected into the joint cleft at a volume of 0.1 ml each. In normal knee joints a single intraarticular injection of IFN-γ dose-dependently decreased the net response rate of C-fibers to noxious stimulation within three hours (0.1 ng by 21±51, 1 ng by 155±32, 10 ng by 171±56 APs/15 s; mean±SEM, respectively), but had no influence on the net response rate to innocuous stimulation. The response rates in Aδ-fibers were only insignificantly changed by IFN-γ. After 7 hours of acute knee joint inflammation in C-fibers a similar dose-dependent decrease in response rate was seen, the highest dose of IFN-γ caused a decrease by 211±61 APs/15 s after three hours of application of the cytokine. Interestingly, in Aδ-fibers recorded from inflamed knee, both 1 ng and 10 ng of IFN-γ caused rather an increase in the net response

rate (1 ng by 135±40, 10 ng by 195±64 APs/15 s, respectively). Simultaneous injection of 10 μg of a blocking IFN-γ receptor antibody together with 10 ng of IFN-γ in normal knee joints prevented the decreasing effect of the cytokine in C-fibers, responses in Aδ-fibers did not change. Thus IFN-γ in slowly conducting nerve fibers both in normal and in inflamed knee joints acts as an antinociceptive cytokine, whereas its effect on fast conducting fibers depends on the situation in the knee joint.

Disclosures: **F. Richter:** None. **S. Wandt:** None. **J. Fink:** None. **M. Rauschelbach:** None. **H. Witzenhausen:** None. **H. Schaible:** None.

Poster

534. Nociceptors: Molecular and Pharmacological Studies

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 534.24/II3

Topic: D.08. Pain

Support: CIHR (INO-77909)

Title: Correlation of the electrophysiological profiles and sodium channel transcripts of individual rat dorsal root ganglia neurons

Authors: ***M. CHAHINE**, O. THÉRIAULT
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Abstract: Voltage gated sodium channels (Na+ channels) play an important role in nociceptive transmission. They are intimately tied to the genesis and transmission of neuronal firing. Five different isoforms (Nav1.3, Nav1.6, Nav1.7, Nav1.8, and Nav1.9) have been linked to nociceptive responses. A change in the biophysical properties of these channels or in their expression levels occurs in different pathological pain states. However, the precise involvement of the isoforms in the genesis and transmission of nociceptive responses is unknown. The aim of the present study was to investigate the synergy between the different populations Na+ channels that give individual neurons a unique electrophysical profile. We used the patch-clamp technique in the whole-cell configuration to record Na+ currents and action potentials from acutely dissociated small diameter DRG neurons (<30 μM) from adult rats. We also performed single cell qPCR on the same neurons. Our results revealed that there is a strong correlation between Na+ currents and mRNA transcripts in individual neurons. A cluster analysis showed that subgroups formed by mRNA quantification have different biophysical properties. In addition, the

firing frequency of the neurons was not affected by the relative populations of Na+ channel subtypes. The synergy between populations of Na+ channel subtypes in individual small diameter DRG neurons gives each neuron a unique electrophysiological profile. The Na+ channel remodeling that occurs in different pathological pain states may be responsible for the sensitization of the neurons.

Disclosures: **M. Chahine:** None. **O. Thériault:** None.

Poster

534. Nociceptors: Molecular and Pharmacological Studies

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 534.25/II4

Topic: D.08. Pain

Support: Clinical Center, NIH

Title: Systems view of axotomy with rna-seq analysis of dorsal root ganglion, dorsal horn and ventral horn

Authors: S. C. GOSWAMI[1], J. R. GROSS[1], *J. K. NEUBERT[2], A. J. MANNES[1], M. J. IADAROLA[1]
[1]Dept. of Perioperative Med., Clin. Center, NIH, Bethedsa, MD; [2]Orthodontics, Univ. Florida, GAINESVILLE, FL

Abstract: The full-spectrum of molecules involved in nociceptive signaling involves many as yet unrecognized molecules. Depending on the cause of pain (e.g. inflammation or nerve injury) the signaling molecules involved may vary. This study focuses on nerve injury and examines the transcriptional changes within the DRG after axotomy. Spinal cord dorsal horn and ventral horn are also examined to elucidate broader system changes, differences, and commonalities between these tissues. In this study we employ RNA-Seq to obtain quantification of transcriptomes at maximal resolution and dynamic range. Rat L4-L5 DRG, lumbar DH, and VH at 0, 1, 3, 10, 30 and 90 day(s) after axotomy were collected and sequenced with the Illumina HiSeq 2000. The RNA-Seq data were mapped with RUM and statistical analyses performed using DESeq. The transcriptome data from the DRG axotomy revealed significant modulation of ~1700 genes (with an adjusted fold difference > 2 and above). Significantly regulated genes are subdivided into two categories: induced (basal expression below 1 RPKM) and upregulated (basal expression > 1 RPKM). Clustering within these categories disclosed several temporal patterns: Peak Day1, Peak

Day 3, Peak Day 10, Bimodal, Transient Decrease and Delayed Increase. Gene enrichment analyses were used to categorize nerve injury changes. GO terms such as negative regulation of catalytic activity, peptidase inhibitor activity for genes in the Peak Day 1 imply an initial attempt to stabilize injury-induced alterations. The DH and VH share some regulated genes with DRG and the general temporal clustering patterns. VH RNA-seq resulted in ~200 significantly regulated (2-fold and above) genes with a strong enrichment for mitotic and cell cycle transcripts at Day 3 consistent with microglial proliferation to effect displacement of synaptic terminals from motoneurons. The DH RNA-Seq dataset revealed ~400 regulated genes (>2-fold). Many of the DH genes regulated at Day 3 also were involved in mitosis. It is unclear however, whether the mitotic changes are due to the same processes in the two tissues. As expected, many other functional groups were discerned and included a strong representation of immune response genes in all three tissues. Understanding molecular changes after axotomy at a fine level of molecular resolution in both PNS and CNS is a key element in understanding the pathophysiological consequences of nerve injury and regeneration mechanisms. The present RNA-Seq study provides a rich repertoire of data to further elucidate the pathways and mechanisms involved nerve-injury models of pain.

Disclosures: **S.C. Goswami:** None. **J.K. Neubert:** None. **J.R. Gross:** None. **A.J. Mannes:** None. **M.J. Iadarola:** None.

Poster

534. Nociceptors: Molecular and Pharmacological Studies

Location: Halls A-C

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Program#/Poster#: 534.26/II5

Topic: D.08. Pain

Support: NIH Grant AR047410

Title: Aminooxyacetic Acid: An aspartate aminotransferase inhibitor with local analgesic properties during chronic inflammation

Authors: *B. R. BOLT, K. E. MILLER
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Abstract: Painful inflammation leads to a local increase in neurotransmitter Glutamate in the rat peripheral afferent terminals (skin). The primary sensory neurons of the dorsal root ganglia (DRG) are glutamatergic utilizing glutaminase (GLS) in the glutamine cycle as a source for

glutamate production, but aspartate aminotransferase (AST; GOT1) also plays a key role by transamination of aspartate and 2-oxoglutarate to produce glutamate along with oxaloacetate. We previously demonstrated that AST, GLS, and glutamate are elevated in the rat DRG and skin during the adjuvant-induced arthritis (AIA) model. Inhibition of GLS in the skin during AIA provides long-lasting analgesia. Since AST contributes to glutamate synthesis in peripheral nerve terminals, we hypothesized that the peripheral inhibition of AST using Aminooxyacetic acid (AOAA) would lead to analgesia during AIA. For the AIA model, peripheral inflammation was induced by intraplantar injection of Complete Freund's adjuvant in the right hind paw. After 4 days of inflammation, rats where treated with subcutaneous administration of AOAA. After AOAA administration, exaggerated thermal and mechanical responses in AIA rats were attenuated by 30 min and lasted for up to 5 hours. The current results further indicate that AST is a major contributor of glutamate synthesis in nociceptive peripheral nerve terminals. AST inhibition appears to be a viable target for producing long lasting local analgesia by decreasing glutamate production and release from peripheral primary afferents.

Disclosures: B.R. Bolt: None. **K.E. Miller:** None.

Poster

534. Nociceptors: Molecular and Pharmacological Studies

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Program#/Poster#: 534.27/II6

Topic: D.08. Pain

Support: AHA 44081

DE017696

Title: Prolactin sex-dependently regulates peripheral and spinal postoperative hypersensitivity

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[1]Endodontics, UT Hlth. Sci. Ctr., SAN ANTONIO, TX; [2]Inserm and Univ. Paris Descartes, Paris, France; [3]UTHSCSA, san antonio, TX

Abstract: Aim of Investigation: Prolactin (PRL) is a multifaceted pituitary hormone dually regulated by estrogen and trauma/stress. PRL levels are sex-dependently elevated in serum and extra-pituitary after pain conditions such as tissue injury, inflammation, surgical procedure, burn and migraine. We recently reported that ablation of PRL receptor (PRLR) leads to reduction

postoperative and inflammatory hypersensitivity in a sex-dependent manner. However, mechanisms mediating these sex-dependent PRL effects are unknown. Here, we investigated how PRL regulates postoperative hypersensitivity at periphery and spinal cord of males and females. Methods: All described experiments were performed on adult males and estrous phase females, which contain moderate PRL levels in blood. Expression of PRLR in dorsal root ganglia (DRG) and spinal cord were assessed using immunohystochemistry (IHC). Functional expression of PRLR long form was assessed by labeling to phspho-STAT5. Classical hindpaw incision model was used as an acute postoperative model. Results: PRLR is expressed in subset of DRG and spinal cord neurons. PRL regulates phosphor-STAT5 in DRG and spinal cord neurons via PRLR long isoform. PRL is able to produce thermal and mechanical hypersensitivity upon injection in hindpaw of male and female naïve rats. These hypersensitivity effects are sex-dependent. We next evaluated role of peripheral and spinal PRLR in postoperative hypersensitivity in males and females using specific PRLR antagonist, αPRL. αPRL when injected peripherally or intrathecally 24h post-surgery did not have an effect on thermal or mechanical hypersensitivity in male rats. The female rats with incision showed significant reduction in both thermal and mechanical hypersensitivity post intrathecal administration of αPRL. The antagonist administration peripherally resulted in inhibition of thermal, but not mechanical postoperative hypersensitivity. Conclusions: Short and long forms of PRLR are expressed in subsets of DRG and spinal cord neurons. PRL is able to cause hypersensitivity in naïve animals, but the effect in sex-dependent. Importantly, peripheral and spinal inhibitory actions of the PRLR antagonist on postoperative thermal and mechanical hypersensitivity are exclusively restricted to females. Compression of presented data with results generated after global ablation of PRLR indicates that PRL can exhibit anti-nociceptive effects in males by acting on unknown brain regions. In contrast, peripheral and spinal mechanisms of PRL action are specific for females.

Disclosures: **M.J. Patil:** None. **V. Goffin:** None. **M. Henry:** None. **A. Akopian:** None.

Poster

535. Somatosensory: Local Cortical Circuits

Location: Halls A-C

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Program#/Poster#: 535.01/II7

Topic: D.09. Tactile/Somatosensory

Support: Studienstiftung des deutschen Volkes

BMBF/FKZ 01GQ1002

Title: The barrel cortex connectome - dense connectivity from sparse reconstructions of neural circuits

Authors: *R. EGGER[1,2], V. J. DERCKSEN[3], D. UDVARY[1,2], H.-C. HEGE[3], M. OBERLAENDER[1,4,5]
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Abstract: In order to gain a mechanistic understanding of sensory signal flow in the rat vibrissal system, it is necessary to determine the activity of neurons in response to sensory inputs, and to identify the spatial distribution of synapses to and from these neurons. While the responses of neurons to whisker stimuli have been characterized extensively, a complete quantitative description of connectivity in the vibrissal system is still lacking. Therefore, we aim to determine all inputs and outputs to and from neurons in rat barrel cortex. Two widely used techniques for investigating neural connectivity are electron-microscopic (EM) reconstruction of brain circuits and tracing of bulk injections. However, so far these approaches have been limited to small circuits of hundreds of neurons (EM reconstruction) or only allow limited extraction of quantitative connectivity data (bulk injections). Here, we present a third, statistical approach for obtaining dense synaptic connectivity of neural networks from representative samples of 3D morphologies of single neurons labeled *in vivo*. To do so, we measure (i) the 3D geometry of rat barrel cortex, (ii) the 3D distribution of excitatory and inhibitory neuron somata, and (iii) the 3D morphology of dendrites and axons of 11 excitatory and 6 inhibitory neuron cell types. Combining these data in a common 3D reference frame allows estimating the 3D distribution of 5 billion boutons from 6,000 thalamocortical and 500,000 intracortical neurons in a volume of 6.5 cubic mm, at a resolution of 50 μm. Putative synaptic innervation at subcellular resolution is predicted from the overlap between reconstructed dendrites and axons. In the past, a variety of results obtained by statistical mapping deviated from direct connectivity measurements. Comparing the resultant cell type-specific innervation probabilities and cell-specific subcellular innervation patterns with previously reported *in vivo* paired-recordings and EM data, we demonstrate the validity and appropriate spatial resolution of this statistical approach. Finally, we show how this statistical approach can be used to generate a dense cell-to-cell connectome of barrel cortex. Analysis of the resultant connectivity reveals cell type- and location-specific connectivity patterns from the microscopic, subcellular level up to the macroscopic scale of the entire barrel cortex, which form the structural basis underlying sensory-evoked signal flow.

Disclosures: R. Egger: None. **D. Udvary:** None. **M. Oberlaender:** None. **V.J. Dercksen:** None. **H. Hege:** None.

Poster

535. Somatosensory: Local Cortical Circuits

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Topic: D.09. Tactile/Somatosensory

Support: Max Planck - Hebrew University Center for Neuroscience

Title: The relationship between microcircuit structure and dynamics in the rat barrel cortex

Authors: *I. D. LANDAU[1], R. EGGER[2], M. OBERLAENDER[3,2], H. SOMPOLINSKY[1,4]
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Abstract: In the study of the cerebral cortex, the roughly uniform six-layer structure and the prominence of functional columns have raised the question of the relationship between structure and function of cortical microcircuits. Nevertheless, experimental work has only slowly begun to systematically unveil the connectivity of local recurrent cortical circuits, and most theoretical work has until now dealt only with simple random connectivity. Even recent realistic numerical studies have used only cell-type connection probabilities accumulated from different experimental techniques, animals, species, and brain regions. The rodent barrel cortex has become an attractive site for experimental work on the structure-function relationship because of its anatomically observable somatotopy and readily isolatable sensory input channels. In this collaborative project, we combine recent anatomical work on the rat barrel cortex which has yielded a coherent, realistic, anatomically constrained cell-to-cell connectivity matrix, with both theoretical and numerical analysis in order to shed new light on the relationship between structure and function in cortical microcircuits. We explore the dynamics of a full-scale spiking neuron network model under realistic cell-to-cell connectivity and identify constraints imposed by the connectivity beginning with one complete Layer 4 Barrel. In particular, we use numerical and theoretical tools to explore the implications of the heterogeneity in the number of connections on the dynamical state. We simulate the network model alongside others with simplified connectivity schemes, and also apply dynamical mean-field theory to a connectivity model that reflects realistic heterogeneity in order to derive analytical results. We address the trade-off between sparsity and irregularity in the balanced state. Furthermore, we probe the signal propagation yielded by the realistic connectivity matrix, exploring the spatial distribution of spontaneous, primary whisker evoked, and secondary whisker evoked firing. We compare the

results of our network model to physiological findings and make further predictions. Our work is an important step toward bridging between theory and experiment, connecting the abstract study of the dynamics of cortical circuits to the increasingly detailed experimental account of their real connectivity structure and the biophysical properties of different cell types.

Disclosures: I.D. Landau: None. **H. Sompolinsky:** None. **R. Egger:** None. **M. Oberlaender:** None.

Poster

535. Somatosensory: Local Cortical Circuits

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Program#/Poster#: 535.03/II9

Topic: D.09. Tactile/Somatosensory

Support: EU Grant 604102 (HBP)

NSF CAREER Award 0952686

Title: A computational model for interacting assembly sequences

Authors: *W. MAASS[1], R. LEGENSTEIN[1], J. MACLEAN[2]
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Abstract: As multineuronal recordings have become more prevalent it is increasingly clear that spatiotemporally patterned spiking activity encodes sensory input (Luczak et al., 2007), motor output (Churchland et al., 2007), and behavioral choice (Harvey et al., 2012). Network dynamics takes the form of trajectories of network states as captured by the assembly phase sequence postulate (Hebb 1949). Despite the utility of attractor networks in computational models the repeated experimental observation of reliable and stereotyped time varying network activity is inconsistent with attractors being the most salient computational primitive. Here we employ a generative model that exhibits stereotyped transitions through network states to evaluate the type of network computations and computational models that are consistent with the existence of stereotypical sequences of network states, and how such sequences could support network computations. In a probabilistic framework, the familiar Hidden Markov Model (HMM) is a sufficient computational (and generative) model for a single assembly sequence. However experimental data indicates that several such sequences can be generated by the same local

neural circuit (Sadovsky and MacLean 2014), and it seems likely that any such sequence interacts with assembly sequences in other parts of a larger neural system. Therefore we propose an extension of the HMM that generates separate, yet potentially interacting, state sequences (Strands) that propagate in parallel. Each strand encodes a local network state and is combined with temporal information such as lifetime and strength of initiation. We show that our Strand Computing Model (SCM) is well-suited for integrating loosely aligned information streams and for supporting inference about a possible common cause of such as multi-modal input streams, or the contextualization of a whole scene. Computations of this type are likely taking place in higher cortical areas (e.g. parietal cortex), but a more abstract computational model has been missing to describe them and to test their utility. We show that the SCM can also be used as a generative model, to produce interacting assembly sequences in large-scale models for cortical systems. Churchland MM, Yu BM, Sahani M, & Shenoy KV (2007). Cur opin neurobiol 17(5), 609-618. Harvey CD, Coen P, & Tank DW (2012). Nature 484(7392), 62-68. Hebb DO (1949) The organization of behavior. New York Wiley. Luczak A, Barthó P, Marguet SL, Buzsáki G, & Harris KD (2007). PNAS 104(1), 347-352. Sadovsky A, MacLean JN (2014), Mouse Visual Neocortex Supports Multiple Stereotyped Patterns of Microcircuit Activity Journal of Neuroscience (In press)

Disclosures: **W. Maass:** None. **R. Legenstein:** None. **J. MacLean:** None.

Poster

535. Somatosensory: Local Cortical Circuits

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Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 535.04/II10

Topic: D.09. Tactile/Somatosensory

Support: HHMI

Title: Circuit mechanisms underlying the thalamocortical transformation during active tactile sensation

Authors: ***J. YU**, D. GUTNISKY, A. HIRES, K. SVOBODA
HHMI Janelia Farm Res. Campus, Ashburn, VA

Abstract: A fundamental challenge in neuroscience is linking behaviorally relevant computations to mechanisms at the level of neural circuits. Tactile information from the whiskers flows from the periphery through the ventral posteromedial nucleus of the thalamus (VPM) to

layer 4 (L4) of the barrel cortex. The microcircuit of each L4 barrel is relatively simple: excitatory and inhibitory neurons within the same cortical column are connected with high probability; the only prominent long-range input originates in the VPM. Here we explored how sensory information is transformed from thalamus to cortex while mice performed an active tactile discrimination task. We recorded intracellularly from L4 neurons in the barrel cortex of mice performing a whisker-dependent tactile task. Whisker trajectories and touches were recorded with high-speed videography. Under baseline conditions during performance of the tactile task, L4 neurons were hyperpolarized, with small-amplitude high-frequency membrane potential fluctuations. Whisker movements produced only small depolarizations (~ 1 mV) that were usually below spike threshold. Membrane potential dynamics associated with spikes was dominated by large-amplitude (> 10 mV) deflections. These bumps occurred almost exclusively within 10 ms after touch. In contrast, GABAergic neurons, including fast-spiking cells, were driven to spike by both touch and whisker movements. The stimulus-selectivity of GABAergic neurons was similar to VPM neurons. We also directly recorded feed-forward inhibition onto excitatory neurons by holding them at the reversal potential for excitatory input. Large inhibitory inputs were coupled to touch and whisker movements. We propose that synchronous touch responses from VPM neurons drive L4 excitatory neurons. Feedforward inhibition, mediated by L4 fast-spiking neurons, selectively suppresses asynchronous input associated with whisker movements.

Disclosures: J. Yu: None. **D. Gutnisky:** None. **A. Hires:** None. **K. Svoboda:** None.

Poster

535. Somatosensory: Local Cortical Circuits

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 535.05/II11

Topic: D.09. Tactile/Somatosensory

Title: Exploring the impact of optogenetic silencing of PV interneurons on barrel cortex microcircuit activity by two photon Ca2+ imaging

Authors: *P.-H. PROUVOT[1,2], G. K. PRAMANIK[2,3], J. W. YANG[3], H. LUHMANN[3], A. STROH[2]
[1]FTN Ag Stroh, Mainz, Germany; [2]ftn & Inst. of Micr. Anat., Mainz Univ., Johannes Gutenberg Univ., Mainz, Germany; [3]Inst. of Physiol., Univ. Med. Ctr. of the Johannes Gutenberg Univ. Mainz, Mainz, Germany

Abstract: Recent studies on optogenetic silencing of PV interneurons in the non-columnar mouse visual cortex suggest a prominent role of this subpopulation in gain control Atallah BV et al (2012). Here, we explore their impact on barrel cortex microcircuit activity *in vivo*, hypothesizing a more specific effect due to its columnar organization.We established a multimodal approach, combining optical and electrophysiological readouts with optogenetics. Excitatory and inhibitory opsins (ChR2 and ArchT) were expressed specifically in PV expressing interneurons in layer II/III of the barrel cortex. We use intrinsic optical imaging (IOI) to target the virus expression to defined barrel related columns. This allowed us to achieve a barrel specific expression of opsins. The synthetic calcium indicator Oregon-green Bapta-1 (OGB-1) was then injected in barrel related columns identified by IOI upon single whisker deflection, typically B1/B2. Employing a custom-built 2 photon microscope, equipped with two independent scanning mirrors for spatio-temporally independent optogenetic modulation, the suprathreshold activity of up to 100 neurons can be imaged at 30 Hz. Electrophysiological recordings from layer V identify the brain state of the animal. This combination of techniques allows us to assess the temporal dynamics and spatio-temporal properties of whisker-evoked activity upon manipulation of PV interneuron activity.

Disclosures: P. Prouvot: None. **G.K. Pramanik:** None. **J.W. Yang:** None. **H. Luhmann:** None. **A. Stroh:** None.

Poster

535. Somatosensory: Local Cortical Circuits

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Topic: D.09. Tactile/Somatosensory

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The research leading to these results has received funding from the European Union Seventh Framework Programme (FP7/20072013) under grant agreement n°604102

Title: Technology innovations for leveraging supercomputers for reconstruction and simulation of large-scale detailed tissue models

Authors: J. G. KING[1], F. DELALONDRE[1], B. MAGALHAES[1], P. KUMBHAR[1], T. EWART[1], A. OVCHARENKO[1], F. CREMONESI[1], M. HINES[2], E. MULLER[1], *F. SCHUERMANN[1], H. MARKRAM[1]
[1]Blue Brain Project, Brain Mind Institute, EPFL, Lausanne, Switzerland; [2]Dept. of Neurobio., Yale Univ., New Haven, CT

Abstract: The Blue Brain Project (BBP) aims to simulate a large-scale morphologically detailed and biologically accurate model of neocortical tissue that includes millions of neurons. Such an endeavor must leverage state-of-the-art high performance computing technology for simulation, as well as at many stages of the model building process, to attain the desired level of biological detail and model fidelity, and a usable time-to-solution at the intended model scale. We describe recent developments of our supercomputer-based software and hardware infrastructure to support these objectives in two directions: Increased problem scale via increased neuron and synapse counts along with greater biological complexity, and exploration of longer biological times allowing better introspection of biological accuracy over longer simulated durations. Increased problem scale: Recent endeavors in our circuit building process have increased the biological complexity of our reconstructed models and require increased capabilities in our simulation software to handle the increased feature set. We describe the latest algorithmic improvements (problem distribution, more efficient memory usage and layouts, and on node/core parallelisms) and present scaling numbers, and continuous efforts to build and simulate increasingly larger models using our recently acquired hardware platform. The later, referred to as BBP4 hosted by the Swiss National Supercomputing Centre (CSCS), ranking 47th in the world top500 ranking (Nov. 2013), consists of a 4-rack IBM Blue Gene/Q (65'536 cores and 64TB of DRAM) as well as a companion analysis and visualization cluster (40 nodes with 16 Intel Xeon cores and 2 K20 GPU per node). Using such a facility we were able to respectively build and simulate circuits of millions morphologically detailed neurons at the time of writing of this abstract. Exploration of longer biological times: We describe a newly developed "SaveState" feature which allows simulations to be saved and resumed. This facilitates common parameter sweep use cases, but also allows the chaining of short blocks of simulations in periods of low demand to attain time-scales relevant for studies of microcircuit plasticity and learning. In addition, performance modeling of the simulation software are used to drive low-level optimizations to reduce time to solution.

Disclosures: **J.G. King:** None. **F. Schuermann:** None. **F. Delalondre:** None. **B. Magalhaes:** None. **P. Kumbhar:** None. **T. Ewart:** None. **A. Ovcharenko:** None. **F. Cremonesi:** None. **M. Hines:** None. **E. Muller:** None. **H. Markram:** None.

Poster

535. Somatosensory: Local Cortical Circuits

Location: Halls A-C

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Title: Multiscale model of glia metabolism linked to an electrical model of cerebral cortex

Authors: *D. KELLER[1], J. S. COGGAN[1], J. G. KING[1], C. CALI[2], H. LEHVASLAIHO[2], H. MARKRAM[1], F. SCHUERMANN[1], P. MAGISTRETTI[2]
[1]Blue Brain Project, Brain Mind Institute, EPFL, Lausanne, Switzerland; [2]KAUST, Thuwal, Saudi Arabia

Abstract: Glia cells are essential for maintaining proper concentrations of metabolically significant molecules in the extracellular space of neural tissue. Of particular interest to energy balance between glia and neurons is the flux of lactate, an anaerobic product of glycolysis. We present a system for modeling the metabolic interaction of glia cells with a large-scale network of spiking neurons. The system models lactate and glucose metabolism within the neurons and glia and tracks their extracellular concentrations, as well as those of pertinent ions such as Na+, Ca2+, and K+. Extracellular concentrations of these ions influence the reversal potential of neurons and release processes. The system also accommodates diffusion of extracellular molecules. Glutamate released from spiking cortical neurons is taken up by glia cells and through this process it modulates metabolism in the glia. Calcium concentrations within neurons and glia are also included in the model and used to predict the production of vasoactive substances. We subject the circuit to various stimuli in order to observe location- and stimulation-dependent effects.

Disclosures: D. Keller: A. Employment/Salary (full or part-time):; EPFL. 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.; The EPFL Blue Brain Project Fund, The ETH Board

Funding to the Blue Brain Project, CADMOS: The financial support for CADMOS and the Blue Gene/Q system is provided by the Canton of Geneva, Canton of Vaud, Hans Wilsdorf Foundation, Louis-Jeantet Foundation, University of Geneva, U. **J.S. Coggan:** None. **C. Cali:** None. **J.G. King:** None. **H. Lehvaslaiho:** None. **H. Markram:** None. **F. Schuermann:** None. **P. Magistretti:** None.

Poster

535. Somatosensory: Local Cortical Circuits

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Title: Morphological analysis and classification of neuronal types in the neocortical microcircuit

Authors: *L. KANARI, G. ATENEKENG, J. W. GRAHAM, R. HATELAND, Y. SHI, J. SHILLCOCK, F. SCHUERMANN, H. MARKRAM
Blue Brain Project, EPFL, Lausanne, Switzerland

Abstract: Abstract: The ability to build and simulate models of the mammalian neocortex relies fundamentally on accurate neuronal morphologies. The shape of neuronal arborizations is a key feature for the classification of neurons and defines amongst other aspects, their physical connectivity and, as a consequence, their functionality (Kalisman, 2003). The extreme diversity of neuron types in the brain renders the direct reconstruction of large numbers of cells from microscopic images infeasible. Generating *in silico* copies of neurons is therefore necessary for modeling large brain regions. For the consistent use of the artificial morphologies in a simulation it is important to ensure that they are statistically close to the biological reconstructions. A workflow has been developed for the extraction of statistical measurements from biological reconstructions of different neuronal types that are used as input for the validation and the classification of artificial or biological morphologies. This morphological analysis process is

implemented in a software tool that allows the user to perform a variety of anatomical operations, ranging from detecting and correcting artifacts in the reconstructed morphologies to viewing, analyzing, comparing and classifying neurons. Here we present the process of measuring the morphological characteristics of a single neuron, comparing them with the statistics of different neuronal populations and objectively classifying the cell into the appropriate morphological class, based on the extracted features. This tool simplifies and automates many of the tasks needed before large-scale neuronal synthesis for brain simulation can become a routine procedure. **References:** 1) *Kalisman N, Silberberg G, Markram H.* **Deriving physical connectivity from neuronal morphology**. Biol. Cybern. 88: 210-218, 2003.

Disclosures: **L. Kanari:** None. **G. Atenekeng:** None. **J.W. Graham:** None. **R. Hateland:** None. **Y. Shi:** None. **J. Shillcock:** None. **F. Schuermann:** None. **H. Markram:** None.

Poster

535. Somatosensory: Local Cortical Circuits

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Title: Predictive in silico reconstruction of neuronal input-output organization in the neocortical microcircuit

Authors: *S. RAMASWAMY, M. REIMANN, J. G. KING, E. B. MULLER, H. MARKRAM
Blue Brain Project, Brain Mind Institute, EPFL, Lausanne, Switzerland

Abstract: The neocortical microcircuit contains a rich diversity of excitatory and inhibitory neurons. Although previous research has revealed the fundamental characteristics of neocortical neuron types, knowledge of their synaptic, laminar, and columnar organization remains very limited. The Blue Brain Project has developed a data-driven process for the in silico reconstruction of a neocortical microcircuit. The reconstruction integrates a vast body of

biological data on ion channel kinetics and distributions, neuronal morphologies and electrical types, synaptic kinetics and dynamics from juvenile rat somatosensory cortex. These data were used to parameterize and validate the in silico anatomy and physiology of monosynaptic pathways arising from the incidental axo-dendritic overlap of diverse pre-post morphological combinations. Experimental protocols of glutamate uncaging through photo-stimulation *in vitro* were mimicked in silico to uncover a gamut of predictions on the complement of intrinsic synapses impinging onto major neocortical neuron types. The reconstruction predicts functional maps for 55 identified neuron types based on afferent and efferent morphological, electrical, and synaptic types showing a high degree of asymmetry in their input-output organization. We describe key organizing principles of neuronal input-output in the local neocortical microcircuit and discuss their roles in governing global circuit activity.

Disclosures: S. Ramaswamy: None. **M. Reimann:** None. **J.G. King:** None. **E.B. Muller:** None. **H. Markram:** None.

Poster

535. Somatosensory: Local Cortical Circuits

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Topic: D.09. Tactile/Somatosensory

Support: EU grant FP7-269921 (BrainScaleS)

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Title: Towards an integrated sensory-motor model of a mouse

Authors: *M.-O. GEWALTIG, C. EROE, B. DENIZDURDURAN, H. MARKRAM
Blue Brain Project, Brain Mind Institute, EPFL, Lausanne, Switzerland

Abstract: We present the first integrated sensory-motor model of a mouse, including a central and a peripheral nervous system, as well as a body. The latter comprises a skeleton along with muscles, skin, and sensory organs. The virtual mouse is embedded in a simulated environment with realistic physics and can therefore be used in a wide range of *in silico* experiments. Thus, we can observe the entire range of neural activity, from the peripheral sensors to the central nervous system, while the virtual mouse is engaged in an experiment. The central nervous system is a brain model that is semi-automatically generated from different data-sets. Cell

positions and inter-region connectivity are generated from the Allen Brain Atlas [1]. Cortical cell types and cell-type specific connectivity are generated from the Blue Brain Project's neo-cortical column model [2]. The final brain model comprises 70 million integrate-and-fire neurons, connected by some 10^{11} synapses, but the precise number can be adjusted. The peripheral nervous system is composed of a spinal-cord, modeled at the population level, and connections between the brain and the sensory organs (whiskers, eyes, ears, and skin). We present a technique to map spatially distributed sensors, such as touch sensors on the skin, to their corresponding regions on the sensory cortex. The result is the first integrated data-driven model of a mouse which also serves as a seed model for the Neurorobotics Platform of the EU-backed Human Brain Project [3]. The model is the starting point for a perpetual refinement cycle where new data is integrated on demand and where each iteration will further our understanding of the nervous system within a closed action-perception loop. At the poster, we will present videos demonstrating the modeling workflow as well as results showing the emergent brain activity of the mouse as it is immersed in a dynamic environment. References: [1] Oh, S. W., Harris, J. a, Ng, L., Winslow, B., Cain, N., Mihalas, S., Zeng, H. (2014). A mesoscale connectome of the mouse brain. *Nature*, *508*(7495), 207–14. ; http://www.brain-map.org/ [2] Ramaswamy, S., Hill, S. L., King, J. G., Schürmann, F., Wang, Y., & Markram, H. (2012). *The Journal of Physiology*, *590*(Pt 4), 737–52. [3] The Human Brain Project; https://www.humanbrainproject.eu/en/neurorobotics-platform

Disclosures: **M. Gewaltig:** None. **C. Eroe:** None. **B. Denizdurduran:** None. **H. Markram:** None.

Poster

535. Somatosensory: Local Cortical Circuits

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Title: Incorporating synthesized meso-scale pyramidal axons into a reconstruction of neocortical tissue

Authors: Y. KIM, J. DYNES, J. KING, *E. B. MULLER, M. REIMANN, J. SHILLCOCK, H. MARKRAM
Blue Brain Project, Brain Mind Institute, EPFL, Lausanne, Switzerland

Abstract: Neuronal morphologies are a vital component of biophysical models of the nervous system because they determine potential connectivity, and provide the geometrical framework for synaptic integration. Pyramidal axons in particular have extensive axonal arborizations extending beyond the local microcircuit (>300µm from soma) which cannot be obtained from standard *in vitro* brain slices. Such axonal morphologies can be obtained from in-vivo labeled neurons, but are significantly more labor-intensive, and are therefore too few in number to allow circuit reconstructions. Here we present a solution to this problem by synthesizing axonal arbors based on available data from in-vivo labeling. We developed a two stage synthesis algorithm where first, spatial clusters of the axons of individual neurons are extracted, and second, axonal arbors are synthesized by employing a wiring/delay cost balancing algorithm, extending previous work to multiple axonal clusters. Employing this technique, we synthesize and incorporate axons of cortical pyramidal cells in layers 2-5 of rat P14 somatosensory cortex into reconstructions of the somatosensory microcircuit. We compare microcircuits with synthesized pyramidal axons to those with axon reconstructions from slice, and highlight differences in multi-synaptic connectivity, long-range connectivity, and effects on in silico simulations of spontaneous and evoked network activity.

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Poster

535. Somatosensory: Local Cortical Circuits

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The research leading to these results has received funding from the European Union Seventh Framework Programme (FP7/20072013) under grant agreement n°604102

Title: Embedding gap junctions in high fidelity models of neocortical microcircuitry

Authors: O. AMSALEM[1], *J. G. KING[2], W. VAN GEIT[2], B. MAGALHAES[2], E. MULLER[2], H. MARKRAM[2], I. SEGEV[1]
[1]Inst. of Life Sciences, Hebrew Univ., Jerusalem, Israel; [2]Blue Brain Project, Brain Mind Institute, EPFL, Lausanne, Switzerland

Abstract: Inhibitory neurons in the neocortex are electrically interconnected through gap junctions (GJs). These connections are established mostly among interneurons of the same type. The function of GJs in the neocortex, and in particular the class-specificity of GJs, is not yet fully understood. Here we present a process for utilizing morphological and physiological data for the parvalbumin+ (PV) basket-cell class (% electrically coupled, coupling strength, number of GJs per cell, spikelet delays, etc.) as constraints for establishing realistic GJs among PV neurons in an in silico reconstruction of a microcircuit of rat P14 somatosensory cortex. Using realistic 3D morphologies, number and distribution for basket cells within the volume of layer 2/3, we found that, when assuming "touch detection" distance of 0 μm between dendrites-to-dendrites and dendrites-to-soma, the number of GJs per basket cell ranged between 40 - 80 with an average of 1.4 GJs per connected cells, both in close agreement with the experimental range. Namely, in this case, Peter's rule holds, in contrast to our previous findings for chemical synapses. Comparing experiments to the in silico reconstruction enabled us to extract the structural connectivity pattern of GJ's in the basket cell network, estimate the conductance of an individual GJ, and of the impact of the GJs load on the soma input resistance. We employ this unique opportunity to explore the effect of GJs, in a major inhibitory sub-network, on spontaneous and evoked dynamics/synchronization in biologically accurate large-scale simulations of neocortical microcircuitry.

Disclosures: O. Amsalem: None. **J.G. King:** None. **W. Van Geit:** None. **B. Magalhaes:** None. **E. Muller:** None. **H. Markram:** None. **I. Segev:** None.

Poster

535. Somatosensory: Local Cortical Circuits

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Topic: D.09. Tactile/Somatosensory

Support: European Union Seventh Framework Programme (FP7/20072013) under grant a greement n°604102

The ETH Board Funding to the Blue Brain Project

Title: The HBP unified portal: Collaborative data-driven multi-scale reconstruction and simulation of neural tissue

Authors: *J. MULLER[1], J. G. BJAALIE[2], L. CORBEIL[3], J.-D. COURCOL[1], D. DARINE[2], F. DELALONDRE[1], S. HILL[1], D. MALLMAN[4], C. MCMURTRIE[3], C. MEZZANOTTE[3], E. MULLER[1], R. NIEDERBERGER[4], B. ORTH[4], F. SCHUERMANN[1], B. SCHULLER[4], M. TELEFONT[1], S. ZANINETTA[1], H. MARKRAM[1]
[1]Blue Brain Project, Brain Mind Institute, EPFL, Lausanne, Switzerland; [2]Inst. of Basic Med. Services, UNIVERSITETET I OSLO, Oslo, Norway; [3]EIDGENOESSISCHE TECHNISCHE HOCHSCHULE ZURICH, Zurich, Switzerland; [4]FORSCHUNGSZENTRUM JUELICH GMBH, Juelich, Germany

Abstract: A central tenant of the EU-FET Flagship “Human Brain Project” (HBP) is that a comprehensive understanding of the brain requires study of the structure and function across all levels of brain organization and cannot be obtained by the study of one level alone. The only approach today that can in principle be developed to simultaneously study the brain across all levels is brain simulations using biophysical in-silico reconstructions of the brain composed of embedded models at each level. Since this effort requires expertise across the spectrum of neuroscience, computer science, physics and mathematics, a massively collaborative scientific effort is crucial to reconstruct such multi-level models. The impact of the social internet and the success of opensource software communities shows that ICT has the capacity to enable the massively collaborative efforts needed. To enable large-scale collaborations in neuroscience, the HBP is developing the Unified Portal (HBP-UP), a web-based collaborative scientific platform intended to bring together all HBP activity. The HBP-UP will be equipped with a layer of powerful collaboration functions and extension frameworks to allow integration of the 6 HBP ICT Platforms. Built on this foundation and leveraging cutting edge HPC facilities, the HBP-UP will allow fluid sharing of data, theories, applications and models prior to publication while still maintaining proper attribution. It is expected that this sharing will be a key factor in the acceleration of neuroscience and the eventual achievement of the HBP’s ambitious goals. We present the current architecture for the HBP-UP along with a tech preview of a number of the key use cases it is meant to address. With this presentation we hope to solicit early feedback from the community.

Disclosures: **J. Muller:** None. **J.G. Bjaalie:** None. **L. Corbeil:** None. **J. Courcol:** None. **D. Darine:** None. **F. Delalondre:** None. **S. Hill:** None. **D. Mallman:** None. **C. McMurtrie:** None. **C. Mezzanotte:** None. **E. Muller:** None. **R. Niederberger:** None. **B. Orth:** None. **F. Schuermann:** None. **B. Schuller:** None. **M. Telefont:** None. **S. Zaninetta:** None. **H. Markram:** None.

Poster

535. Somatosensory: Local Cortical Circuits

Location: Halls A-C

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Program#/Poster#: 535.14/II20

Topic: D.09. Tactile/Somatosensory

Support: BrainGain, a Smart Mix Program of the Netherlands Ministry of Economic Affairs and the Netherlands Ministry of Education, Culture and Science

Neuroseeker, a European Community Seventh Framework Programme (FP7/2007-2013) under grant agreement no. 600925

Title: Sensory and Cognitive electrophysiology in rats: Tactile stimulation and micro-ECoG recordings in freely moving animals

Authors: ***G. DIMITRIADIS**, A. M. FRANSEN, E. MARIS
Donders Inst. For Brain, Cognition and Behaviour, Nijmegen, Netherlands

Abstract: We have developed a setup for rats that allows for controlled sensory input to an animal engaged in a task while recording both electrophysiological signals and behavioral output. We record electrophysiological signals using a novel high-density micro-electrocorticography (micro-ECoG) grid that covers almost the whole somatosensory system. We dealt with the well-known difficulty that the rat uses its whisker system in an active (motor-controlled) way to explore its environment by designing a head-mounted device that stimulates the rat's snout in a way unaffected by whisker movements. We replicate the spatial specificity of early evoked responses in somatosensory and auditory cortex. We validate our setup by replicating (1) the functionally nonspecific spread of neural activity following tactile stimulation, and (2) the effects of anesthesia on the tactile evoked responses. We also demonstrate for the first time that the ECoG can be used to record evoked responses in a signal that reflects neural output (spiking activity), and illustrate the usefulness of our setup by demonstrating that these evoked responses are modulated by both the phase of pre-stimulus oscillations and by

expectation. Compared with high-density wire recordings, micro-ECoG offers a much more stable signal without readjustments, and a much better scalability. Compared with head-fixed preparations, our head-mounted stimulator allows to stay closer to the rat's natural way of collecting sensory information. For perceptual and cognitive research, our setup provides a unique combination of possibilities that cannot be achieved in other setups for rodents.

Disclosures: G. Dimitriadis: None. **A.M. Fransen:** None. **E. Maris:** None.

Poster

535. Somatosensory: Local Cortical Circuits

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Topic: D.09. Tactile/Somatosensory

Support: BBSRC grant BB/K010123/1.

Title: Effect of bicuculline on the temporal dynamics of the evoked local field potential: Implication on the interpretation of EEG

Authors: M. BRUYNS-HAYLETT[1], J. LUO[1], A. KENNERLEY[3], S. HARRIS[3], L. BOORMAN[3], N. VAUTRELLE[3], C. MARTIN[3], B. WHALLEY[1], M. JONES[4], J. BERWICK[3], *Y. ZHENG[2]
[2]Sch. of Systems Engin., [1]Univ. of Reading, Reading, United Kingdom; [3]Univ. of Sheffield, Sheffield, United Kingdom; [4]Univ. of Sheffield, sheffield, United Kingdom

Abstract: Increasing evidence from *in vivo* intracellular studies has demonstrated that proportionally balanced neural excitation and inhibition is a fundamental principle underlying a wide range of cortical activities. In particular, it has been shown that excitatory and inhibitory synaptic inputs are co-tuned, with inhibition lagging excitation by several milliseconds. However this temporal lag has not yet been demonstrated conclusively in extracellular field potential recordings. In this study, we investigated neural excitatory and inhibitory post-synaptic activity in the barrel cortex of anaesthetised rodents in response to electrical stimulation of the contra-lateral whisker pad. We manipulated the balance between neural excitation and inhibition by micro-injection of bicuculline, a competitive gamma-aminobutyric acid (GABAA) receptor antagonist at subconvulsive concentrations using a laminar linear 16-channel electrode array with an 'infusion-port' connected to a syringe pump. This multi-channel electrode allowed measurement of changes in the stimulus-evoked local field potential (LFP) before and after local

cortical bicuculline infusion *in vivo*. We found that for a range of dosages of bicuculline (6.25-50mM), the temporal dynamics of the evoked LFP did not change within the first 7-8 ms after the onset of stimulation and suggests that this initial segment of the evoked LFP reflects evoked temporal dynamics of excitatory post-synaptic activity of the local neural population alone. As the onset latency of the LFP response is ~4 ms, our data imply that, at the neural population level, inhibitory post-synaptic activity lags that of the excitatory activity by ~3-4ms and agrees well with findings from single neuron, patch clamp recordings (Wehr and Zador 2003, Okun and Lampl 2008). Furthermore the evoked LFP from the uppermost channel of the 16-channel micro-electrode (~50μm below the cortical surface) exhibited similar temporal characteristics as the event related potential (ERP) observed in human electroencephalography (EEG), with well-defined positive (e.g., p1, p2) and negative deflections (e.g., n1). Bicuculline had little effect on the amplitude and latency of p1 in the uppermost LFP channel, indicating, for the first time, that p1 may be solely associated with the excitatory post-synaptic activity of the local neural population.

Disclosures: **M. Bruyns-Haylett:** None. **J. Luo:** None. **A. Kennerley:** None. **S. Harris:** None. **L. Boorman:** None. **N. Vautrelle:** None. **C. Martin:** None. **B. Whalley:** None. **M. Jones:** None. **J. Berwick:** None. **Y. Zheng:** None.

Poster

535. Somatosensory: Local Cortical Circuits

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 535.16/II22

Topic: D.09. Tactile/Somatosensory

Support: BBSRC BB/K010123/1

Title: Investigating the shift in balance between neural excitation and inhibition due to bicuculline infusion using a two-compartment model of local field potentials

Authors: ***J. LUO**[1], M. BRUYNS-HAYLETT[1], J. BERWICK[2], A. KENNERLEY[2], L. BOORMAN[2], S. HARRIS[2], E. MILNE[2], D. COCA[3], S. BILLINGS[3], Y. ZHENG[1]
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Abstract: Abstract Local field potentials (LFPs) reflect the synaptic activity of neural assemblies surrounding the recording electrode. Using a single compartment configuration, we previously derived a model of the LFP which decomposed LFP into co-tuned and temporarily shifted excitatory and inhibitory components. However the model was only applicable to LFP recorded in layer IV of the neocortex where cortical pyramidal neurons receive most of their synaptic inputs (Zheng et al 2012). In the present study, we extended the LFP model using a two-compartment (2C) representation. The model was used to fit simultaneous LFP recordings from both layer I and IV of the neocortex, decomposing the signals into their respective excitation and inhibitory components. Model validation was performed using the LFP data recorded after the infusion of bicuculline, a competitive gamma-aminobutyric acid (GABAA) receptor antagonist, which diminishes or reduces the fast inhibitory post-synaptic activity in the vicinity of the infusion site. In other words, the estimated excitatory component of the LFP by the 2C LFP model prior to bicuculline infusion was compared to the LFP recording immediately after the administration of bicuculline as inhibition was mostly eliminated by the manipulation. Furthermore the change in the balance between neural excitatory and inhibitory post-synaptic activities over the recovery phase of bicuculline infusion was captured by the 2C LFP model parameters, both in terms of the amplitude of each component, and more interestingly in terms of the temporal lag of inhibition with respect to excitation. Reference: Zheng, Y, J J Luo, S Harris, A Kennerley, J Berwick, S A Billings, J Mayhew (2012) “Balanced excitation and inhibition: model based analysis of local field potentials.”NeuroImage 63(1), 81-94.

Disclosures: J. Luo: None. **M. Bruyns-Haylett:** None. **J. Berwick:** None. **A. Kennerley:** None. **L. Boorman:** None. **S. Harris:** None. **E. Milne:** None. **D. Coca:** None. **S. Billings:** None. **Y. Zheng:** None.

Poster

535. Somatosensory: Local Cortical Circuits

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Support: NIH, NINDS, Grant R01NS045130

NIH, NINDS, F32-NS078895

Title: *In vivo* optogenetic manipulation of cells within the neurovascular unit leads to local changes in neural activity

Authors: *T. C. BROWN, C. BURLEY, K. SALEHI, T. KISHKOVICH, C. DEISTER, C. MOORE
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Abstract: Communication and plasticity within or between cells in the neurovascular unit (neurons, glia, vascular endothelial and smooth muscle cells, and pericytes, among others) may play an important role in how neural networks adapt during sensory processing, encode synaptic changes, and/or contribute to the maintenance of cerebral metabolism. Disrupted signaling within the neurovascular unit has been linked to breakdown of the blood-brain barrier (BBB), and several pathological conditions, including diabetes, multiple sclerosis, Alzheimer's disease, epilepsy, and stroke. However, the mechanisms and precise role that neurovascular coupling plays in neural processing remains unclear. To this end, we have developed and further characterized multiple Vascular Optogenetic (VasOpto) and two-photon imaging methods that allow simultaneous visualization and bidirectional manipulation of cells within the neurovascular unit. Preliminary data show that bidirectional optogenetic manipulation of neocortical vascular endothelial or smooth muscle cells can lead to dilation and/or constriction of cerebral vessels comparable to the vascular changes evoked by physiological sensory input to the mouse primary somatosensory cortex. Utilizing these tools, experiments have revealed that somatosensory and VasOpto evoked vascular events lead to rapid changes in local neural activity, and that neurons can be tuned to unique aspects of the vascular response (dilation, constriction, peak, or other vascular specific parameters). Approximately 15-20% of neurons (N = 227 cells labeled w GCaMP5 under control of the synapsin promoter, all localized to layer I/II) were significantly modulated in firing by vibrissal stimulation, and a comparable number were similarly driven by VasOpto dilation. Ongoing and future studies are aimed at teasing apart the cell-type specificity of these interactions and comparing the neural responses associated with different VasOpto evoked vascular events, including dilation and constriction.

Disclosures: T.C. Brown: None. **C. Burley:** None. **K. Salehi:** None. **T. Kishkovich:** None. **C. Deister:** None. **C. Moore:** None.

Poster

535. Somatosensory: Local Cortical Circuits

Location: Halls A-C

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Support: Ecole des Neurosciences de Paris ("Network for Viral Transfer")

Fondation pour la Recherche sur le Cerveau / Rotary Club de France

Agence Nationale de la Recherche ("IHU Institut de Neurosciences Translationelles de Paris")

Title: Orexin-dependent activation of layer vib enhances cortical network activity and integration of non-specific thalamocortical inputs

Authors: *A. H. HAY[1,2], S. ANDJELIC[2,3], S. BADR[2], B. LAMBOLEZ[2]
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Abstract: The deepest part of neocortical layer VI (layer VIb) is the only lamina reactive to orexins. Furthermore, layer VI of the parietal cortex receives dense projections from the orexin-sensitive rhomboid, a non-specific thalamic nucleus involved in cortical arousal. This convergence of structures involved in arousal onto layer VIb neurons prompted us to investigate how layer VIb modulates cortical arousal. We used patch-clamp and single cell reverse transcription-polymerase chain reaction to explore the sensitivity to neurotransmitters of arousal and the intracortical projections of layer VIb neurons. We found that the majority of neurons were excited by nicotinic agonists and orexin B through the activation of nicotinic receptor subunits α4, α5 and β2 and the OX2R orexin receptor, respectively. We found that a low dose of nicotine potentiated orexin effect specifically on layer VIb neurons and used this paradigm to explore their cortical projections. Co-application of nicotine and orexin increased the frequency of excitatory post-synaptic currents in ipsilateral layers I, II/III, V and VIa neurons and in contralateral layer V, with a minimal effect in ipsilateral layer IV. The ability of layer VIb to relay rhomboid thalamocortical inputs was tested using optogenetics. In infragranular layers of the parietal cortex, photostimulation of channelrhodopsin-expressing rhomboid fibers induced robust reponses that were not pre-synaptically affected by orexin. Activation of layer VIb neurons by orexin enhanced the reliability of layer VIa responses to rhomboid inputs and increased spike timing precision. These results indicate that layer VIb acts as an excitatory feedforward loop in the neocortex that potentiates cortical arousal.

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Poster

535. Somatosensory: Local Cortical Circuits

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 535.19/II25

Topic: D.09. Tactile/Somatosensory

Title: Synaptic properties of inverted pyramidal cells in layer vi of the rodent barrel cortex

Authors: *R. STEGER[1], L. BLACHORSKY[2], J. C. BRUMBERG[3,4]
[1]Grad. Ctr., Forest Hills, NY; [2]Neurosci. Major, [3]Psychology and Neurosci. Major, Queens College, CUNY, Flushing, NY; [4]Psychology and Biol., The Grad. Center, CUNY, New York, NY

Abstract: Within the nervous system, the cortex is the area of the brain where higher order sensory, motor and cognitive processing occurs. Around 70% of the neurons found in the mammalian cortex are upright pyramidal cells (UPCs). However, some of these pyramidal cells have their dendrites pointing in the opposite direction (towards the white matter); they appear inverted. Thus far, there has only been very limited research concerning the function of these inverted pyramidal cells (IPCs). Previous research in our lab has revealed numerous differences in the intrinsic physiology between IPCs and UPCs including input resistance and action potential threshold. In the present study, we characterized the synaptic inputs onto IPCs and UPCs. Coronal slices from mouse barrel cortex were prepared and whole cell recordings were done from layer VI IPCs and UPCs. Minimal stimulation techniques were used to activate axons passing through the underlying white matter. Single pulse experiments revealed that while the peak amplitude of the evoked excitatory postsynaptic potentials (EPSP) is similar in both IPCs and UPCs, the EPSP rise time was significantly shorter in UPCs than IPCs. Both pairs of electrical stimulation, and stimulus trains (8 pulses) delivered to the cortical white matter demonstrated synaptic depression over a wide range of frequencies (2-20Hz) in both IPCs and UPCs. These results may indicate that IPCs and UPCs may receive similar synaptic inputs but due to their intrinsic differences may process them differently.

Disclosures: **R. Steger:** A. Employment/Salary (full or part-time):; Quantitative Research Fellow, City University of New York. **L. Blachorsky:** None. **J.C. Brumberg:** A. Employment/Salary (full or part-time):; Professor Queens College and the The Graduate Center, CUNY.

Poster

535. Somatosensory: Local Cortical Circuits

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

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Topic: D.09. Tactile/Somatosensory

Support: FAPERJ

CNPq

Title: Cebus monkeys have a relatively complex somatosensory cortex: An architectonic study of the anterior parietal cortex and area 5 of Broadmann

Authors: *A. MAYER[1], M. N. S. LUIZ[1], N. B. KEHER[1], R. E. BITTENCOURT-NAVARRETE[2], R. GATTASS[1], J. G. FRANCA[1]
[1]Inst. de Biofísica Carlos Chagas Filho, Federal Univ. of Rio De Janeiro, Rio De Janeiro, Brazil; [2]Federal Univ. of Juiz de Fora, Governador Valadares, Brazil

Abstract: Cebus monkeys stand out from other New World monkeys by their ability to perform fine hand movements and use tools. Those behaviors are dependent on the processing and integration of somatosensory information performed by different areas of the parietal cortex. Although a few studies have examined and parceled the areas of cebus monkey parietal cortex, mainly using electrophysiological criteria, very little is known about its anatomical organization. In this study, we used SMI-32 immunohistochemistry, myelin and Nissl preparations to characterize the architecture of the cortical areas of the anterior parietal cortex, plus area 5, in the cebus monkey. Six adult cebus monkeys had their brains sectioned parasagitally, coronally or horizontally at 40 or 50 μm. In all cases, alternate sections were stained with cresyl violet (Nissl). In two cases, additional histological series were processed for myelin and/or for immunoreactivity against SMI-32 antibodies (SMI-32 IR). The analysis was performed on images of the whole section, collected using a 5x objective in a microscope equipped with the Neurolucida system (MBF, Inc). Six cortical areas were identified between the precentral gyrus and the anterior bank of the intraparietal sulcus. Area 3a showed moderate SMI-32-IR cell bodies and thin apical dendrites in layer 3, very strong myelination in layers 4-6, and a distinctive homogenous appearance in Nissl preparations, with a poorly developed layer 4. Area 3b exhibited light SMI-32-IR, moderate density of horizontal myelinated fibers in layer 4, dense horizontal fibers in infragranular layers (IG), and well-developed layers 4 and 6 in Nissl preparations. Area 1 presented a typical palisade-like appearance, especially in SMI-32-IR and myelin stained sections, due to dense immunolabeled cell bodies with long apical dendrites in supragranular layer (SG), and dense thick vertical fibers in IG. Area 2 showed moderate density of SMI-32-IR cell bodies with short apical dendrites in layer 3, moderate density of thick and short vertical fibers in IG, and a relatively homogeneous appearance in Nissl stained sections. Area 5d presented an immunostaining pattern and myeloarchitecture similar to area 2, but with much higher density of SMI-32-IR cell bodies and myelinated fibers. In contrast, area 5v exhibited a moderate density of SMI-32-IR cell bodies with characteristic long apical dendrites in SG and visible bands of Baillarger. Our results show that cebus monkeys have a relatively

complex somatosensory cortex, comparable to that of humans. This certainly is one of the key neurobiological features that allow cebus monkeys to perform outstanding manual behaviors.

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Poster

535. Somatosensory: Local Cortical Circuits

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Topic: D.09. Tactile/Somatosensory

Support: NIH P01 NS074972

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Epilepsy Foundation Grant 260691

Title: Tyrosine-protein kinase Kit (c-Kit) protein as a marker for supragranular neurogliaform cells in the neocortex

Authors: I. KRUGLIKOV[1], Z. TALBOT[1], L. BAYER[1], A. Z. AHMED[1], R. MACHOLD[1], *B. RUDY[2]
[1]NYU Neurosience Inst., New York, NY; [2]Physiol. & Neurosci., NYU Sch. of Med., NEW YORK, NY

Abstract: Layer 1 (L1) is an enigmatic layer of cerebral cortex that has been suggested as a site for integration of information carried by local, inter-areal and subcortical projections. L1 is populated exclusively by inhibitory interneurons whose activity controls electrogenesis in dendrites of deeper pyramidal cells. Neurogliaform cells (NGFCs) are known to be enriched in L1; however their function is not completely understood in part because of a lack of a specific genetic marker. Here we characterize cortical eGFP expression in c-Kit (CD117) BAC transgenic mouse line (Tallini et al. PNAS 2009). In supragranular layers of somatosensory cortex of adult animals, c-Kit eGFP expression does not overlap with Satb2 marker of pyramidal cells in immunohistochemical assay. It also does not overlap with three major markers of cortical interneurons -- parvalbumin, somatostatin and VIP. Taken together these data suggest labeling of a separate population of interneurons. To clarify this further, we performed whole-cell electrophysiological recordings of intrinsic properties and synaptic outputs of c-Kit interneurons.

Majority of c-Kit interneurons exhibited firing pattern characterized by a stuttering action potential discharge with or without delay to the first spike. A minority of c-Kit cells exhibited a firing pattern characterized by an initial burst of a few spikes. Anatomically, interneurons with stuttering firing patters display both elongated and local NGFCs morphologies, while bursting cells have sparser local as well as multiple translaminar downward axons. Synaptic outputs of c-Kit interneurons, assessed by paired recordings in L2 pyramidal cells, displayed properties of slow GABAA inhibitory currents. Preliminary recordings of c-Kit interneurons in awake behaving animals, using 2-photon targeted patching, show correlation of spiking activity as well as sub-threshold inputs with voluntary whisker movement. In conclusion, expression of c-Kit eGFP in supragranular cortical layers is biased towards NGFCs. L1 c-Kit cells in somatosensory cortex show motor-related activity.

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Poster

535. Somatosensory: Local Cortical Circuits

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 535.22/II28

Topic: D.09. Tactile/Somatosensory

Title: Mouse barrel connectomics

Authors: *K. M. BOERGENS, P. BASTIANS, M. BERNING, B. COWGILL, I. YU, N. MARAHORI, M. HELMSTAEDTER
Structure Of Neocortical Circuits, Max Planck Inst. of Neurobio., Planegg, Germany

Abstract: The input module of one presumed cortical column, the layer 4 “barrel” of mouse primary somatosensory cortex, contains about 2,000-3,000 neurons, of which more than 90% are spiny excitatory neurons. Their pairwise synaptic connectivity is about 30 percent. Beyond this statistical knowledge, the circuit structure of this neuronal ensemble is unknown. Here, we used serial blockface scanning electron microscopy to acquire a dataset of about 575 µm x 600 µm x 250 µm size and a nominal voxel size of about 11 nm x 11 nm x 28 nm of fully stained tissue targeted to the C2/C3 barrels in mouse S1 cortex. We are currently investigating the specificity of inhibitory innervation, the targets of thalamocortical axons and the structure of excitatory circuitry in this dataset.

Disclosures: **K.M. Boergens:** None. **P. Bastians:** None. **M. Berning:** None. **B. Cowgill:** None. **I. Yu:** None. **N. Marahori:** None. **M. Helmstaedter:** None.

Poster

535. Somatosensory: Local Cortical Circuits

Location: Halls A-C

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Program#/Poster#: 535.23/II29

Topic: D.09. Tactile/Somatosensory

Title: Structural distinguishability of neocortical circuit models

Authors: *E. KLINGER[1], T. KRETSCHMAR[1], F. THEIS[2], M. HELMSTAEDTER[1]
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Abstract: Despite almost 100 years of intense research on neuronal circuits in the cerebral cortex, knowledge about the structure and computational capacity of cortical circuits is still poor today. Many largely varying types of such models have been proposed and studied, ranging from strictly layered feed-forward connectivity to largely randomly wired random-pool models. With novel high-resolution circuit mapping techniques being available, circuit models should become testable. Here, we analyzed three types of previously proposed cortical circuit models for their realistic implementation in a concrete cortical circuit, layer 4 of one cortical column in mouse somatosensory cortex. We find that only for a limited range of network parameters such models can be stably implemented under biologically plausible constraints. We are developing structural descriptors of such circuit models and investigate their distinctive power given densely mapped neocortical circuit data. We identified a set of observables that distinguish between feed-forward and random circuit types in the presence of connection noise. Such circuit analysis tools will allow coarse class distinctions of cortical models when dense connectomics data becomes available for networks in the cerebral cortex.

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Poster

535. Somatosensory: Local Cortical Circuits

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 535.24/II30

Topic: D.09. Tactile/Somatosensory

Title: Target-specificity of long-range cortical input to mouse barrel cortex

Authors: *F. P. DRAWITSCH, Y. HUA, M. HELMSTAEDTER
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Abstract: Long-range cortico-cortical projections are known to target pyramidal neurons at their apical dendrites in layer 1. Little is known about the cellular specificity of such long-range projections. In mouse barrel cortex, all pyramidal neurons from layers 2, 3 and 5 extend their dendrites into layer 1, where they receive long-range input from other cortical regions such as primary motor cortex (M1) and secondary somatosensory cortex (S2). Here, we investigate the specificity of such long-range projections to the ensemble of pyramidal cells in one cortical column. We develop methods for correlated viral-injection based long-range axon labeling and light-microscopic imaging with high-resolution SBEM-based dense circuit reconstruction in layer 1. These methods include a fixation protocol for combined LM and EM imaging and large-volume staining, and computational methods to correlate light and EM images at the level of single axons. We are investigating whether long-range input from one cortical source is target-specific for subgroups of pyramidal neurons, and whether clustered innervation of dendritic branches occurs.

Disclosures: F.P. Drawitsch: None. **Y. Hua:** None. **M. Helmstaedter:** None.

Poster

535. Somatosensory: Local Cortical Circuits

Location: Halls A-C

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Program#/Poster#: 535.25/JJ1

Topic: D.09. Tactile/Somatosensory

Title: Comparative analysis of local circuits in six- and three-layered sensory cortices

Authors: *P. BASTIANS[1], M. HEMBERGER[2], G. LAURENT[2], M. HELMSTAEDTER[1]
[1]MPI of Neurobio., Martinsried, Germany; [2]Max Planck Inst. for Brain Res., Frankfurt am Main, Germany

Abstract: Primary visual, auditory and somatosensory mammalian cortices are six-layered, and receive topographically organized sensory input. In contrast three-layered cortices are non-focally innervated from the sensory periphery. Some animals only possess three-layered cortices, such as turtle. Here we analyze by which principles local cortical circuits differ between six-layered and three-layered sensory cortices. Using serial block-face electron microscopy (SBEM), we compare neuronal circuits in six-layered primary somatosensory "barrel" cortex from mouse with dorsal cortex from turtle. As one focus, we investigate the innervation specificity of inhibitory neurons in both systems, testing whether non-topographic sensory innervation is mirrored by non-local inhibition.

Disclosures: **P. Bastians:** A. Employment/Salary (full or part-time):; Max Planck Institute of Neurobiology. **M. Helmstaedter:** None. **G. Laurent:** None. **M. Hemberger:** None.

Poster

535. Somatosensory: Local Cortical Circuits

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Program#/Poster#: 535.26/JJ2

Topic: D.09. Tactile/Somatosensory

Support: NSERC

Title: Influence of area 5 on motor cortical excitability during the pre-movement phase of a GO/NO-GO task in humans

Authors: *T. N. MACKENZIE, A. J. NELSON
McMaster Univ., Hamilton, ON, Canada

Abstract: Anatomical and physiological studies have implicated Brodmann's area 5 in fine motor control in the upper limb. However, the relationship between area 5 and ipsilateral motor cortex (M1) during movement planning remains unclear. The present study investigated the functional connectivity between putative area 5 and ipsilateral M1 in humans using paired-pulse Transcranial magnetic stimulation (TMS). Twelve right-handed individuals were studied. The right arm was engaged in the task while the left arm remained at rest. The GO/NO-GO task

involved an auditory warning cue followed at various intervals by a second auditory 'GO' or 'NO-GO' cue. For 'GO' trials participants were instructed to make a mouse click while 'NO-GO' trials required participants to withhold their response. Paired-pulse TMS involved a conditioning stimulus (CS) over putative area 5 followed by a test stimulus (TS) over M1. Both the CS and TS were delivered using customized 50 mm figure-of-eight branding coils, each connected to a Magstim 2002. Paired-pulse TMS was delivered at two timeframes (125 and 175 ms) and at two interstimulus intervals (ISIs) (6 and 8 ms) for GO and NO-GO trials. Eight conditions were tested in total: Task (GO/NO-GO) x timeframe (125, 175 ms) and ISI (6, 8 ms). Ten CS-TS trials were performed for each condition. In addition, TS alone trials were obtained for GO/NO-GO x timeframe. CS was set at 90% RMT. TS was set at the intensity to achieve ~ 1 mV motor evoked potential (MEP) in the first dorsal interosseous. The peak-to-peak amplitude of MEPs were averaged for each condition and normalized to TS alone (i.e. MEPCS-TS/MEPTS). Preliminary data indicate that MEPs are modestly facilitated for 'GO' compared to 'NO-GO' at the 125 ms timeframe for both 6 and 8 ms ISIs. These data suggest that area 5 modulates the excitability of M1 in a task-dependent manner and at specific time intervals within the pre-movement period, similar to the relationship found between right inferior frontal cortex and M1.

Disclosures: T.N. Mackenzie: None. **A.J. Nelson:** None.

Poster

535. Somatosensory: Local Cortical Circuits

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Topic: D.09. Tactile/Somatosensory

Support: Swedish Research Council (2010-3250)

Internal funds from Karolinska Institutet

Title: Neurotensin decreases the frequency of the cortical slow oscillation and excites a neuronal population residing in the white matter

Authors: *L. CASE, C. BROBERGER
Karolinska Institutet, Stockholm, Sweden

Abstract: The peptide, neurotensin (NT), has been ascribed a wide variety of CNS actions, including analeptic effects. Within the cortex, where vigilance state is ultimately set, NT receptors are abundantly expressed. There is little information available, however, on direct NT actions within the cortical network. One prominent cortical manifestation of deep sleep is the so-called “slow oscillation” (SO; <1Hz) consisting of alternating UP- and DOWN-states. The SO can be reproduced in cortical slice preparations. We have explored electrophysiological actions of NT in slices of somatosensory and visual cortex prepared from 2-13 week-old rats. Robust SO activity was observed in extracellular multi-unit recordings from slices maintained in an interface chamber. In the presence of NT (0.2-1μM), UP-states decreased in frequency and eventually disappeared but returned upon washout. To determine the site of action of NT on the SO, NT was next focally applied. A decrease in SO frequency was seen only when NT was applied in the deepest layer, L6b, and in the white matter (wm). Cortical responsiveness to thalamic input differs between states of vigilance. To test if NT can induce such differences the peptide was bath applied to thalamocortical slices. The ventrobasal thalamus was electrically stimulated with two pulses at 50Hz and whole-cell recordings of pyramidal and fast spiking (FS) interneurons were performed in L6 of the somatosensory cortex. Thalamic stimulation reliably evoked UP-states in control conditions. In the presence of NT, however, all spontaneous and evoked network activity was abolished. In addition, the decay slope of the thalamic input was significantly increased during NT application providing greater temporal separation of inputs. Whole-cell recordings were performed to characterize the cells responsive to NT. Single cell responses to NT were rare in most cortical layers. Robust excitation in the form of depolarization and induction of action potentials could, however, be recorded in a subpopulation residing within the wm. In L6, pyramidal cells did not respond to NT but FS cells exhibited a small depolarization and increased synaptic input. These data suggest that NT-ergic regulation of vigilance state involves direct actions on discreet components of the cortical network. These actions may comprise recruitment of wm neurons leading to decorrelation of the cortical network. Decreased global synchrony may facilitate accurate sensory information processing associated with wakefulness.

Disclosures: **L. Case:** None. **C. Broberger:** None.

Poster

535. Somatosensory: Local Cortical Circuits

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Topic: D.09. Tactile/Somatosensory

Support: McKnight Foundation

NIH DA 017188

Title: Layer 2/3 neurons in mouse barrel cortex receive direct synaptic input from the posterior-medial nucleus of the thalamus

Authors: *N. AUDETTE[1], J.-S. JOUHANNEAU[2], J. F. A. POULET[2], A. L. BARTH[3]
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Abstract: Neurons in somatosensory (barrel) cortex respond to stimulation of principal (center) and adjacent (surround) whiskers. Although principal whisker stimulation is an effective stimulus for many neurons, neurons within a column can also respond to stimulation of multiple surround whiskers, and surround responses are not restricted to a single non-principal whisker. The source of inputs that generates multiwhisker, surround responses has been debated: this information could arise through horizontal connections between barrel columns in the cortex, or could be generated directly from thalamic inputs from the posterior medial nucleus of the thalamus (PoM) that exhibit multiwhisker, surround responses. *In vivo* under urethane anaesthesia, layer 2/3 neurons show short-latency subthreshold responses to optogenetic stimulation of PoM. To test whether layer 2/3 neurons receive direct synaptic input from PoM, whole-cell voltage-clamp recordings were carried out during optogenetic stimulation of PoM fibers in acute brain slices. In the presence of the Na channel blocker TTX to block recurrent activity and the K channel antagonist 4-AP to assist in axon depolarization, short-duration (5 ms) light stimulation elicited glutamatergic responses, evidence for PoM synapses onto layer 2 neurons. The strength of PoM input varied across different cell populations within and across layers. Thus, the multiwhisker receptive field properties of PoM neurons recorded *in vivo* may be sufficient to generate surround receptive field responses in neurons from barrel cortex.

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Poster

536. Plasticity After Spinal Cord Injury I

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Topic: D.10. Spinal Cord Injury and Plasticity

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NYS SCI Trust Fund (XYC)

Title: In rats with transection of the dorsal column ascending tract soleus H-reflex conditioning impairs locomotion

Authors: *L. CHEN, Y. CHEN, Y. WANG, J. R. WOLPAW, X. Y. CHEN
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Abstract: In normal rats, right soleus H-reflex (HR) conditioning affects locomotor EMG activity and kinematics but does not disturb key locomotor features (e.g., right/left symmetry in timing and hip height (J Neurosci 25:6898-6906, 2005)). According to the *negotiated equilibrium* hypothesis (Neuroscientist 16:532-549, 2010), these features are preserved through an iterative process, a *negotiation*, in which the old behavior (i.e., locomotion) repeatedly induces compensatory plasticity that preserves its key features despite the plasticity induced by the new behavior (i.e., a larger or smaller H-reflex). Ascending sensory input is thought to guide the brain in inducing this compensatory plasticity. If this is correct, HR conditioning in rats in which transection of the dorsal column ascending tract (DA) has abolished much of this sensory input should disturb key locomotor features. We have tested this prediction. In 18 anesthetized Sprague-Dawley rats, the DA was transected bilaterally at T8-9, EMG electrodes were implanted in both solei, and a stimulating cuff was placed on the right posterior tibial nerve. At least 40 days later, control HR data were collected, and then for 50 days rats were exposed to right soleus HR up- or down-conditioning (HRup and HRdown rats) or continued in the control mode (Control rats). Locomotor EMG activity, H-reflexes, and kinematics were assessed before and after the 50 days. The results show: (1) DA transection itself does not disturb key locomotor features; (2) In DA rats as in normal rats, prolonged control-mode exposure does not affect the HR or locomotion, and HR up- or down-conditioning increases or decreases, respectively, the HR in the conditioning protocol and during locomotion and the right locomotor burst; (3) Unlike in normal rats, HR up- or down-conditioning in DA rats causes right/left asymmetry in locomotor timing and hip height. Thus, HR conditioning in DA rats disrupts key features of locomotion. These results are consistent with the prediction of the negotiated equilibrium hypothesis that when a new behavior (e.g., a larger or smaller HR) changes spinal neurons and synapses that also serve an old behavior (e.g., locomotion), the compensatory plasticity that

preserves the key features of the old behavior is guided by sensory input to the brain. When DA transection has removed much of this input, HR conditioning produces abnormal (i.e., asymmetrical) locomotion. A related abstract (Y Chen et al) addresses the locomotor impact of DA transection after HR conditioning has occurred.

Disclosures: L. Chen: None. **Y. Chen:** None. **Y. Wang:** None. **J.R. Wolpaw:** None. **X.Y. Chen:** None.

Poster

536. Plasticity After Spinal Cord Injury I

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Topic: D.10. Spinal Cord Injury and Plasticity

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NS22189(JRW)

NS061823(XYC&JRW)

HD032571(AWE)

NYS SCI Trust Fund (XYC)

Title: Impact of dorsal column ascending tract transection on locomotion after successful H-reflex conditioning in rats

Authors: *Y. CHEN, L. CHEN, R. L. LIU, Y. WANG, J. R. WOLPAW, X. Y. CHEN
Lab. Neural Injury and Repair, Wadsworth Ctr, NYS Dept Hlth. & SUNY, ALBANY, NY

Abstract: In normal rats, right soleus H-reflex (HR) conditioning affects locomotor EMG activity and kinematics but does not disturb key locomotor features (e.g., right/left symmetry in step-cycle timing and hip height (J Neurosci 25:6898-6906, 2005)). According to the *negotiated equilibrium* hypothesis (Neuroscientist 16:532-549, 2010), key features are preserved through an iterative process, a *negotiation*, in which the old behavior (i.e., locomotion) repeatedly induces compensatory spinal cord plasticity that preserves its key features despite the plasticity induced by the new behavior (i.e., a larger or smaller HR). Ascending locomotor sensory input guides the brain in inducing this compensatory plasticity. The hypothesis is supported by our recent finding

that, in rats in which dorsal column ascending tract (DA) transection has eliminated much ascending locomotor sensory input, HR conditioning disrupts key locomotor features (L Chen et al. abstract). We are now studying the locomotor impact of DA transection after HR conditioning has occurred. In anesthetized Sprague-Dawley rats, EMG electrodes are implanted in both solei, and a stimulating cuff is placed on the right posterior tibial nerve. At least 30 days later, control HR data are collected, and rats are exposed to right soleus HR up- or down-conditioning (HRup or HRdown) for 50 days. The DA is then transected bilaterally at T8-9 under anesthesia, and up- or down-conditioning continues for 50 more days. Locomotor EMG activity, H-reflexes, and kinematics are assessed before conditioning, just before DA transaction, and 50 days later. The initial results are consistent with previous data indicating that HR conditioning in normal rats does not disrupt key locomotor features; and they suggest that, when the DA is transected and conditioning continues, key locomotor features are disturbed. Confirmation of these early results by further data would provide additional support for the hypothesis that ascending sensory input is essential for inducing the compensatory plasticity in the spinal cord that ensures preservation of an old behavior (i.e., locomotion) when a new behavior (i.e., a larger or smaller HR) changes the spinal cord.

Disclosures: Y. Chen: None. **L. Chen:** None. **R.L. Liu:** None. **Y. Wang:** None. **J.R. Wolpaw:** None. **X.Y. Chen:** None.

Poster

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Topic: D.10. Spinal Cord Injury and Plasticity

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HD032571(AWE)

NYS SCI Trust Fund (XYC)

Title: Inferior olive to cerebellum to sensorimotor cortex to spinal cord: A hierarchy of plasticity probably underlies down-conditioning of the H-reflex

Authors: *X. Y. CHEN, Y. CHEN, L. CHEN, Y. WANG, J. R. WOLPAW
Lab. Neural Injury and Repair, Wadsworth Ctr, NYS Dept Hlth. & SUNY, ALBANY, NY

Abstract: Monkeys, humans, rats, and mice can gradually increase or decrease the soleus H-reflex (HR) in response to an operant conditioning protocol. This simple motor skill is associated with multi-site plasticity in the spinal cord and brain. Physiological and anatomical studies, primarily in down-conditioned animals, indicate that this plasticity operates as a hierarchy in which plasticity in the brain guides and maintains plasticity in the spinal cord that is directly responsible for the HR change. These studies (recently reviewed in Front Integr Neurosci (doi: 10.3389/fnint.2014.00025)) suggest that acquisition and maintenance of an operantly conditioned decrease in the HR may occur as follows: 1: Before conditioning, sensorimotor cortex (SMC) produces continually varying corticospinal tract (CST) activity that excites GABAergic interneurons (INs) in the ventral horn that have terminals on soleus motoneurons (MNs). Via $GABA_B$ receptors, this input affects voltage-gated Na channels in the MN membrane and thereby affects MN firing threshold, which affects the probability that the primary afferent EPSP (which is largely responsible for the HR) will excite the MN to contribute to the HR. CST collaterals provide efference copy input to the cerebellum (CB) via mossy fibers (MFs). 2: With the onset of down-conditioning, the reward network in the cerebrum sends a reward/no reward signal to the inferior olive (IO), which produces corresponding climbing fiber (CF) input to the CB (and/or CB nuclei). 3: The conjunction of MF input (reflecting the CST activity) and CF input (indicating whether reward occurred) induces CB plasticity. This plasticity can survive ~7 days without the IO input. 4: The CB plasticity produces input to SMC that induces plasticity that increases the probability of CST activity that leads to a positive shift in MN firing threshold and thus reduces the HR. This plasticity can survive ~40 days without the CB input. 5: The altered CST activity induces plasticity in the GABAergic INs and their terminals and in MN Na channels that results in the positive shift in MN firing threshold that reduces the HR and increases reward probability. This spinal cord plasticity can survive 5-10 days without the CST activity. In sum, the data support the hypothesis that the reward contingency produces IO input to the CB that combines with CST-induced MF activity to induce and maintain CB plasticity that induces and maintains SMC plasticity that induces and maintains spinal GABAergic IN plasticity that induces and maintains MN plasticity that is directly responsible for the smaller HR. Similar hierarchies may underlie other motor learning that involves spinal cord plasticity.

Disclosures: X.Y. Chen: None. **Y. Chen:** None. **L. Chen:** None. **Y. Wang:** None. **J.R. Wolpaw:** None.

Poster

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Topic: D.10. Spinal Cord Injury and Plasticity

Support: HD36020(XYC)

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NS061823(XYC&JRW)

HD032571(AWE)

NYS SCI Trust Fund (XYC)

Title: H-reflex conditioning appears to affect motoneuron axon initial segment size and voltage-gated sodium channel labeling

Authors: *Y. WANG, L. CHEN, Y. CHEN, J. R. CHEN, X. Y. CHEN
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Abstract: Monkeys, humans, rats, and mice can gradually increase or decrease H-reflex (HR) size in response to an operant conditioning protocol (Encyclopedia of Neuroscience 7:225-233, 2009 & Front Integr Neurosci (doi: 10.3389/fnint.2014.00025) for review). HR down-conditioning is associated with a positive shift in motoneuron firing threshold (and a decrease in axonal conduction velocity) that could reflect a change in voltage-gated sodium (Na_v) channels in the motoneuron membrane (J Neurophysiol 74:867-871, 1995). To further evaluate this possibility, we are exploring the effects of HR conditioning on axon initial segment (AIS) size (measured as AIS length and hillock diameter) and on AIS Na_v content. Rat soleus motoneurons are labeled by retrograde transport of a fluorescent tracer; and AIS size and Na_v content are determined by blinded assessment of immunofluorescent labeling of the anchored protein ankyrin G (ANK3) and Na_v channels, respectively. To date, four groups of rats have been studied: rats in which down-conditioning has reduced the H-reflex by ≥20% (successful HRdown) (n=9); rats in which down-conditioning has not reduced the H-reflex by ≥20% (failed HRdown) (n=6); rats in which up-conditioning has increased the H-reflex by ≥20% (successful HRup) (n=7); and unconditioned (naïve control) rats (n=7). Analysis of these initial results (i.e., ANOVA followed by Tukey Test) indicates that: (1) In successful HRdown rats, AIS hillock diameter and Na_v labeling are significantly less ($p<0.001$ and $p<0.0001$, respectively) than in naïve rats. (2) In failed HRdown rats, AIS length, hillock diameter, and Na_v labeling are not significantly different ($p>0.06$ for all) from those of naïve rats. (3) In successful HRup rats, AIS length is significantly greater ($p<0.01$) than in naïve rats. These initial results may provide new insight into the mechanisms directly underlying operantly conditioned decrease in H-reflex size. Their connections to the corticospinal tract activity that is essential for acquisition and

maintenance of down-conditioning, and to the increases in GABAergic interneurons in the ventral horn and GABAergic terminals on motoneurons that are associated with successful down-conditioning (reviewed in Encyclopedia of Neuroscience 7:225-233, 2009 & Front Integr Neurosci (doi: 10.3389/fnint.2014.00025)), remain to be defined.

Disclosures: Y. Wang: None. **L. Chen:** None. **Y. Chen:** None. **J.R. Chen:** None. **X.Y. Chen:** None.

Poster

536. Plasticity After Spinal Cord Injury I

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Topic: D.10. Spinal Cord Injury and Plasticity

Support: NS22189 (JRW)

NIH (HD36020 (XYC)

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NIH HD032571 (AWE)

NYS SCI Trust Fund (XYC)

Title: Long-term single-neuron data from behaving rats undergoing operant conditioning of the H-reflex: Initial data

Authors: *J. R. WOLPAW, W. T. BAXTER, Y. CHEN, J. S. CARP, X. Y. CHEN
Lab. Neural Injury & Repair, Wadsworth Ctr, NYS Dept Hlth., ALBANY, NY

Abstract: H-reflex (HR) operant conditioning changes the spinal cord and brain (Front Integ Neurosci (doi: 10.3389/fnint.2014.00025) for review). It depends on the corticospinal tract, and thus on neuronal activity in sensorimotor cortex (SMC). To define this activity and explore corticospinal interactions during conditioning, we have developed automated methods for recording and analyzing single-neuron and local field potential (LFP) data from rat SMC 24 hr/day throughout one or more exposures to HR conditioning. Sprague-Dawley rats are implanted under anesthesia with EMG electrodes in right soleus, a stimulating cuff on right posterior tibial nerve, and a 32-microwire array (2.1x0.9 mm; 300-μm interelectrode spacing)

fixed to the skull over left hindlimb area of SMC and extending 1.3-1.5 mm into cortex. EMG and nerve-cuff wires pass subcutaneously to a flexible head-mounted tether and through a commutator to amplifiers and stimulator; and EMG activity is stored (5 kHz). The array wires pass to a head-mounted preamplifier/digital-multiplexer (Blackrock), the output of which passes through the tether and commutator to a digital demultiplexer and then to a Cerebus NSP unit (Blackrock) for online visualization and to a computer for storage (30 kHz). The tether, which allows the rat to move freely about the cage, is in place continuously. Beginning 10-15 days after implantation, whenever EMG activity remains in a given range for 2.3-2.7 s, nerve stimulation just above M-response threshold elicits the HR. In control mode (first 10-20 days), no reward occurs. In HRup or HRdown mode (next 50 days), reward occurs when HR size is > or < criterion, respectively. The rat may then be switched to the opposite mode for 50 more days. EMG and cortical data from 0.5 s pre- to 0.2 s post-stimulation are stored. To date, data have been collected from 8 rats exposed to the HRup or HR down mode and 0-3 reversals over 88-297 days. While action potentials (spikes) are most frequent and largest in the first 2-4 weeks, they are generally detectable throughout data collection. After 400-5000 Hz bandpass filtering, trials in which pre-stimulus RMS averages 4-40 μV are analyzed for spikes, which are defined as negative deflections 0.1-0.5 ms wide and ≥5 times the average trial RMS. We are determining pre-stimulus firing rates and post-stimulus responses, their correlations with concurrent background EMG level and H-reflex size, and the relationships among these parameters; and we are assessing the impact on these measures of HRup or HRdown conditioning. The results should help to illuminate the role of SMC in acquisition and maintenance of this simple motor skill (i.e., a larger or smaller H-reflex).

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Poster

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Topic: D.10. Spinal Cord Injury and Plasticity

Support: Villum Kann Rasmussen Fonden (DK)

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Title: Trial-to-trial and session-to-session variability in the human soleus stretch reflexes

Authors: *Y. MAKIHARA[1,2], P. P. SILVA[2], L. ARENDT-NIELSEN[2], A. K. THOMPSON[3,4,5,6], N. MRACHACZ-KERSTING[2]
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Abstract: The purpose of this study was to systematically examine the variability of soleus (SOL) spinal stretch reflex (SSR) size within and across days in neurologically normal subjects. First, we evaluated the variability in the SSR size in 12 different stretch conditions using coefficient of variation (CV). Then, with one of the 12 stretch conditions, the SSR was measured at 4 different times within a 24-hour period (8:00, 12:00, 16:00, and 20:00) and across 4 different days at the same time of day. All the measurements were made while the sitting subject maintained a fixed stable level of SOL and tibialis anterior background EMG. At the beginning of each session, a recruitment curve of SOL H-reflex and M-wave was obtained. Then, for the first part of the study, twenty SOL SSRs were elicited in each of the 12 stretch conditions (4 deg of dorsiflexion at a velocity of 150 deg/sec, 6 deg at 75, 150, 200, 250, and 280 deg/sec, 8 deg, 10 deg, and 12 deg at 200 and 250 deg/sec), using a custom joint perturbation device. The mean rectified values were obtained for first ("M1") and second ("M2") components of the SSR. M1 is originated primarily from group Ia afferents and typically occurs 46-57ms after the onset of perturbation; M2 is presumably mainly mediated by group II afferents, and typically occurs 59-78ms after perturbation onset. The M1 and M2 SSR sizes were normalized by the maximum M-wave size, and the mean and CV were calculated for each condition. For the second part, the evaluation of the within- and across-day variability, the SSR was elicited using 6 deg perturbation at 250 deg/sec. Twenty responses were averaged together to calculate the average M1 and M2 SSR sizes for each time or day. We found that the M1 with 6 deg perturbation at 200, 250, and 280 deg/sec was significantly larger that that with 6 deg at 75 deg/sec. The M1 with 6 deg at 280 deg/sec was significantly larger than that with 6 deg at 150 deg/sec. The CVs of 6 deg at 150, 200, 250, and 280 deg/sec were significantly smaller than that of 6 deg at 75 deg/sec. The CV of 6 deg at 280 deg/sec was significantly smaller than that of 6 deg at 150 deg/sec. M2 did not present any significant size or variability differences among tested conditions. There was no difference in the extent of M1 or M2 size variation within- or across-days ($p>0.05$, 2-way ANOVA); the mean CVs of 6 deg perturbation at 250 deg/sec over 8 measurement times were 0.21(±0.01) for M1 and 0.40(±0.02) for M2. The present study shows that SOL SSR sizes do not change systematically within- or across-days. It also suggests that a stretch parameter should include ≥6 deg of dorsiflexion at a ≥200 deg/sec to elicit reliable SOL SSRs. This study provides a framework for the evaluation of human SOL SSRs.

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Poster

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Topic: D.10. Spinal Cord Injury and Plasticity

Support: NIH RO1 NS054025

NIH RO1 NS080180-01A1

Title: Phrenic motoneuron loss and diaphragm function following cervical spinal cord injury

Authors: *L. N. LITTLE[1], S. HUSSEY[1], E. E. GONZALEZ-ROTHI[2], L. M. MERCIER[1], D. D. FULLER[2], M. A. LANE[1], P. J. REIER[1], P. J. REIER[1]
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Abstract: White matter injury and repair is a dominant emphasis in spinal cord injury (SCI) research. In contrast, the functional contributions of gray matter damage to post-SCI outcomes, especially at cervical or lumbar enlargements, are less well understood. We and others have described respiratory deficits following cervical contusions at the level of the phrenic nucleus (C3-C5/6) which could be attributed to a significant loss of phrenic motoneurons (PhMNs) by 24hrs post-injury (p.i.). To determine the potential for using a neuroprotective treatment, the initial goal of this study was to determine the early time-course of PhMN loss. Lateralized C3/4 spinal contusions were performed on adult, female Sprague-Dawley rats with the Infinite Horizon pneumatic impactor preset to 200 kilodynes (i.e., moderate injury). Ipsilateral PhMN cell counts were obtained at 0, 4, 8 and 24 hours after injury by retrograde labeling with Cholera Toxin β applied to the corresponding hemidiaphragm. Immediately after injury, there is an ~15% loss of PhMNs with no further significant decline thereafter. This result demonstrates an absence of secondary PhMN degeneration during the first 24hrs post-injury. Other published data also suggest minimal PhMN loss between 24 and 6wks (p.i.). The second goal of this study is to establish a model of restricted ventral gray matter damage at the level of the phrenic nucleus without concomitant direct white matter involvement. The antimetabolite, 6-aminonicotinamide (6-AN), has been shown to cause initial astrocyte toxicity and considerable destruction of spinal ventral gray matter. Intraspinal injection of 6-AN (0.025 M) was performed at the level of C3/4. Preliminary histological examination revealed loss of neurons, including cells in the region of the PhMN pool, within the targeted ventral horn 2 weeks later. Ventilatory data are currently being obtained to test the hypothesis that a focal gray matter lesion alone can contribute to respiratory insufficiency.

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Poster

536. Plasticity After Spinal Cord Injury I

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Topic: D.10. Spinal Cord Injury and Plasticity

Support: NIH 1R01NS080180-01A1 (DDF)

State of Florida Brain and Spinal Cord Injury Research Program (DDF, PJR)

Title: Electrical stimulation of the diaphragm following cervical spinal cord injury in rats

Authors: *K. A. STREETER[1], E. J. GONZALEZ-ROTHI[1], G. FITZPATRICK[1], G. ARMSTRONG[1], P. J. REIER[2], E. J. FOX[1], D. D. FULLER[1]
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Abstract: Respiratory insufficiency and complications are the leading causes of death following cervical spinal cord injury (SCI), presenting a large clinical problem within the human population. The traditional approach for managing severe hypoventilation post-SCI is mechanical ventilation (MV). However, an emerging therapy utilizing direct diaphragm pacing via intramuscular electrical stimulation appears to offers advantages over MV. Indeed, pacing is highly likely to mitigate diaphragm muscle atrophy and result in improved quality of life. However, a careful study examining the impact of diaphragm pacing on respiratory motor output and if diaphragm pacing can provide a neurorehabilitative impact following SCI, is lacking. Thus, we aimed to: 1) develop an anesthetized rat model of diaphragm pacing, and 2) examine the impact of diaphragm pacing on respiratory motor output following incomplete SCI. Initial results established the feasibility of direct diaphragm stimulation in rats in phase with respiratory activity (by triggering of off inspiratory activity) and optimized pacing parameters (frequency of 40Hz; stimulation intensity 1-15MA). To test the hypothesis that direct diaphragm stimulation restores activity to the paralyzed hemi-diaphragm and intercostals following C2 spinal hemi-section (C2Hx), rats were studied at 1 or 6 weeks post-C2Hx. At each time point rats were anesthetized and ventilated and bilateral diaphragm and intercostal (T2/T3) EMG activity was recorded. Our preliminary results indicate that intermittent diaphragm pacing while being ventilated (3 x 10 min pacing -in phase with respiratory activity-separated by 10 min no pacing)

at 1 week post-C2Hx induces a small, but highly variable recovery of left diaphragm and intercostal EMG activity compared to controls not receiving intermittent pacing. However, at 6 weeks post-C2Hx, intermittent diaphragm pacing elicits a robust recovery of left diaphragm and intercostal EMG activity while right diaphragm and intercostal EMG activity remains relatively unaffected. Together these results indicate that direct diaphragm pacing has the potential to influence the neural control of respiratory muscles following cervical SCI.

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Poster

536. Plasticity After Spinal Cord Injury I

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Support: NIH 1R01NS080180-01A1 (DDF)

State of Florida Brain and Spinal Cord Injury Research Program (DDF and PJR)

NIH Diversity Supplement NS80180 (EGR)

Title: Impact of high frequency epidural stimulation on respiratory function following incomplete cervical spinal cord injury

Authors: *E. J. GONZALEZ-ROTHI[1], S. M. TURNER[1], K. A. STREETER[1], G. M. FITZPATRICK[1], P. J. REIER[2], D. M. BAEKEY[3], D. D. FULLER[1]
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Abstract: Injury to the cervical spinal cord disrupts descending bulbospinal pathways innervating the phrenic motor pool (C3-C5/6), resulting in diaphragm paralysis and impaired ventilatory function. High frequency epidural stimulation (HF-ES) is a promising therapy for activating paralyzed muscles after spinal cord injury (SCI), and can effectively drive the phrenic motor system in a physiologic respiratory pattern following spinal transection. However, the impact of HF-ES on phrenic motor output has not been examined in cases of incomplete spinal lesion. This is an important translational consideration, as incomplete SCIs are far more common clinically. We hypothesize that thoracic HF-ES will enhance respiratory motor output when descending drive to the phrenic motor pool is partially spared. Thus, in ongoing studies, we are

using our established rodent model of cervical SCI (C2 hemisection) to evaluate the effects of thoracic (T2) HF-ES on respiratory motor output. In terminal neurophysiology experiments, phrenic and hypoglossal motor output were recorded from anesthetized, vagotomized, and mechanically ventilated rats at 2 or 12 weeks post-injury. Using a bipolar silver wire electrode, short duration (60s) bouts of HF-ES (300Hz) were administered to the ventrolateral spinal cord (ipsilateral to the side of injury). Preliminary results indicate that HF-ES enhances both tonic and phasic bursting in the ipsilateral phrenic nerve. The magnitude of this response correlates with the intensity of stimulation, and facilitation of medullary (hypoglossal) output occurs only high stimulation intensities. These initial results indicate that HF-ES can increase phrenic motor output after incomplete cervical SCI. This approach may be paired with conventional respiratory rehabilitation approaches or with pharmacological interventions to improve respiratory function in individuals with chronic cervical SCI.

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Poster

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State of Florida Brain and Spinal Cord Injury Research Program (DDF)

Canadian Institutes for Health Research (JJG)

Title: Ampakine administration potentiates intermittent hypoxia-induced long-term facilitation in mice

Authors: *S. TURNER[1], M. K. ELMALLAH[1], E. J. GONZALEZ-ROTHI[1], J. GREER[2], D. FULLER[1]
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Abstract: Respiratory plasticity is a persistent change in the neural control system based on prior experience which may involve structural and/or functional alterations arising from mechanisms distributed throughout the respiratory control system. One well-studied model of respiratory

plasticity is long-term facilitation (LTF). LTF is triggered by intermittent hypoxia and induces a long-lasting (>60 min post-hypoxia) increase in respiratory motor output. More recent work indicates that hypoxic exposure paradigms and LTF have rehabilitative value in conditions associated with respiratory compromise (e.g., spinal cord injury). We are investigating if a novel pharmacological approach can enhance the functional impact of intermittent hypoxia on respiratory motor activity. Ampakines are compounds that have been used in clinical trials to stimulate breathing during conditions associated with impaired respiratory neuromotor activity. Ampakines selectively enhance function of glutamatergic AMPA receptors, and these receptors are widely distributed in brainstem and spinal respiratory control networks. In ongoing experiments we are using an anesthetized mouse model to test the hypothesis that ampakine pretreatment can enhance the functional impact of intermittent hypoxia on respiratory motor output. Preliminary results suggest that intermittent hypoxia (3 x 1 min hypoxia, 10% O_2; separated by 3 min of hyperoxia, 50% O_2) enhances respiratory motor output 60 min post-hypoxia compared to time controls receiving a similar surgery but not experiencing intermittent hypoxia. Delivery of ampakine CX717 (15mg/kg) 10 min prior to intermittent hypoxia considerably increases the magnitude of LTF. These preliminary data suggest that ampakines may synergistically increase respiratory motor output following intermittent hypoxia.

Disclosures: S. Turner: None. **M.K. ElMallah:** None. **E.J. Gonzalez-Rothi:** None. **J. Greer:** None. **D. Fuller:** None.

Poster

536. Plasticity After Spinal Cord Injury I

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 536.11/JJ15

Topic: D.10. Spinal Cord Injury and Plasticity

Support: NIH Grant NINDS R01-NS08111

Drexel University College of Medicine

Title: Supraspinal respiratory neuroplasticity within reticular nuclei following cervical spinal cord injury

Authors: *T. BEZDUDNAYA, V. MARCHENKO, T. J. WHELAN, M. A. LANE
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Abstract: Spontaneous respiratory recovery after spinal cord injury (SCI) has been reported in a multitude of studies, demonstrating the neuroplastic potential of the respiratory network. These changes appear throughout the entire central and peripheral nervous system. However, the extent of respiratory recovery is minor and long-term deficits persist post-SCI. Understanding the mechanisms of recovery is an important step in developing new therapeutic strategies to improve breathing following cervical SCI. The present work attempts to elucidate the anatomical and functional changes that occur within medulla that are associated with respiration following high cervical SCI. Specific focus is on the phrenic motor system that mediates diaphragm activity, using the adult Sprague-Dawley rat. To first define the spinal and supraspinal circuitry associated with phrenic function, a retrograde transynaptic tracer (pseudorabies virus; PRV) was applied to the hemi-diaphragm, and animals perfuse-fixed 60-72 hours later. Analysis of tissue revealed neuronal labelling throughout the spinal cord, brainstem and brain. The earliest labelling within brainstem was seen within the raphe and reticular nuclei suggesting a close synaptic integration with spinal phrenic circuitry. For electrophysiological studies we used decerebrate, unanesthetized and artificially ventilated rats. We systematically mapped medulla for the presence of inspiratory and expiratory activity. To examine the extent of supraspinal plasticity following cervical SCI, animals received a C2 lateral hemisection (C2Hx). This injury disrupts descending bulbospinal and ascending spinobulbar pathways between the medulla and spinal phrenic neurons, resulting in immediate hemidiaphragm paralysis. Acute partial recovery of ipsilateral phrenic activity was observed at 4-6 hours after C2Hx. In injured animals, electrophysiological mapping was conducted once recovery of ipsilateral phrenic nerve activity reached a plateau. These experiments revealed an increase in respiratory activity within the reticular nuclei after C2Hx. Ongoing studies are using retrograde and anterograde tracing methods to define connectivity between respiratory neurons in the medulla and the spinal cord. The recruitment of respiratory activity within reticular nuclei may indicate their involvement with post-injury respiration and neuroplastic changes within respiratory network after cervical spinal cord injury.

Disclosures: T. Bezdudnaya: None. **V. Marchenko:** None. **T.J. Whelan:** None. **M.A. Lane:** None.

Poster

536. Plasticity After Spinal Cord Injury I

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Topic: D.10. Spinal Cord Injury and Plasticity

Support: NIH Grant R01NS076589

VA Grant 3397626

Title: Assessment of sensory function after human spinal cord injury

Authors: *R. A. MACKLIN[1], P. H. ELLAWAY[2], M. A. PEREZ[1]
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Abstract: The current gold standard for assessing sensory function in humans with spinal cord injury (SCI) is the International Standards for Neurological Classification of Spinal Cord Injury (ISNCSCI). However, increasing evidence supports the view that new and more sensitive quantitative outcome measurements of sensory function are needed to supplement the ISNCSCI. The purpose of our study was to compare sensory deficits revealed by the ISNCSCI and by the sensory electrical perceptual threshold (EPT) test in individuals with chronic incomplete cervical SCI. The ISNCSCI examines responses of cutaneous afferents to light touch and pin prick and the EPT examines cutaneous sensitivity to electrical stimulation. During EPT testing, electrical stimulation was delivered using a constant current high voltage electrical stimulator (model DS7A, Digitimer, Ltd.) at a repetition rate of 3 Hz (monophasic pulses of 0.5 ms duration). Threshold was defined as lowest stimulus intensity at which subjects reported sensation in dermatomes C2 to T4. In age-matched controls, we found that female subjects required less stimulus intensity compared to males, regardless of age and the dermatome tested. Older males required more stimulus intensity that younger males in all conditions tested. In SCI participants, the EPT test detected asymmetries between left and right dermatomes in all patients tested, which were not revealed using the ISNCSCI. Importantly, the EPT test revealed deficits between 2 to 5 segments lower than what was found with the ISNCSCI examination. Our findings highlight the need of combining ISNCSCI scores with additional outcome measurements to increase the sensitivity of sensory testing after human SCI.

Disclosures: R.A. Macklin: None. **P.H. Ellaway:** None. **M.A. Perez:** None.

Poster

536. Plasticity After Spinal Cord Injury I

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

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Topic: D.10. Spinal Cord Injury and Plasticity

Support: PVA Research Foundation Grant 2955

NIH Grant R01NS076589

VA Grant 3397626

Title: Reduced motor cortical maps during voluntary activity after incomplete spinal cord injury

Authors: *T. TAZOE, M. A. PEREZ
Dept. of Physical Med. and Rehabil., Ctr. for the Neural Basis of Cognition, Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Motor cortical representations are reorganized after spinal cord injury (SCI). However, the extent to which voluntary muscle contraction influences the reorganization of muscle representations in motor cortex after SCI is unknown. Here we examined motor cortical maps in distal and proximal upper limb muscles in patients with chronic incomplete cervical SCI and age-matched controls at rest and during ~5% of isometric maximal voluntary contraction (MVC). Transcranial magnetic stimulation was guided by the Brainsight Frameless Nagivation system to acquire cortical motor maps of the first dorsal interosseous (FDI) and biceps brachii (BB) muscles. At rest, SCI patients exhibited larger cortical motor maps of both FDI and BB compared with controls. The overlap between the FDI and BB motor maps was also larger in SCI patients than controls. We found that the area of cortical motor maps was not affected by voluntary muscles contraction in controls. In contrast, in SCI patients, the areas of individual FDI and BB motor maps and their overlap were decreased during 5% of MVC compared to rest. In either group, the center of gravity of the motor maps was not different at rest or at 5% of MVC regardless of the muscle tested. Our findings demonstrate that a different form of reorganization takes place in motor cortical representations of upper-limb muscles at rest and voluntary activity after incomplete SCI. We speculate that the reduced size of motor cortical maps during voluntary activity may represent maladaptive plasticity during a motor behavior.

Disclosures: T. Tazoe: None. **M.A. Perez:** None.

Poster

536. Plasticity After Spinal Cord Injury I

Location: Halls A-C

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Topic: D.10. Spinal Cord Injury and Plasticity

Support: NIH Grant R01NS076589

VA Grant 3397626

Title: Impaired corticospinal excitability during inhibition of voluntary movement after tetraplegia

Authors: *P. FEDERICO, M. A. PEREZ
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Abstract: The ability to stop voluntary movement is impaired in individuals with incomplete spinal cord injury (SCI). The extent to which the corticospinal system is involved in this process after SCI remains unknown. Here, we tested patients with chronic (≥1 year) incomplete cervical (C4 to C7) SCI and uninjured age-matched controls during a GO and NOGO task. Using transcranial magnetic stimulation we examined motor evoked potentials (MEPs) in the first dorsal interosseous (FDI) muscle during 5-10% of maximal voluntary contraction into index abduction and instructed by a visual stimulus on a computer screen to increase voluntary activity with the index finger as fast as possible (GO trial - green light) or not at all (NOGO trial - red light). During GO trials, index finger reaction time was shorter in controls (303.2 ± 48.6 ms) compared to patients (372.8 ± 54.4 ms) at matched levels of background electromyographic activity. The number of errors during the task did not differ between patients (2.9 ± 2.7 %) and controls (1.7 ± 1.1 %). Importantly, we found that the size of MEPs in the FDI muscle decreased significantly during NOGO compared to GO trials in control subjects but to a lesser extent in SCI patients. Thus, our findings indicate that the ability to suppress corticospinal drive during an ongoing voluntary visuomotor task is impaired in humans with SCI. Since changes in MEP size might involve cortical and subcortical mechanisms it is possible that impaired modulation at these levels contributed to our results.

Disclosures: P. Federico: None. **M.A. Perez:** None.

Poster

536. Plasticity After Spinal Cord Injury I

Location: Halls A-C

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Topic: D.10. Spinal Cord Injury and Plasticity

Support: PVA Research Foundation Grant 2968

NIH Grant R01NS076589

VA Grant 3397626

Title: Temporal pattern of corticospinal volleys influences motor function after tetraplegia

Authors: *J. CIRILLO, F. J. CALABRO, M. A. PEREZ
Dept. of Physical Med. and Rehabil., Ctr. for the Neural Basis of Cognition, Univ. of Pittsburgh, Pittsburgh, PA

Abstract: How do corticospinal volleys descend from the cerebral cortex and reach spinal motoneurones in humans with incomplete spinal cord injury (SCI)? In an intact system, a single shock over the primary motor cortex evokes temporally organized descending volleys in the corticospinal tract, which can generate a burst of activity in spinal motoneurons. Using noninvasive cortical stimulation, we measured indirect (I) wave corticospinal volleys targeting an intrinsic finger muscle in surface electromyographic recordings in humans with chronic anatomically incomplete SCI. We demonstrate that corticospinal discharge frequency and amplitude decreased in all corticospinal volleys after SCI. Late but not early corticospinal volleys had increased latencies and duration, which correlated with the magnitude and onset of hand voluntary motor output. Our results for the first time identify deficits in specific I-wave corticospinal volleys after SCI, which influence residual voluntary motor output. We argue that dysfunction of these volleys may underlie some functional deficits in motor disorders affecting corticospinal tract function.

Disclosures: J. Cirillo: None. **F.J. Calabro:** None. **M.A. Perez:** None.

Poster

536. Plasticity After Spinal Cord Injury I

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Topic: D.10. Spinal Cord Injury and Plasticity

Support: NIH Grant 1R01-NS069568-01A1

NIH Grant 1F32NS084680-01A1

Title: Molecular adaptations in the periaqueductal gray in a rat model of chronic neuropathic pain: Focus on dopamine D1 receptors

Authors: *P. J. VOULALAS, Y. JI, L. JIANG, R. MASRI
Div. of Prosthodontics, Univ. of Maryland Sch. of Dent., Baltimore, MD

Abstract: Spinal cord injury (SCI) in humans often leads to the development of chronic neuropathic pain, due to persistent changes in brain areas mediating the behavioral, sensory, and affective components of pain. We have used a rat model of neuropathic pain to investigate molecular alterations in the periaqueductal gray (PAG) following SCI. We hypothesized that chronic neuropathic pain is associated with maladaptive changes in receptor expression and phosphorylation in the PAG. Male Sprague-Dawley rats were subjected to SCI or sham surgery. Electrolytic lesions to the spinal cord (10 μA DC pulses, 4 X 10s) were at C6, designed to lesion parts of the spinothalamic tract. Von Frey filaments and the Rat Grimace Scale were used to assess pain-like behaviors in animals following surgery. At 21 days post SCI or sham surgery, rats were sacrificed and the PAG isolated from 2 mm punches from frozen sections. Semi-quantitative western blot analysis was conducted on cytoplasmic and membrane fractions isolated from PAG extracts to assess changes in the expression of the following proteins: Glutamate receptor type A, subunit 1 (GluA1), phosphorylated GluA1 at serine 831 (GluA1-pS831), cannabinoid receptor 1 (CB1), metabotropic glutamate receptor 5 (mGlu5), phosphorylated ERK2 (pERK2), protein phosphatase 1 (PP1), and D1 dopamine receptor (D1). Data were statistically analyzed using ANOVA: $p < 0.5$ was considered significant. Immunoblot analysis revealed a significant decrease in synaptosomal GluA1 protein in SCI rats compared to Shams (Sham: 100 ± 7; SCI: 64 ± 2; $p = 0.003$; $n = 4$), with GluA1-pS831 significantly elevated in the same SCI rats ($p = 0.02$). There were no detectable changes in CB1 or mGlu5 protein, but D1 levels were reduced 3-fold in SCI rats compared to Shams (Sham: 100 ± 16; SCI: 33 ± 4; $p = 0.017$; $n = 7$). In addition, two mediators of D1 signaling exhibited different expression profiles following SCI. While pERK2 levels were unchanged ($p = 0.32$; $n=4$), PP1 levels were significantly increased after SCI, compared to Shams ($p = 0.0004$; $n = 4$). These results suggest that glutamatergic and dopaminergic neurotransmission in the PAG may be compromised due to reduced expression of D1 and GluA1 following SCI. Since dopaminergic and glutamatergic neurotransmission increases activity of output neurons in the PAG and enhances descending inhibition, we hypothesize that this reduction in D1 and GluA1 may contribute to suppression of descending pain pathways, and the emergence of pain-like behaviors in SCI animals. These results derived from a rat model of neuropathic pain are predicted to advance our mechanistic understanding of neuropathic pain, facilitating development of therapies for humans.

Disclosures: P.J. Voulalas: None. **L. Jiang:** None. **Y. Ji:** None. **R. Masri:** None.

Poster

536. Plasticity After Spinal Cord Injury I

Location: Halls A-C

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Topic: D.10. Spinal Cord Injury and Plasticity

Support: NIH Grant 1R01-NS069568-01A1

NIH Grant 1F32NS084680-01A1

Title: Post-translational modifications of cortical GluA1 receptors in a rat model of spinal cord injury pain

Authors: *L. JIANG[1], P. VOULALAS[1], Y. JI[1], R. MASRI[1,2]
[1]EPOD, Univ. of Maryland Dent. Sch., Baltimore, MD; [2]Anat. and Neurobio. Dept., Univ. of Maryland Med. Sch., Baltimore, MD

Abstract: Injury to the spinal cord (SCI) is devastating and leads to catastrophic consequences including the development of chronic neuropathic pain (SCI-pain). Previous studies suggested that SCI is associated with maladaptive plasticity not only at the site of injury (spinal segments) but also in supraspinal structures (thalamus and cerebral cortex) involved in pain processing. Little is known about the molecular and cellular mechanisms leading to maladaptive plasticity in the cortex and how they contribute to the development of SCI-pain. Here, we study changes in AMPA-type glutamate receptor (GluAR) expression in conditions of SCI-pain. We hypothesize that SCI-pain results from *maladaptive homeostatic plasticity caused by post-translational modifications of GluARs in the primary somatosensory cortex (S1).* To test our hypothesis, we used an animal model of SCI-pain and injured the anterolateral quadrant of the spinal cord at the level of C6. In these animals, we extracted brain tissue from the hindlimb representation of S1 before surgery, and at 3, 7, 14, 21, and 35 days after SCI or sham surgery (control). We performed western blot analysis to quantify total protein levels of GluA1 subunits relative to a loading control (β-actin) and to quantify protein levels of phosphorylated serine residue 831 (pS831) and pS845 relative to total GluA1. In all animals we also assessed pain-like behaviors using the Grimace Scale test at the same 6 time points post SCI. ANOVA followed by Dunnett's multiple comparison test was used for statistical comparison. A $p < 0.05$ was considered significant. We found that pS831 levels gradually reduced after SCI and pS831 was maximally dephosphorylated at day 21 (>2-fold) compared to controls. No significant changes were found in pS845 levels or in pS831 levels in S1 of control animals ($p > .05$). The temporal pattern of changes in pS831 levels mimicked the temporal pattern for the development of pain-like

behaviors suggesting that the dephosphorylation of pS831 is causally related to the development of SCI-pain. Our results suggest that SCI-pain is associated with maladaptive plasticity caused by dephosphorylation of pS831 in S1. We propose that this dephosphorylation preferentially affects inhibition, which will result in hyperactivity in cortical pain networks.

Disclosures: L. Jiang: None. **P. Voulalas:** None. **Y. Ji:** None. **R. Masri:** None.

Poster

536. Plasticity After Spinal Cord Injury I

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 536.18/JJ22

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

Support: Travis Roy Foundation

Title: Unilateral, but not bilateral motor cortex electrical stimulation promotes corticospinal function after injury

Authors: *T. T. BETHEA[1], Y. HAROONIAN[1], G. DRUMMOND[1], A. KHALILI[1], J. B. CARMEL[1,2]
[1]Burke-Cornell Med. Res. Inst., White Plains, NY; [2]Weill Med. Col. of Cornell Univ., New York, NY

Abstract: In our previous studies, we have applied electrical stimulation to the motor cortex on the uninjured hemisphere after unilateral corticospinal tract (CST) injury. Stimulation promoted axon outgrowth, functional connections, and behavioral recovery even after chronic injury. Here we hypothesized that stimulation of the injured CST would also promote CST function by causing collateral sprouting of axons above the lesion or in sparse spared axons below the lesion. We performed two studies. For both studies, we used ladder-walking as an assay of skilled motor function. Female rats were trained on the task, and baseline error rates were recorded. Performance was measured each week thereafter without additional training. Electrical stimulation was performed after lesion using epidural electrodes over forelimb area of motor cortex. Real or sham stimulation was delivered 6 hours per day for 10 days, using our published paradigm. In the first study, we completely severed the CST at the medullary pyramid and stimulated motor cortex in the lesioned hemisphere. Electrical stimulation was performed four weeks after CST injury. The error rates of rats with electrical stimulation diminished over the four weeks after starting stimulation and fell to the baseline error rates. The error rate in rats with

sham stimulation remained high. In the second study, we stimulated the motor cortex bilaterally after dorsal column injury. This lesion severs 95% of CST axons in the spinal cord, as well as sensory axons. Real or sham stimulation was initiated 2 weeks after injury. The error rates were variable, and there was no change in rats with either real or sham stimulation. Anatomical analysis of spared axons below the lesion showed no difference between groups. Lengths above the lesion, counted using stereological techniques also show no difference. Thus, there was striking discord in the positive effects of unilateral stimulation after unilateral CST injury and the null effects of bilateral stimulation after bilateral injury. The difference in patterns of injury and stimulation need to be compared directly to better understand the effects and potential use of motor cortex electrical stimulation.

Disclosures: T.T. Bethea: None. **Y. Haroonian:** None. **G. Drummond:** None. **A. Khalili:** None. **J.B. Carmel:** None.

Poster

536. Plasticity After Spinal Cord Injury I

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Topic: D.10. Spinal Cord Injury and Plasticity

Support: NIH HL111598

NIH HL080209

NIH HL69064

NIH NS065067

The Craig H. Neilsen Foundation

Title: PIAS1 may play a role in repetitive acute intermittent hypoxia induced down-regulation of spinal inflammatory gene expression in rats

Authors: *B. J. DOUGHERTY[1], S. S. SPRINGBORN[1], A. S. ROOPRA[2], K. K. BOWEN[1], G. S. MITCHELL[1], J. J. WATTERS[1]
[1]Comparative Biosci., [2]Neurosci., Univ. of Wisconsin - Madison, Madison, WI

Abstract: Repetitive acute intermittent hypoxia (rAIH; 10 episodes of 10.5% O2/day, 3 days/wk, 4 wks) promotes spinal motor plasticity and elicits functional recovery of breathing capacity after cervical injuries. rAIH also elicits anti-inflammatory effects, down-regulating cyclooxygenase-2, tumor necrosis factor family members, chemokines and interleukins-1β and -6 (plus others) in the ventral cervical spinal cord of adult rats. To explore possible transcriptional drivers of these changes, whole genome Vista analyses were utilized to identify over-represented transcription factor binding sites in the upstream 5kb promoter regions of rAIH down-regulated genes. Binding sites for Signal Transducer and Activator of Transcription 1 and 2 (STAT1/2) and nuclear factor kappa B (NF-κB) were over-represented ($p<0.005$), suggesting that rAIH may decrease STAT1/2 and NF-κB transcriptional activity. Here we localize expression of protein inhibitor of activated STAT-1 (PIAS1) within the ventral cervical spinal cord; PIAS1 is a small ubiquitin-related modifier E3 ligase known to negatively regulate DNA binding activities of STAT1/2 and NF-κB. PIAS1 was highly expressed in identified phrenic motor neurons, suggesting a potential role in the anti-inflammatory actions of rAIH and, quite possibly, in rAIH-induced spinal motor plasticity. An understanding of mechanisms giving rise to the anti-inflammatory actions of rAIH may broaden its therapeutic potential in diverse clinical disorders associated with either systemic or neural inflammation.

Disclosures: B.J. Dougherty: None. **S.S. Springborn:** None. **A.S. Roopra:** None. **K.K. Bowen:** None. **G.S. Mitchell:** None. **J.J. Watters:** None.

Poster

536. Plasticity After Spinal Cord Injury I

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Topic: D.10. Spinal Cord Injury and Plasticity

Support: NIH Grant NS072206

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Title: Chronic morphine exposure activates mTOR pathway through μ-opioid receptor in dorsal horn neurons

Authors: *L. SUN[1], L. LIANG[2], S. WU[2], X. GU[2], B. LUTZ[2], A. BEKKER[3], Y. TAO[2]
[1]Anesthesiol. Department, NJMS, Rutgers, Newark, NJ; [2]Rutgers New Jersey Med. Sch., Newark, NJ; [3]Rutgers New Jersey Med. Sch., Demarest, NJ

Abstract: Our previous research found that blocking spinal mTOR activity dramatically attenuated morphine-induced tolerance and hyperalgesia. This observation led us to hypothesize that spinal mTOR is activated during chronic morphine exposure. Here, we first investigated the phosphorylation levels of mTOR and its downstream effectors S6K1 and 4E-BP1 in dorsal horn after repeated morphine injections. Results showed that phosphorylated mTOR (p-mTOR), p-S6K1, and p-4E-BP1 were undetectable or expressed at low levels in saline-treated rats, while intrathecal morphine led to significant, time-dependent increases in the levels of p-mTOR, p-S6K1, and p-4E-BP1 in lumbar enlargement segments. Total expression of mTOR, S6K1, and 4E-BP1 protein in dorsal horn was not altered during the observation period. The number of cells positive for p-mTOR, p-S6K1, and p-4E-BP1 was also dramatically increased exclusively in dorsal horn neurons. Then, we defined which opioid receptors triggered this morphine-induced activation of mTOR and its downstream effectors in dorsal horn neurons during chronic morphine exposure. Intrathecal co-injection of CTOP (1 ng, a selective μ opioid receptor antagonist) blocked morphine-induced increases in p-mTOR, p-S6K1, and p-4E-BP1 in dorsal horn. μ opioid receptor knockout mice failed to display an increase in dorsal horn p-mTOR after repeated subcutaneous injections of morphine. Furthermore, cultured dorsal horn neurons exposed to morphine (20 μM) or DAMDO (20 μM) exhibited time-dependent increases in p-mTOR, p-S6K1, and p-4E-BP1, but not total expression of these three proteins. These increases were attenuated by CTOP (10 μM), but not η-BNI (20 μM; a selective κ opioid receptor antagonist) or naltrindole (20 μM; a selective δ opioid receptor antagonist). Double labeling revealed that μ opioid receptor mRNA co-expressed with mTOR and morphine-induced p-mTOR in dorsal horn neurons. Our findings indicate that the μ opioid receptor triggers activation of the dorsal horn mTOR pathway during chronic morphine exposure. This previously unknown μ opioid receptor-triggered mTOR pathway in dorsal horn neurons may be involved in morphine-induced spinal protein translation changes under the conditions of chronic morphine tolerance and hyperalgesia. Thus, inhibiting mTOR pathway may have potential clinical implications.

Disclosures: L. Sun: None. **L. Liang:** None. **S. Wu:** None. **X. Gu:** None. **B. Lutz:** None. **A. Bekker:** None. **Y. Tao:** None.

Poster

536. Plasticity After Spinal Cord Injury I

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Topic: D.10. Spinal Cord Injury and Plasticity

Support: NIH Grant 1R01NS071056-01

Title: Pharmacological inhibition of histone deacetylase-6 as a treatment for spinal cord injury

Authors: *M. METCALFE
Biol., Burke Med. Res. Inst., White Plains, NY

Abstract: Metcalfe MJ, Tanner J, Rivieccio MA, Brochier CA, Brown J, Hill CE, and Langley B. During spinal cord injury ascending and descending axonal pathways are damaged resulting in interrupted motor and sensory communication between the brain and body and functional loss. In addition to this, neuronal death and limited axonal regeneration restricts the amount of functional repair that takes place. Few therapeutic options exist for treatment after spinal cord injury and they are not very successful in promoting regeneration and the recovery of function. Histone deacetylase 6 (HDAC6) is a class IIb deacetylase that has preference for non-histone proteins, including -tubulin, HSP90 and cortactin. We previously showed that chemical or genetic inhibition of HDAC6 allows axons to grow on non-permissive substrates *in vitro*, in a manner independent of transcription (Riveccio et al., 2009); however, the molecular mechanisms that lead to this growth, and whether it occurs *in vivo* have yet to be determined. In this study we investigate -tubulin, a constituent of microtubules, as a molecular target of HDAC6 deacetylase activity in mediating the growth potential of CNS neuron axons, and whether HDAC6 inhibition can promote functional recovery after spinal cord injury. For spinal cord injury studies, we use a rat contusion model and we assess functional recovery by Basso, Beattie, Bresnahan open field locomotor test (BBB), grid walk, and catwalk tests. After 8 weeks post-injury, treated rats showed promising improvements in locomotor paradigms compared to control rats. We propose that inhibiting HDAC6 could promote axon growth and functional recovery following mechanical injury.

Disclosures: M. Metcalfe: None.

Poster

536. Plasticity After Spinal Cord Injury I

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 536.22/JJ26

Topic: D.10. Spinal Cord Injury and Plasticity

Support: Ege University School of Medicine Research Fund (2010-T-00023)

Title: Effect of hyperbaric oxygen therapy on Fos expression in neuroropathic pain following spinal cord injury

Authors: *G. SENGUL[1], A. KESER[2], M. ERTURK[3], T. DAğCı[2], B. BALKAN[2], F. AYDıN[4]
[2]Physiol., [3]Anat., [1]Ege Univ. Sch. Med., Izmir, Turkey; [4]Neoks Hyperbaric Oxygen Therapy Ctr., Izmir, Turkey

Abstract: Neuropathic pain caused by traumatic spinal cord injury is an important clinial problem. The aim of this study was to investigate the changes in the number of neurons containing Fos protein after HBO treatment following spinal cord injury (SCI). Rivlin and Tator clip method was used to induce SCI at T9-T11 in Sprague-Dawley male rats (n=16). Rats received HBO at 2.80 ATA for 60 min daily. Groups were: 1)Control(SCI, no HBO), 2)Preoperative HBO for 5 days, 3)Postoperative HBO for 5 days, 4)preoperative and postoperative HBO(5 days and 5 days after SCI). HBO applied preoperatively, postoperatively and pre+postoperatively induced Fos expression significantly in spinal cord sections 1cm proximal and 1 cm distal to SCI. The increase was in proportion with the duration of HBO treatment. Fos expression was mostly found in laminae 1-2, followed by laminae 3 -4, and laminae 7-8. In a previos study on oxidative stress following SCI (Dayan et al., 2012), we found that HBO treatment decreased superoxide dismutase, glutathione peroxidase, catalase and nitric oxide synthase in the spinal cord tissue, and significantly improved BBB scores. In this project, we had expected a decrease in Fos expression parallel to these improvements; however, we have observed an increase. This leads us to the conclusion that HBO treatment induced Fos expression following SCI is related to other mechanisms, not due to mechanisms related to pain.

Disclosures: G. Sengul: None. **A. Keser:** None. **M. Erturk:** None. **T. Dağcı:** None. **B. Balkan:** None. **F. Aydın:** None.

Poster

537. Plasticity After Spinal Cord Injury II

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Topic: D.10. Spinal Cord Injury and Plasticity

Support: KAKENHI 25702033

KAKENHI 23500617

Title: Plasticity of indirect cortico-motoneuronal excitations in relaxed hand muscles in humans

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Abstract: We previously reported that repetitive combined stimulation (RCS) of pyramidal tract and peripheral nerve could induce long-term potentiation (LTP) in indirect cortico-motoneuronal (C-M) excitations in biceps brachii (BB) of human subjects, which are mediated by cervical propriospinal neurons (PNs). However, the LTP could be induced only when the target muscle was voluntarily contracted, which limits possible clinical use. In animal studies, PNs are known to project various forelimb motoneurons. Because RCS could induce plastic changes in synapses from pyramidal tract to PNs, we hypothesized that the LTP of C-M excitations could also be induced in non-target muscles in upper limb, which are relaxed during RCS. RCS intervention (0.2 Hz, 10 min) was the same as in the previous study. With EMG recording from right BB under weak contraction, transcranial magnetic stimulation (TMS) to the arm area of left motor cortex (M1) was delivered with right ulnar nerve stimulation. Inter-stimulus interval for the combined stimulation was set at 10 ms, where inputs by both stimuli to reach PNs simultaneously. Stimulus strengths were determined to observe the maximum spatial facilitation in BB by the simultaneous inputs. To observe LTP in non-target muscles, motor evoked potentials (MEPs) were obtained from flexor digitorum superficialis, extensor digitorum communis and first dorsal interosseous muscles, by TMS to the optimal point in M1, which was ~1 cm lateral to the arm area. As previously reported, MEPs in BB were potentiated after RCS, which lasted for ~65 min. Furthermore, the potentiation could be observed in hand muscles, which showed similar time course to that in BB. When the stimulation point in M1 was moved laterally by 2-3 cm, where MEPs could be evoked in hand muscles but less efficiently, the potentiation was not observed after RCS. These results show that LTP could be induced in muscles without contraction, if RCS induces LTP in another muscle under weak contraction. Differences between stimulation positions indicate that the LTP was caused by plastic changes in synapses, which are activated by TMS during RCS.

Disclosures: **T. Nakajima:** None. **S. Suzuki:** None. **H. Ohtsuka:** None. **T. Endoh:** None. **Y. Masugi:** None. **S. Irie:** None. **T. Komiyama:** None. **Y. Ohki:** None.

Poster

537. Plasticity After Spinal Cord Injury II

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Foundation for Physical Therapy - Promotion of Doctoral Studies I Scholarship

Title: Decreased spinal inhibition during muscle lengthening in humans with incomplete spinal cord injury may contribute to increased central activation during eccentric contractions

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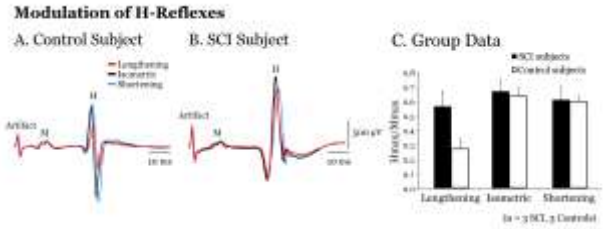
Abstract: Recent data suggest individuals with chronic motor incomplete spinal cord injury (SCI) generate greater central activation of the knee extensors during eccentric maximal voluntary contractions (MVCs) than during isometric or concentric MVCs. This activation pattern is markedly different from that of healthy adults, who demonstrate reduced central activation during eccentric MVCs in several different muscle groups. Inhibition of Ia-α motoneuron excitability during muscle lengthening in healthy adults, as determined by H-reflex testing, may contribute to central activation deficits. Currently, there are no published data on H-reflex modulation during dynamic muscle length changes in humans with SCI. The aims of the current study are to assess patterns of central activation in the ankle plantarflexors using the interpolated twitch technique, and to compare modulation of soleus H-reflexes during isometric, shortening, and lengthening muscle actions between SCI and healthy subjects. We hypothesize that differences in muscle activation patterns between the two groups are associated with divergent modulation of Ia input onto motor pools. Preliminary results in 5 males with incomplete SCI demonstrate central activation ratios (CARs) in the plantarflexors are greater during eccentric MVCs (0.78±0.13) than isometric (0.64±0.17) or concentric (0.55±0.29) MVCs. In addition to CAR testing, H-reflex modulation during isometric, shortening, and lengthening muscle actions was assessed in a subset of the SCI subjects (n=3) and 3 healthy males. SCI subjects demonstrated decreased spinal inhibition of H-reflexes during muscle lengthening compared to controls. These data suggest contrasting patterns of muscle activation between SCI and healthy subjects may be related to differences in modulation of Ia-α motoneuron excitability

across different types of muscle length changes. Increased understanding of mechanisms underlying altered muscle activation patterns in SCI patients may aid future development of more effective training protocols.



Disclosures: **H.E. Kim:** None. **D.M. Corcos:** None. **T.G. Hornby:** None.

Poster

537. Plasticity After Spinal Cord Injury II

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NEILSEN FOUNDATION 83607

Title: Locomotor training modifies soleus motoneuron excitability in human spinal cord injury

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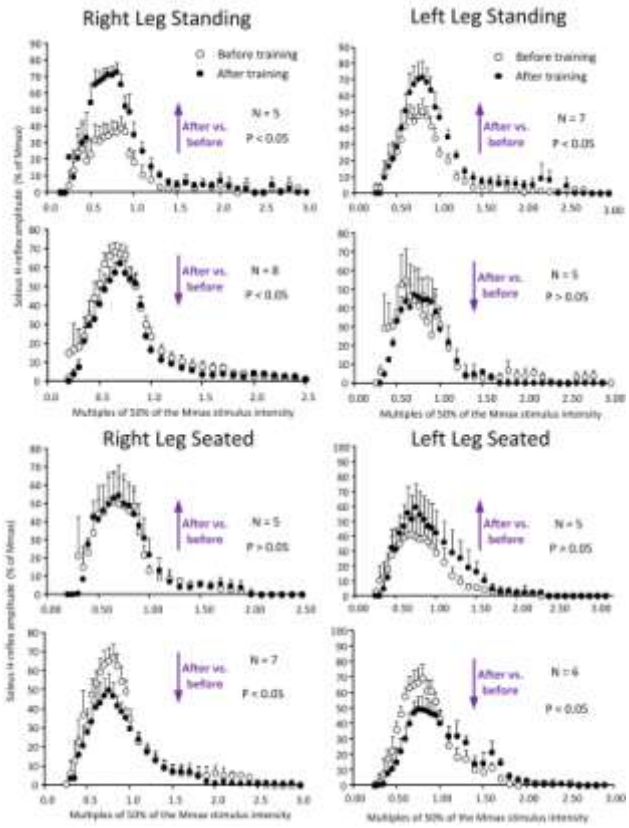
Abstract: The objective of this study was to describe changes in soleus motoneuron excitability after locomotor training in humans with spinal cord injury (SCI). We hypothesized that locomotor training alters spinal reflex excitability in a body position-dependent manner. Fifteen people who had either chronic motor complete or incomplete SCI received an average of 45 daily locomotor training sessions. The soleus H-reflex and M-wave recruitment curves were assembled using data collected in the right and left legs, with subjects seated and standing, before and after training. The soleus H-reflexes and M-waves, measured as peak-to-peak amplitudes, were normalized to the maximal M-wave (Mmax). Stimulation intensities were normalized to the 50 % Mmax stimulus intensity. A sigmoid function was also fitted to

normalized soleus H-reflexes on the ascending limb of the recruitment curve. Locomotor training modified the reflex excitability of soleus motoneurons in people with motor complete and incomplete SCI, while seated and during standing. After training, H-reflex excitability was decreased during seated position testing, and was increased during standing, in both limbs. Changes in reflex excitability coincided with changes in H-reflex stimulation threshold, and at 50 % maximal H-reflex, while the slope remained unaltered. Adaptations of the intrinsic properties of soleus motoneurons and Ia afferents, excitability profile of the soleus motoneuron pool, oligosynaptic inputs, and corticospinal inputs may all contribute to these changes. The findings of this study demonstrate that the injured human spinal cord can adjust reflex excitability levels after locomotor training, based on demands of the motor task.



Disclosures: **M. Knikou:** None. **A.C. Smith:** None. **W.Z. Rymer:** None.

Poster

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Topic: D.10. Spinal Cord Injury and Plasticity

Support: Helen Hayes Hospital Foundation

New York State Spinal Cord Injury Research Trust Fund C023685

NIH NS069551

Title: Operant up-conditioning of the tibialis anterior motor evoked potential in neurologically normal subjects

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Abstract: The corticospinal tract (CST) plays a key role in motor control and motor skill learning and re-learning after CNS damage. The corticospinal motor evoked potential (MEP) to transcranial magnetic stimulation (TMS) that reflects the excitability and connectivity of CST is decreased after CNS damage, and increases during recovery of motor function. Thus, strengthening remaining CST function might help restoring motor function in people after CNS damage. As the first step in testing the hypothesis that operant up-conditioning of the ankle dorsiflexor MEP can strengthen CST connectivity and alleviate foot-drop in people with CNS damage, we are up-conditioning the tibialis anterior (TA) MEP in people with no known neurological conditions. The protocol consists of 6 baseline (3/wk) and 24 up-conditioning or control sessions (3/wk), similar to a human soleus H-reflex conditioning protocol previously used (J Neurosci 2009:29:5784-92). In all sessions, TA MEPs are measured while the sitting subject provides 15-20% maximum voluntary contraction (MVC) level of TA background EMG with ankle, knee, and hip joint angles fixed. In all the trials of baseline sessions, all the trials of control sessions, and the first 20 trials of conditioning sessions, the subject is given no feedback as to MEP size (i.e., control MEPs). In 225 conditioning trials of conditioning sessions (i.e., conditioning MEPs), the subject is encouraged to increase the TA MEP, and receives immediate feedback as to whether MEP size was above a criterion (i.e., whether the trial was a success). TMS is kept at 10% above threshold. To date, 6 subjects have completed the up-conditioning protocol and 4 subjects have completed the control protocol. Four of 6 up-conditioning subjects increased the TA MEP significantly. Among these subjects, the final MEP size (averaged over conditioning sessions 22-24) was 147±12(SE)% of the baseline value. In contrast, MEP size did not change in the 4 control subjects; the final MEP size was 92±12(SE)% of the baseline value. Initial gait analysis indicates that locomotor EMG activity and kinematics did not change in either up-conditioning subjects or control subjects. The absence of locomotion change after MEP conditioning in normal subjects is different from our preliminary findings in people with

multiple sclerosis (Favale et al., SfN abstract, 2014), in whom successful TA MEP up-conditioning alleviated foot drop, increased TA MVC, and increased walking speed. Lack of functional impact in normal subjects may reflect compensatory plasticity that prevents the plasticity underlying MEP change from disturbing normal locomotion (Neuroscientist 2014: Epub ahead of print)

Disclosures: J.A. Brangaccio: None. **B.M. Favale:** None. **G. Fiorenza:** None. **A. Thompson:** None.

Poster

537. Plasticity After Spinal Cord Injury II

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Program#/Poster#: 537.05/JJ31

Topic: D.10. Spinal Cord Injury and Plasticity

Support: Helen Hayes Hospital Foundation

NIH NS69551

Title: Operant up-conditioning of the tibialis anterior motor evoked potential in neurologically normal subjects

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Abstract: The corticospinal tract (CST) is important in motor control and in guiding the spinal cord plasticity that is associated with learning motor skills and re-learning them after CNS trauma or disease (Neuroscientist 2010:16:532-549; 2014:Epub ahead of print). Damage to the corticospinal pathway often results in weak ankle dorsiflexion and spasticity, and thereby limiting the mobility of people with multiple sclerosis (MS). Thus, strengthening the corticospinal function may improve locomotion. This pilot study investigated whether operant up-conditioning of the tibialis anterior (TA) motor evoked potential (MEP) can enhance the corticospinal excitability and connectivity and alleviate locomotor problems in people with chronic stable MS. MEP up-conditioning protocol consisted of 6 baseline and 24 up-conditioning sessions (3/wk for 10 wks). In all sessions, TA MEPs at 10% above threshold were measured

while the sitting subject provided a pre-set level (typically 30% of maximum voluntary contraction (MVC) level at the beginning of study) of TA background EMG with ankle, knee, and hip joint angles fixed. During the baseline sessions, MEPs were simply measured. During 225 conditioning trials of each of the 24 conditioning sessions, the subject was encouraged to increase MEP, and was given immediate feedback indicating whether MEP size was above a criterion (i.e., whether the trial was a success). In 3 of 4 subjects with MS, over the course of conditioning, TA MEP and MVC increased significantly by 35-77% and 28-61%, respectively, and locomotor EMG activity improved bilaterally and their foot drop became less severe. All three of them spontaneously reported better leg movement during walking. Twenty-five-feet walking times improved more than 20% in all but one subject. To our pleasant surprise, these improvements were also observed in one of the subjects, whose disease progress was noted near the end of the conditioning period. In addition, in one of them, extensive follow-up sessions occurred for the following 3 years, during which increases in TA MEP and MVC were maintained. These case studies suggest that while the disease itself may continue to gradually progress, CNS plasticity can be induced and maintained, and thereby generate functional improvement in people with chronic stable MS. Operant up-conditioning of MEP could provide a promising new therapy for improving locomotion in people with MS or other CNS disorders.

Disclosures: **B. Favale:** None. **J. Velez:** None. **P. Falivena:** None. **A.K. Thompson:** None.

Poster

537. Plasticity After Spinal Cord Injury II

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Program#/Poster#: 537.06/JJ32

Topic: D.10. Spinal Cord Injury and Plasticity

Support: NIH NS069551

NIH NS22189

NIH NS061823

Title: Operant conditioning of spinal reflexes in people with chronic CNS damage

Authors: *A. THOMPSON[1,2,3,4], J. R. WOLPAW[1,2,3,4]
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Hlth., Albany, NY; [3]Dept. of Neurology, Neurolog. Institute, Columbia Univ., New York, NY; [4]Dept. of Biomed. Sciences, State Univ. of New York, Albany, NY

Abstract: People with chronic CNS damage often suffer motor disabilities due to spasticity and poor muscle control, even after conventional therapy. Abnormal spinal reflex activity commonly contributes to these problems. Operant conditioning protocols can modify specific spinal reflex pathways and thereby restore more normal reflexes. We recently showed that down-conditioning the soleus H-reflex during standing can markedly improve locomotion in people with spasticity due to chronic incomplete spinal cord injury (SCI) (J Neurosci 2013:33:2365-2375). Furthermore, the beneficial reflex change initiates widespread plasticity that improves locomotor function in both legs (Neuroscientist 2014:Epub ahead of print). We are currently testing several different reflex conditioning protocols in people with chronic CNS damage: (1) We are down-conditioning the soleus H-reflex during walking in people with spastic hyperreflexia due to chronic incomplete SCI to test the hypothesis that down-conditioning during the mid-late swing phase (when the H-reflex is very small or absent normally but very large in these individuals) will restore more normal locomotor reflex modulation, and thereby improve locomotion. (2) We are down-conditioning the soleus H-reflex in people after stroke to determine whether reflex conditioning is possible after stroke. (3) We are down-conditioning the soleus stretch reflex in people with chronic incomplete SCI to compare the effects and time course of stretch reflex conditioning with those of H-reflex conditioning. All three protocols consist of 6 baseline and 30 conditioning sessions (J Neurosci 2013:33:2365-2375). Each session includes 3 blocks of 75 reflex trials, without (control trials in baseline sessions) or with (conditioning trials in conditioning sessions) encouragement to reduce reflex size with the aid of visual feedback. Before and after the 30 conditioning sessions, 10-m walking speed and locomotor EMG are assessed. To date, 3 of 4 Protocol-1 subjects >3yrs after incomplete SCI, 2 of 3 Protocol-2 subjects >2yrs. after stroke, and 1 of 2 Protocol-3 subjects >10yrs after incomplete SCI successfully reduced the reflex. Their 10-m walking speeds improved by 8-30%, and their locomotor EMG modulation improved. We are currently studying additional subjects with each protocol. Confirmation of these initial results should increase understanding of reflex conditioning protocols and broaden the range of their potential therapeutic applications.

Disclosures: A. Thompson: None. **J.R. Wolpaw:** None.

Poster

537. Plasticity After Spinal Cord Injury II

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 537.07/JJ33

Topic: D.10. Spinal Cord Injury and Plasticity

Title: Afferent regulation of the upper limb motor cortex following incomplete cervical spinal cord injury

Authors: *A. BAILEY, P. MI, A. J. NELSON
Kinesiology, McMaster Univ., Hamilton, ON, Canada

Abstract: Aaron Bailey*, Peter Mi, and Aimee J. Nelson Department of Kinesiology, McMaster University, Hamilton, Ontario Incomplete spinal cord injury (SCI) to the cervical spine impairs the transmission of afferent and efferent volleys along the remaining neural pathways. Impairment in somatosensory afferent transmission can alter the gating effect on the Transcranial magnetic stimulation (TMS) elicited motor evoked potential (MEP) seen in uninjured participants (i.e. short and long-latency afferent inhibition). The purpose of the present study was to investigate the afferent regulation of the flexor carpi radialis (FCR) muscle in individuals with incomplete SCI. Eight individuals with incomplete SCI (mean age = 31±7.13, 7 males, ASIA range B,C,D with lesions between C4-C7) and aged-matched uninjured controls (mean age = 30.67±7.09, 3 males) were studied. Afferent regulation was studied bilaterally by electrical stimulation of the median nerve at the elbow (1.2x motor threshold) followed by TMS pulses delivered over the FCR representation to evoke MEP at half-maximum amplitude. Afferent regulation was studied while FCR was at rest and also during 15-20% of maximum voluntary contraction. The interstimulus intervals between the nerve stimulation and TMS pulse included: 15, 20, 25, 35, 45, 55, 65 and 200 ms. Ten trials were recorded at each ISI and the resulting MEP was normalized to those obtained in the absence of conditioning (i.e. TS alone). Afferent regulation was measured using the area of the MEP and calculated as the $MEP_{nerve-TMS}/MEP_{TMSalone.}$ Preliminary results indicate that afferent input elicits greater inhibition of the MEP in controls versus SCI at latencies of ~15 ms corresponding to the short-latency afferent inhibition interval for the FCR muscle. Afferent modulation of MEPs is similar for controls and SCI at intervals from 35 - 65 ms. These results indicate alterations in the afferent regulation of the motor cortical output to FCR muscle that coincide with the cortical arrival of the somatosensory afferent volley.

Disclosures: A. Bailey: None. **P. Mi:** None. **A.J. Nelson:** None.

Poster

537. Plasticity After Spinal Cord Injury II

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Program#/Poster#: 537.08/JJ34

Topic: D.10. Spinal Cord Injury and Plasticity

Title: Short and long-interval intracortical inhibition in flexor carpi radialis muscle in incomplete spinal cord injury

Authors: *Y. P. MI, A. BAILEY, A. J. NELSON
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Abstract: Peter Y. Mi*, Aaron Bailey, Aimee J. Nelson. Corticospinal output as measured using Transcranial magnetic stimulation (TMS) is typically reduced in individuals with spinal cord injury (SCI). These changes may be due to alterations at the spinal level and possibly by changes within primary motor cortex. Two TMS-evoked circuits, short- and long-interval intracortical inhibition (SICI and LICI) are measures of the intracortical inhibitory mechanisms within primary motor cortex. The present study measured SICI and LICI in 8 individuals with incomplete SCI (mean age = 31±7.13, 7 males, ASIA range B,C,D with lesions between C4 and C7) and aged-matched controls (mean age = 30.67±7.09, 3 males). SICI and LICI measures were obtained from the flexor carpi radialis (FCR) muscle bilaterally in SCI and in the dominant limb in controls. SICI and LICI were obtained with the muscle at rest and also during a 15 - 20% maximum voluntary contraction i.e. active. SICI recruitment curves were generated using six conditioning stimulus (CS) intensities: 60, 70, 80, 90, 100, 110% of rest and active motor threshold. For SICI the ISI between the CS and the test stimulus (TS) was set at 2 ms. LICI recruitment curves were generated using five CS intensities: 90, 100, 110, 120, 130% of rest and active motor threshold. For LICI the ISI between the CS and the TS was set at 150 ms. For both circuits, 30 trials were obtained at each CS intensity (15 with test stimulus alone, 15 with conditioning preceding test stimulus). SICI and LICI were measured using the area of the MEP using the formula: $MEP_{CS\text{-}TS}/MEP_{TSalone}$. Preliminary data reveals a decrease in SICI at rest in SCI compared to controls. However, in ‘active’, SICI appears to be similar across groups. LICI obtained at rest is similar between SCI and controls. However, LICI appears to be greater in SCI compared to controls during ‘active’. The data suggests some degree of reduction in $GABA_A$ function in the cortex resulting in the reduction of SICI as well as a persistent $GABA_B$ function during movement which causes dysfunction of the LICI circuit.

Disclosures: Y.P. Mi: None. **A. Bailey:** None. **A.J. Nelson:** None.

Poster

537. Plasticity After Spinal Cord Injury II

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Program#/Poster#: 537.09/JJ35

Topic: D.10. Spinal Cord Injury and Plasticity

Support: NIH Grant NS047567

Title: Effects of zolmitriptan and N-methyl-D-aspartate on the sensory input processing of deep dorsal horn neurons in mice with acute spinal cord injury

Authors: *T. THAWEERATTANASINP[1], C. J. HECKMAN[2], V. M. TYSSELING[3]
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Abstract: Spinal cord injury (SCI) results from a physical insult to the spinal cord, which can cause various motor dysfunctions. A muscle spasm is one of the common dysfunctions sustained in chronic SCI patients. It is characterized by a sudden, involuntary contraction of a muscle, which can interfere with standing, seating, walking, and other physical movements. SCI causes loss of tonic serotonin (5-hydroxytryptamine; 5-HT) inhibition of the excitatory deep dorsal horn neurons (DHN), likely contributing to the exaggerated excitatory drive to the spinal motoneurons. The enhancement of excitatory input over inhibitory one to spinal motoneurons then results in muscle spasms. Recent studies in rodents and humans with chronic SCI revealed that activation of 5-HT1B/1D receptors can reduce muscle spasms without affecting the intrinsic properties of spinal motoneurons. Moreover, the majority of 5-HT1 receptors concentrate highly in the spinal dorsal horn, where the early processing of sensory input from the body occurs. This evidence suggests the involvement of DHNs in producing muscle spasms after chronic SCI. We hypothesize that DHNs become hyperexcitable after SCI by showing prolonged plateau potentials and strong persistent inward currents, thus amplifying synaptic input to the downstream motoneurons. Moreover, if the 5-HT deficiency is the cause of the hyperexcitable DHNs, the selective 5-HT1B/1D receptor agonist zolmitriptan should reduce the neuron hyperexcitability. We have tested these hypotheses by using the *in vitro* sacral cord preparation from the adult mice (Mus musculus) for the model of acute SCI. We analyzed the effects of zolmitriptan and N-methyl-D-aspartate (NMDA) on the extracellularly recorded responses of DHNs located in spinal lamina III-V and ventral root activity. The synaptic activation of DHNs and ventral root activity was achieved via dorsal root stimulation at four stimulus strengths. Compared with controls, bath application of zolmitriptan (1 μM) increased spike threshold of DHNs, thus generating fewer spontaneous or evoked spikes at a given stimulus strength. However, not on all DHNs did we observe the inhibitory effect of zolmitriptan. Surprisingly,

NMDA had dual effects on the firing activity of DHNs. While NMDA (100 μM) facilitated bursting in one DHN population, it suppressed the evoked responses in the other. These preliminary results suggest the differential sensory input processing of DHNs in acute SCI. A future study of chronic SCI is necessary to see how sensory processing of DHNs would change when muscle spasms arise. It is also interesting to see how the interplay between 5-HT and NMDA receptor systems would affect DHNs in SCI.

Disclosures: T. Thaweerattanasinp: None. **C.J. Heckman:** None. **V.M. Tysseling:** None.

Poster

537. Plasticity After Spinal Cord Injury II

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Topic: D.10. Spinal Cord Injury and Plasticity

Support: Aix Marseille Universite

IRME

ISRT

Title: Proteolytic cleavage of sodium channels and co-transporters KCC2: Mechanism leading to spasticity after spinal cord injury

Authors: V. PLANTIER, F. GACKIÈRE, C. BROCARD, S. LIABEUF, L. VINAY, *F. BROCARD
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Abstract: Pathophysiological mechanisms of spasticity are quite diverse but alterations in intrinsic properties of motoneurons play a central role. Aside an upregulation of the persistent sodium current (I_{NaP}) in motoneurons, disinhibition of motoneurons due to of the down-regulation of the co-transporter KCC2 appears to be an important substrate for spasticity. We postulated that the up- and down-regulation of I_{NaP} and KCC2 respectively not only have a synergistic effect in generating spasticity but also share a common upstream mechanism responsible for their dysregulations. In this study, neonatal Wistar rats underwent a T8 spinal cord injury at birth. One week later, characteristic spastic-like responses were observed. This hyperexcitability of the spinal cord emerged with a concomitant upregulation of I_{NaP} and depolarization of the chloride equilibrium potential (E_{Cl}), that were both associated with a

proteolytic cleavage of voltage-gated sodium channel (Nav) and a down-regulation of KCC2 expression, respectively. In uninjured rats, the pharmacological inhibition of KCC2 by DIOA (25 μM) combined with an upregulation of I_{NaP} by veratridine (75 nM) reproduced spastic-like responses. The *in vitro* biochemical assays identified the calcium-dependent protease, calpain, to be the main proteolytic factor. Acute or chronic administration of the calpain inhibitor MDL28170 decreased proteolytic cleavage of Nav channels, rescued KCC2, reduced I_{NaP} in motoneurons, hyperpolarized E_{Cl}, and attenuated spastic-like responses. Altogether, this study establishes a causal link between the proteolytic cleavage of Nav channels and KCC2 and the alterations of I_{NaP} and E_{Cl} in motoneurons. These two mechanisms act synergistically to induce spasticity.

Disclosures: V. Plantier: None. **F. Gackière:** None. **C. Brocard:** None. **S. Liabeuf:** None. **L. Vinay:** None. **F. Brocard:** None.

Poster

537. Plasticity After Spinal Cord Injury II

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Topic: D.10. Spinal Cord Injury and Plasticity

Support: NIH Grant NS027910

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US Army MRAA W81XWH-12-1-504

Title: Injury discharge immediately following a T10 spinal contusion is critical for the development of DRG neuron hyperexcitability

Authors: *J. DU[1], E. T. WALTERS[2], S. M. CARLTON[1]
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Abstract: Spinal cord injury (SCI) patients often live with chronic pain and mechanisms underlying its generation are unknown. Our previous studies using a rat T10 contusion model demonstrated that the dorsal root ganglion (DRG) cells, including nociceptors, at and below the

level of the injury, are hyperexcitable 3 days and 1-5 months post-contusion, with a significant increase in the number of fibers with spontaneous activity (SA). The goal of the present study was to elucidate whether aberrant electrical activity initiates DRG neuron hyperexcitability. We investigated in male SD rats whether signals coming to the DRG from the spinal cord via the dorsal roots (DRs) contribute to development of SA in the DRG after SCI. To determine whether electrical activity generated in the spinal cord is transmitted to the DRG, we recorded from DRs immediately (within 5 min), 30 min, 7 weeks, and 2 months following contusion. The greatest electrical activity (generated in the cord, recorded in the DR) occurred immediately following contusion; at all other time points, SCI activity was not different from sham activity. To determine whether intact DRs are necessary for the development of SA in the DRG, L4/5 DRs were cut on one side and then rats were contused. SA was recorded in ipsilateral and contralateral DRs on days 3, 7 and 35 post-injury. At all time points, the percentage of units with SA in the rhizotomized DRs was significantly decreased compared to contralateral intact DRs, and was not different from sham. These data suggest that signals conveyed from the cord in the DRs play a major role in inducing the SA that develops in DRGs after contusion. To determine whether injury discharge is key to the induction of SA in the DRG, lidocaine (LIDO) was applied to the DRs from 45 min before to 45 min after contusion. Compared to vehicle, 3 days post-contusion the LIDO rats showed a significant decrease in the proportion of units with SA and their discharge rate was not different from sham. These findings strongly suggest that an electrical signal, generated in the spinal cord at the time of contusion and transmitted to the DRG via the DRs, is critical for the development of SA in the DRG after spinal contusion.

Disclosures: J. Du: None. **E.T. Walters:** None. **S.M. Carlton:** None.

Poster

537. Plasticity After Spinal Cord Injury II

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 537.12/KK2

Topic: D.10. Spinal Cord Injury and Plasticity

Support: DoD SC090469

Craig H. Neilsen 284874

Title: Cardiovascular responses to cutaneous nociceptive input after cervical spinal cord injury: Role of pain afferent types and their plasticity

Authors: *H. LEE[1,2], J. CHUNG[1,2], K. E. TANSEY[1,2,3]
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Abstract: In normal, anesthetized rats, stimulation of segmental (T6 - L1) dorsal cutaneous nerves (DCNs) generates different cardiovascular responses depending on which pain afferent types are activated. Stimulation of A delta fibers alone (0.5 mA) generates increases in heart rate (HR) but relatively limited decreases in blood pressure (BP), with this effect on BP being greater at higher stimulation frequencies (10 Hz vs 1, 2 or 5 Hz). Stimulation of both A delta and C fibers together (5 mA) generates the same increase in HR but now a greater drop in BP, especially at higher stimulation frequencies (2, 5, & 10 Hz). The temporal relationships between BP and HR changes show an initial drop in BP followed by an increase in HR followed by a recovery in BP and then a recovery in HR. Following C7 crush spinal cord injury (SCI), we have found three cardiovascular responses based on BP, a depressor (normal) response where DCN stimulation at A delta and C fiber strength (5 mA) almost always generates a drop in BP, a pressor (autonomic dysreflexia) response where DCN stimulation almost always generates an increase in BP, and a dysautonomia response where there are mixed depressor and pressor responses to DCN stimulation at different spinal levels. We have now investigated the BP vs. HR relationships within these groups and found that the depressor response after injury is different than the normal response in that the final recovery of increased HR is delayed. This finding is also true in the dysautonomia response. In the pressor response, there are simultaneous increases in both BP and HR and both BP and HR are delayed in their return to baseline. In this last group, the effect is much greater in response to rostral DCN stimulation than to caudal DCN stimulation. To relate these cardiovascular responses to pain afferent anatomical plasticity in the spinal cord dorsal horn, we transganglionically labeled A and C fibers with CTB and IB4 respectively in the T7 and T13 DCNs. In all injury cardiovascular responses, we found increased A fiber sprouting from both DCNs relative to uninjured animals. C fibers were fewer than, or the same as, in uninjured animals in both the depressor and dysautonomia response groups but showed sprouting in the pressor response group, significantly more so at T7 than at T13. Taken together, the normal animal data and the spectrum of cardiovascular responses and anatomical plasticity after cervical SCI, it would seem that A delta afferent input and plasticity preferentially affect HR responses and C fiber input and plasticity preferentially affect BP responses. This may mean that A delta effects could be predominantly cardiac while C fiber effects could be predominantly on vascular tone.

Disclosures: H. Lee: None. **J. Chung:** None. **K.E. Tansey:** None.

Poster

537. Plasticity After Spinal Cord Injury II

Location: Halls A-C

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Topic: D.10. Spinal Cord Injury and Plasticity

Support: Craig H. Neilsen Foundation postdoctoral fellowship

NIH NIAMS R01AR053608

NIH NINDS NS047567

Title: Chronic SSRI treatment following incomplete spinal cord injury changes 5HT-2c receptor activity

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Abstract: Deprivation of spinal serotonin after spinal cord injury (SCI) causes significant plasticity in spinal circuitry. Recent studies demonstrated an increase in the expression of the constitutively active isoform of the 5-HT2c receptor following chronic sacral SCI in rats. This increase was functionally tied to the development of tail spasms. Most clinical cases of SCI, however, involve incomplete injuries in which some descending systems, including raphespinal systems, remain partially intact. In this study we examined whether such clinically relevant cases of incomplete SCI also involve the development of constitutively active 5-HT2c receptors. We also asked whether the development of constitutively active 5-HT2c receptors might be modulated by chronic inhibition of serotonergic reuptake with SSRIs. To test this, we used both *in vivo* and *in vitro* testing on two groups of mice with incomplete thoracic SCIs, one control group and one with chronic treatment of Prozac. *In vivo*, we used open field locomotor scores and EMG responses to the flexor withdrawal stimulus to examine behavior and hyperreflexia/spams. *In vitro*, we recorded sacral spinal reflexes to assess the presence of constitutive activity. We show that constitutive activity does increase in mice with incomplete thoracic SCI, but to a lesser degree as compared to the previously published complete studies. In addition, we show that chronic treatment with SSRIs significantly alters adaptations in 5-HT receptors following incomplete SCI. These results reinforce previous work showing an increase in 5-HT2c receptor constitutive activity after spinal cord injury and also suggest that this increase can be modulated through pharmacological intervention.

Disclosures: **V.M. Tysseling:** None. **D.A. Klein:** None. **R. Imhoff:** None. **C. Heckman:** None. **M.C. Tresch:** None.

Poster

537. Plasticity After Spinal Cord Injury II

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Program#/Poster#: 537.14/KK4

Topic: D.10. Spinal Cord Injury and Plasticity

Support: NHMRC Grant 628765

Title: Changes in electrophysiological properties of interneurons over time following incomplete spinal cord injury

Authors: ***M. M. RANK**[1], J. R. FLYNN[1], M. P. GALEA[2], R. CALLISTER[1], R. J. CALLISTER[1]
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Abstract: Following incomplete spinal cord injury (SCI) some spontaneous recovery of function occurs. In rodent models of SCI, particularly in mice, the degree of spontaneous recovery exceeds that observed in humans, or even in other animal models of SCI (eg. cat). The specific cellular mechanisms underlying this spontaneous recovery of function, as the animals transition from the acute to chronic stages of injury, are unknown. For mice such information is important as this species is increasingly used to study the involvement of various genes in recovery from SCI. Here we characterize the changes to intrinsic cellular and synaptic properties of spinal interneurons occurring in the acute (4 wks post SCI) and chronic (10 wks) stages of SCI in an adult mouse hemisection model of injury. Male mice (C57Bl/6; ~P63) received a spinal hemisection (T9-10) and were allocated to acute and chronic groups. After the recovery period, mice were sacrificed and horizontal spinal cord slices (T6-T12, 250 μm) were prepared for whole cell patch clamp analysis. Recordings were made from deep dorsal horn (DDH) interneurons located within two spinal segments of the SCI. Input resistance and rheobase current were decreased in chronic (n=108 neurons) versus acute (n=65) SCI mice and resting membrane potential was more depolarized in interneurons from chronic SCI mice ($p<0.05$). In response to square step depolarising current injection DDH interneurons exhibit four action potential discharge patterns: tonic firing, initial bursting, delayed firing and single spiking. The proportion of neurons exhibiting each discharge pattern differed significantly in acute versus

chronic SCI mice; specifically the proportion of tonic firing neurons is increased and delayed firing neurons decreased in chronic SCI. Moreover, the expression of several voltage-gated ion channels (I_{Ar}, I_{As} and I_{Ca}) known to underlie differences in AP discharge categories in DDH interneurons also differed in acute versus chronic groups. Acute SCI mice exhibit a higher proportion of I_{Ar} and chronic mice a higher proportion of I_{Ca}. Spontaneous excitatory postsynaptic currents (sEPSCs) were recorded to assess excitatory synaptic drive. sEPSC amplitude decreased, sEPSC rise and decay time increased, while sEPSC frequency did not change over time post-injury. Together these data indicate that DDH interneurons in the chronic stages of SCI are characterized by altered excitability, reduced excitatory synaptic drive, and either altered expression of glutamate receptor subtypes or receptor location on their somatodendritic trees.

Disclosures: **M.M. Rank:** None. **J.R. Flynn:** None. **M.P. Galea:** None. **R. Callister:** None. **R.J. Callister:** None.

Poster

537. Plasticity After Spinal Cord Injury II

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

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Topic: D.10. Spinal Cord Injury and Plasticity

Support: NIH Grant NS079147

Title: Assessment of Hebbian plasticity in the spinal cord

Authors: ***A. J. FUGLEVAND**[1], M. J. RAMIREZ[2]
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Abstract: A fundamental process underlying learning and memory is a long-lasting change in the effectiveness of synaptic communication among neurons. One principle that governs such changes in synaptic 'strength' was proposed by Hebb in the 1940s. Hebb postulated that synaptic connections among neurons that are co-active become stronger whereas synaptic connections among neurons that are active at different times are weakened. While a substantial body of research has largely confirmed aspects of Hebb's rule underlying plasticity in various brain structures, little is known about the capability of neurons in the spinal cord to exhibit Hebbian plasticity. The goal of this project was to determine whether the strength of synaptic connections between corticospinal neurons and spinal motor neurons (MNs) could be changed by a period of

training that involved their repetitive co-activation. As such, we measured the extent of short-term synchronized spiking between MNs supplying two hand muscles before and following 4 weeks of training on a computer game that required precise matching of the forces exerted by the two muscles. Because such synchronization reflects the strength of synaptic inputs projecting across the two motor nuclei, training-related increases in synchrony were assumed to reflect Hebbian-like increases in the strength of common synaptic inputs. Despite marked improvements in game performance, no changes in synchrony were observed. Given the constraints of the training protocol and the indirect assay to evaluate changes in synaptic strength, these results should not be taken to indicate that synapse-specific and activity dependent changes in synaptic strength cannot occur in the spinal cord. Nevertheless, it still seems feasible that the molecular machinery needed to support Hebbian plasticity is absent at MN synapses.

Disclosures: A.J. Fuglevand: None. **M.J. Ramirez:** None.

Poster

537. Plasticity After Spinal Cord Injury II

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 537.16/KK6

Topic: D.10. Spinal Cord Injury and Plasticity

Support: University of Helsinki Funds

Title: The use of F-response in defining interstimulus intervals appropriate for LTP-like plasticity induction in lower limb spinal paired associative stimulation

Authors: *A. SHULGA[1,2], P. LIOUMIS[1], E. KIRVESKARI[1,2], S. SAVOLAINEN[3], J. P. MÄKELÄ[1], A. YLINEN[1,2]
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Abstract: In spinal paired associative stimulation (PAS), orthodromic volleys are induced by transcranial magnetic stimulation (TMS) in upper motor neurons, and antidromic volleys by peripheral nerve stimulation (PNS) in lower motor neurons of human corticospinal tract. The volleys arriving synchronously to the corticomotoneuronal synapses induce long term potentiation -like plasticity in the spinal cord. For clinical use of spinal PAS, it is important to develop protocols that reliably induce facilitation of corticospinal transmission. Due to variability in conductivity of neuronal tracts in neurological patients, it is beneficial to estimate

interstimulus interval (ISI) between TMS and PNS on individual basis. Spinal root magnetic stimulation has previously been used for this purpose in spinal PAS targeting upper limbs. However, at lumbar level this method does not take into account the conduction time of spinal nerves of the cauda equina in the spinal canal. For lower limbs spinal PAS, we propose estimating appropriate ISIs on the basis of F-response and motor-evoked potential (MEP) latencies. The use of navigation in TMS and ensuring correct PNS electrode placement with F-response recording enhances the precision of the method. Our protocol induced over two-fold MEP amplitude facilitation in healthy subjects, being effective in all subjects and nerves tested. We report for the first time the individual estimation of ISIs in spinal PAS for lower limbs. Estimation of ISI on the basis of F and MEP latencies is sufficient to effectively enhance corticospinal transmission by lower limb spinal PAS in healthy subjects.

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Poster

537. Plasticity After Spinal Cord Injury II

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 537.17/KK7

Topic: D.10. Spinal Cord Injury and Plasticity

Support: Adelson Program in Neural Repair and Rehabilitation

Title: Comparison of two TMS plasticity protocols across two TMS labs

Authors: ***A. D. WU**[1,2], D. J. EDWARDS[4,5], M. IACOBONI[2,3], C. DEBLIECK[1,2], M. CORTES[4,5], R. R. RATAN[4,5], B. DOBKIN[1]
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Abstract: Transcranial magnetic stimulation (TMS) can be used in many modulation protocols to induce lasting changes in motor evoked potentials (MEP). These effects resemble changes seen with plasticity paradigms in animal models and have been proposed as probes of plasticity in human subjects. However, data on reproducibility of protocols between TMS labs are sparse, but essential if these tools are to be used in study of plasticity changes in patients. We tested a standard (paired-associative stimulation, PAS) and a novel (dual-TMS) modulation protocol across two TMS labs (UCLA, Burke-Cornell). The goal was to establish uniform methods of

assessing plasticity in human subjects which can be applied to the study of candidate drugs, manipulating plasticity, for brain and spinal cord injury recovery studies. PAS protocol involved 200 pairs of ulnar nerve stimulation and contralateral motor cortex TMS with an interstimulus interval (ISI) of 25 msec at 0.25 Hz. Dual-TMS protocol involved 90 pairs of TMS over right posterior parietal cortex (PPC) followed by left dorsal premotor cortex (PM) with 1 msec ISI at 0.1 Hz. Outcome MEPs were recorded from right FDI muscle for 60 min every 10 min after each protocol. For PAS, 24 control subjects (mean 26yo [18-56 yo], 11M) were tested. MEP amplitudes increased over baseline following PAS (20, 30, 40 min, p=0.02, 0.005, 0.04). However, we found no significant MEP facilitation analyzing data from each site separately. No significant differences between sites were found at any time point. For dual-TMS, 19 control subjects (mean 27 yo [18-47 yo], 8M) were tested. MEP amplitudes increased over baseline following dual-TMS (30, 40, 50 min, p=0.04, 0.03, 0.04). We found significant MEP facilitation at 40 min (p=0.03) and trends without significance at 50, 60 min (p=0.08, 0.05) at UCLA (n=11) and at Burke at 30 min (p=0.09, n=8). No significant differences between sites were found at any time point. Data suggest PAS and dual-TMS modulation as tests for plasticity effects in producing post-modulation MEP facilitation using combined data from both sites. PAS effects are compatible with spike-timing mechanisms. The short ISI cortico-cortical dual-TMS effects support a hypothesis of increased synchrony and functional connectivity in a parietal-premotor circuit and its downstream influence on the motor cortex. Data suggest that MEP data from two sites are comparable, but limited sample size make this tentative. Future work will increase sample sizes to ensure between site reliability of the protocol across sites and compare within-subject reliability between sites in anticipation of looking at plasticity following stroke in humans.

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Poster

537. Plasticity After Spinal Cord Injury II

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 537.18/KK8

Topic: D.10. Spinal Cord Injury and Plasticity

Title: Characterization of thoracic (Th9), complete transection spinal cord injury model of muscle spasticity in the rat

Authors: *J. A. CORLETO[1], M. BRAVO-HERNANDEZ[3], O. KAKINOHANA[2], M. HEFFERAN[4], M. MARSALA[2]
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Abstract: José A. Corleto, Mariana Bravo-Hernández, Osamu Kakinohana, Camila Santucci, Michael P. Hefferan, Martin Marsala University of California, San Diego, La Jolla, CA, USA
The development of muscle spasticity is a serious complication associated with traumatic spinal cord injury. It is believed that the mechanism leading to development of muscle spasticity is the result of the loss of descending facilitatory inhibition, increased sensory input and/or loss of local segmental inhibitory interneurons. Here, we characterize a thoracic 9 (T9) complete transection model in Sprague-Dawley (SD) rats to study the onset of muscle spasticity following SCI. Adult SD rats were used to perform a T8 laminectomy followed a complete, T9 transection of the spinal cord. To identify the presence of spasticity, two different qualitative tests were employed: i) changes in ankle resistance and corresponding EMG activity were measured in the gastrocnemius muscle in fully awake animals using a computer-controlled ankle rotational system, and ii) gastrocnemius muscle EMG response was recorded after applying a progressively increased paw pressure using von Frey filaments. The changes in H-reflex and rate dependent depression (RDD) in T9-transected rats were also measured. In the pharmacological part of the study, the effect of systemic (IP) treatment with: i) Baclofen (10 mg/kg), ii) Tizanidine (1 mg/kg), or iii) NGX424 (1 mg/kg) was studied. A time-dependent and muscle stretch-velocity-dependent development of spasticity response was seen between 1-3 months after spinal transection. Treatment with baclofen, tizanidine, and NGX424 led to significant suppression of otherwise increased EMG activity measured during ankle rotation. Analysis of the H-reflex showed a significant increase in the H wave at 3 months post-transection when compared to naïve non-injured animals. Similarly, an average 60% loss in rate dependent depression was measured at high stimulation frequencies (3, 5, and 10 Hz) in spastic animals. Analysis of tactile stimulus-evoked EMG response showed the presence of tactile hypersensitivity at intervals longer than 4 weeks after spinal transection. This hypersensitivity was effectively blocked by systemic treatment with baclofen (10-20 mg/kg, ip). These data demonstrate that rats with complete spinal transection develop a consistent and prominent muscle spasticity/spinal hyper-reflexia at intervals of 1-3 months after injury. The potent anti-spastic effect measured after treatment with clinically effective antispastic agents also demonstrates the validity of this model in screening new anti-spasticity agents.

Disclosures: J.A. Corleto: None. **M. Bravo-Hernandez:** None. **O. Kakinohana:** None. **M. Hefferan:** None. **M. Marsala:** None.

Poster

537. Plasticity After Spinal Cord Injury II

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Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 537.19/KK9

Topic: D.10. Spinal Cord Injury and Plasticity

Support: Craig H Neilson Foundation. 191152

NJCSCR 07-3063-SCR-E-0

Title: Multi muscle neuromuscular stimulation of the lower limbs: Effect on Motor Pools

Authors: *G. F. FORREST[1], E. JOHNSON[1], A. RAMANUJAM[2], E. GARBARINI[2], R. LAMB[2]
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Abstract: Acute spinal cord injury often leads to rapid muscle atrophy in the paralyzed limbs. Recently we have shown that an intense novel form of multi-muscle neuromuscular stimulation combined with dynamic stand retraining task may potentially restore muscle structure and function after sub acute to chronic, motor-complete SCI. Recovery of stepping and standing after spinal cord injury is often measured by studying motor pool activation of the lower extremity during standing or stepping with very little attention given to the importance of the amount of leg muscle mass and its corresponding effect on neuromuscular control for the reorganization of the lower or upper extremity neural circuitry. Recently, we have also shown that a large dose of repetitive task specific stand and step training can improve trunk function as well as standing, stepping or walking overground for sub acute to chronic SCI. However, little is known on the flexor and extensor motor pool recruitment changes or neural changes during stepping when there has been an significant increase in lower limb muscle volume/strength (through multi muscle neuromuscular stimulation with mechanical loading) of a previous atrophied muscle. We will present data for several individuals with a cervical and thoracic, motor complete spinal cord injury who have undergone a large number of standardized repetitive task specific training sessions with mechanical loading combined with lower extremity multi neuromuscular stimulation. These data will evaluate the changes in leg and trunk extensor and flexor muscle activation pools or motor patterns during active standing perturbations and stepping concomitant with the changes in lower limb muscle volume. In addition, these data will be compared to those individuals who received the lower extremity multi neuromuscular stimulation without mechanical loading. For an increase in muscle volume in the lower extremity extensors and flexors in both the neuromuscular stimulated groups (loaded and unloaded groups) it was found

that there was a significant increase in flexor and extensors muscle activation amplitude during continuous stepping in the neuromuscular stimulated and mechanical loaded group only. It was noted that the the phasic coordination of the flexor and extensor muscle activation pools were inappropriate for stepping. Multi muscle lower limb hypertrophy/strength gains may require repetitive task specific training to benefit the reorganization of the neural circuitry after severe human spinal cord injury.

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Poster

537. Plasticity After Spinal Cord Injury II

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Topic: D.10. Spinal Cord Injury and Plasticity

Support: Neilsen Foundation

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Paul G. Allen Family Foundation grant to Chet T. Moritz

Title: Optogenetic mapping of forelimb movement in the rat cervical spinal cord

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Abstract: In a previous study, the Moritz Laboratory meticulously mapped the responses of forelimb muscle activation and movements in response to intraspinal stimulation at various sites along the cervical spinal cord. A variety of complex movements were elicited depending on the site of stimulation. In the current study, we perform a similar assessment using a novel type of

stimulation called optogenetics. This technique allows for the targeted activation of cells stably transfected with the light-activatable channel channelrhodopsin (Chr2) with blue light. In the current study, rats were pre-transfected with AAV-CamkII2alpha-Chr2-mcherry virus with a viral injection into the gray matter of the hemicord along spinal segments C3 through T1. Several weeks later, rats underwent a mapping procedure in which forelimb muscle activation and movements were assessed across a systematic range of sites along the surface of the cord while stimulating those sites optogenetically. Similar to what was reported in our previous work, we found that optogenetic stimulation activated different muscles and elicited different movements depending on the site being stimulated. Further, we found that this optogenetic system has the intriguing capability to elicit movements when stimulated with a two micron microLED from above the surface of the spinal cord. In combination, these findings will assist in our future plans to use optogenetics as therapeutic stimulation for enhancing functional recovery after spinal cord injury.

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Poster

537. Plasticity After Spinal Cord Injury II

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Topic: D.10. Spinal Cord Injury and Plasticity

Support: US Army MRAA W81XWH-12-1-504

Title: Inhibition of Nav1.8 channels reduces pain-related behavior after spinal cord injury

Authors: *M. A. ODEM[1], J. K. HADDEN[1], R. J. CROOK[1], J. DU[2], S. M. CARLTON[2], Q. YANG[1], E. T. WALTERS[1]
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Abstract: Chronic pain is an intractable problem for many people with spinal cord injury (SCI). Unexpectedly, we found in a rat model of contusive SCI at T10 that this central neuropathic pain is associated with persistent hyperexcitability and spontaneous activity (SA) in the cell bodies of primary nociceptors recorded *in vitro* and *in vivo* below the injury level (Bedi et al. J Neurosci, 30:14870, 2010; Wu et al. Pain, 154:2130, 2013). Primary afferent neurons, including most

nociceptors, are the only neurons that express the voltage-gated sodium channel, Nav1.8. We found recently (Yang et al., unpublished) that chronic hyperreflexia and nociceptor SA induced by SCI can be reversed by antisense knockdown of Nav1.8 channels, and that SCI-induced SA in dissociated nociceptors can also be suppressed by the selective Nav1.8 antagonist, A-803467. We are now testing the prediction that A-803467 will also ameliorate hyperreflexia and other pain-related behavioral changes after SCI. Injection of A-803467 (100 mg/kg i.p.) caused a significant reversal of hindlimb hyperreflexia assessed with heat and mechanical test stimuli 6-8 weeks after SCI, but had no significant effect on hindlimb reflexes in control rats given sham surgery. Ongoing experiments are testing the effects of A-803467 on operant tests of spontaneous pain and evoked pain. Spontaneous pain is assessed with a conditioned place preference test. Evoked pain is assessed with an operant task in which a rat has to choose between escaping from an aversive bright light and crossing a floor of potentially painful probes. In addition, preliminary results indicate that application of A-803467 to exposed DRG in an anesthetized rat reduces chronic SCI-induced SA generated in lumbar DRG *in vivo*. These results suggest that drugs selectively inhibiting Nav1.8 channels may provide a promising approach for treating persistent neuropathic pain after SCI.

Disclosures: M.A. Odem: None. **J.K. Hadden:** None. **R.J. Crook:** None. **J. Du:** None. **S.M. Carlton:** None. **Q. Yang:** None. **E.T. Walters:** None.

Poster

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Program#/Poster#: 537.22/KK12

Topic: D.10. Spinal Cord Injury and Plasticity

Support: Craig H Neilsen Foundation

Title: Sympathetic preganglionic and afferent stimulation-evoked responses in paravertebral thoracic chain ganglia

Authors: *M. HALDER[1], M. SAWCHUK[2], S. HOCHMAN[3]
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Abstract: Paravertebral sympathetic postganglionic neurons (SPNs) located in thoracic chain sympathetic ganglia represent the predominant sympathetic control of vascular function in the upper and middle extremities. These ganglia are practically inaccessible for *in vivo* studies, and

consequently have barely been studied directly (Blackman and Purves J Pysiol 1969; Lichtman et al J Physiol 1980). We developed an *in vitro* adult mouse model to examine evoked population responses following stimulation of segmental sympathetic preganglionic and primary afferents axons. The approach retains the adult mouse thoracic sympathetic chain ganglia *in situ* with connections to dorsal root ganglia, dorsal roots and ventral roots. Dorsal roots were stimulated to examine primary afferent evoked responses, and ventral root stimulation was used to recruit axons of sympathetic preganglionic neurons. Responses in individual ganglia were recorded with suction electrodes attached to cut inter-ganglia nerve bundles. We first characterized the multisegmental axonal composition in individual chain ganglia. When recording from T12 ganglia, we observed orthodromic responses following stimulation of T7-T12 ventral roots and antidromic responses from T4-T12 dorsal roots. In addition to recordings of compound action potentials, dorsal and ventral root stimulation evoked synaptically-mediated spiking responses were seen and subsequently blocked in a high Mg2+/low Ca2+ solution or in the presence of ionotropic receptor antagonists. Both dorsal and ventral root stimulation evoked responses both showed sensitivity to ionotropic glutamatergic and cholinergic receptor antagonists while ventral root-evoked synaptic actions were also facilitated in the presence of the nitric oxide donor DEANO. The observation of considerable synaptic actions arising from visceral afferents was unexpected. To further support this observation, we undertook post-experiment immunolabeling within the chain and found CGRP+ puncta in a position to interact synaptically with SPNs. Overall, we conclude that most thoracic ganglia receive widely divergent input from multiple spinal segments. Moreover, the visceral afferents that within chain ganglia can project many spinal segments prior to entry into spinal cord and also appear to issue collaterals within the ganglia and have synaptic actions on SPNs.

Disclosures: **M. Halder:** None. **S. Hochman:** None. **M. Sawchuk:** None.

Poster

538. Circuit Connectivity

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Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Support: GM103507

KSCHIRT 13-14

Title: Conditional silencing of adult rat spinal locomotor circuitry induces hopping

Authors: *A. POCRATSKY[1], A. S. RIEGLER[2], J. R. MOREHOUSE[2], D. A. BURKE[2], J. T. HARDIN[3], R. M. HOWARD[2], D. S. K. MAGNUSON[2], S. R. WHITTEMORE[2]
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Abstract: Identifying the functional role of spared neural pathways post-spinal cord injury can help design targeted rehabilitation strategies to enhance recovery. Methods to functionally dissect locomotor circuitry primarily consist of non-specific spinal lesions or a limited number of neuron-specific, fate-mapped transgenic strains. A conditional two viral vector system was recently developed, allowing specific neuronal pathways to be functionally silenced based solely on their anatomy. Targeted neurons are silenced *in vivo* through reversible expression of enhanced tetanus neurotoxin (eTeNT) that proteolytically cleaves vesicle-associated membrane protein 2, which is essential for synaptic vesicle exocytosis. Here, we conditionally silenced L2 interneurons with descending projections to L5. Those neurons were double-infected by bilateral injections of HiRet-TRE-EGFP.eTeNT at L5 and the tetracycline-responsive AAV2-CMV-rtTAV16 at L2. Doxycycline (DOX, 15 mg/ml) was given ad libitum to induce eTeNT expression. Behavioral, kinematic, gait, and electrophysiological assessments were performed pre-injections, before DOX-induced neurotransmission silencing, during DOX treatment (DOXON), and post-DOX. DOXON was repeated one month later to assess reproducibility. Silencing descending L2 interneurons induced a hop-like phenotype in the hindlimbs. Hopping was quantified by a significant increase in average hip excursion and changes to locomotor-related measures, including hindlimb swing, stance, and stride during volitional and treadmill-based stepping. L2-L5 interneuron silencing switched the step sequence pattern from alternate to cruciate wherein forelimb stepping precedes hindlimb, as opposed to alternation. These DOXON functional changes were replicated one month after DOX washout. Current studies focus on long ascending propriospinal neurons (LAPNs), a neural pathway thought to functionally interconnect hind- and forelimb central pattern generators. Bilateral injections of eTeNT at C6 and rtTAV16 at L2 doubly-infected LAPNs. DOX treatment induced a symmetrical hop-like gait involving both fore- and hindlimbs during volitional locomotion. Ongoing behavioral, kinematic, gait, and electrophysiological analyses are being performed to quantitatively describe this locomotor behavior. This approach enables delineation of the functional contribution of propriospinal pathways in normal locomotor function, after spinal cord injury, and importantly after targeted rehabilitative therapy.

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Poster

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Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Support: NSF Grant IOS 1120291

GSU Brains & Behavior Fellowship

Title: Proprioceptive feedback affects antidromic activity in sensory afferents of crayfish legs

Authors: B. CHUNG[1], J. BACQUE-CAZENAVE[1], D. CATTAERT[2], *D. H. EDWARDS[1]
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Abstract: In both vertebrates and invertebrates, primary afferent depolarization (PAD) leads to antidromic excitation of afferents. Antidromic afferent spikes serve as a hallmark of PAD and may act to inhibit generation of orthodromic spikes in the afferent axon. Antidromic spikes have been recorded in the coxobasal chordotonal organ (CBCO) stretch receptor afferents of the crayfish leg during walking, but their role in producing or modifying the crayfish walking motor program is unclear. We have recorded antidromic spikes in CBCO afferents in an *in vitro* preparation of the thoracic nerve cord in which one of the leg proprioceptive feedback loops has been closed. Spikes in the leg levator and depressor motor nerves drive levator and depressor muscles of a computational neuromechanical model of the crayfish leg, and movement of the leg stretches and releases the intact CBCO stretch receptor organ to produce appropriately timed feedback to the central nervous system. This preparation can be in either a quiescent state, where neurons fire tonically and imposed leg lifts evoke downward resistance reflexes, or in an active state, where Levator/Depressor (Lev/Dep) burst pairs occur and leg lift evokes an assistance reflex. In six preparations, spikes from the Lev, Dep, and CBCO nerves were recorded when the feedback loop was closed and when it was open during both quiescent and active states. After sorting spikes among 18 motorneurons (MNs) in the Lev nerve, 13 MNs in the Dep nerve, and 36 afferents in the CBCO nerve, we used cross-correlation analysis to identify afferents excited antidromically by MNs. We found that nearly all CBCO afferents (e.g., 28/36 in one preparation) were excited antidromically, and that a single Lev MN excited all of them, while a few other Lev and Dep MNs and the Common Inhibitor MN each excited only a few afferents (<5). Twice as many afferents were excited antidromically when the preparation was quiescent (25/36) than when active (12/36), and in both states only the larger units were excited when the feedback loop was closed. In the active state under closed loop, the preparation produced rhythmic pairs of

Lev/Dep bursts at a frequency of 1/10s, and antidromic excitation was restricted to larger afferents firing strongly at the onset of each Lev MN burst. All these afferents were sensitive to CBCO release and excited Dep MNs in quiescent preparations to produce resistance reflexes. This result suggests that antidromic excitation of these afferents at the beginning of the Lev burst in closed loop will prevent resistance reflexes that would interfere with the leg rise. In open loop, antidromic activity was weaker and more evenly distributed throughout the burst duration.

Disclosures: B. Chung: None. **D.H. Edwards:** None. **J. Bacque-Cazenave:** None. **D. Cattaert:** None.

Poster

538. Circuit Connectivity

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Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Support: DA1182/1-1

Title: Experimental and theoretical studies concerning an inter-segmental neuronal network controlling locomotion, and application of the results to locomotion with more than six legs

Authors: *M. J. GRABOWSKA, T. I. TÓTH, A. BÜSCHGES, A. BORGMANN, S. DAUN-GRUHN
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Abstract: In legged animals, inter-segmental coordination is required to perform locomotion. This is true for insects, e.g. the stick insect, as well as for invertebrates with more than six walking legs, like crabs and crayfish. In all of these animals, inter-segmental neural connections, as well as local sensory signals, are assumed to play a crucial role (Barnes 1974; Sillar, Clarac and Bush 1987; Cruse and Müller 1986; Clarac 1982). Daun-Gruhn and Tóth (2011) designed an inter-segmental network model to mimic some basic aspects of the inter-leg coordination in the stick insect. As an initial step, they focused on the local segmental networks with central pattern generators (CPG) as their core elements that generate rhythmic activity of the thoraco-coxal motoneuron pools, i.e. the protractor-retractor neuro-muscular system (ThC-CPG), in three adjacent segments. The three ipsilateral segmental ThC-CPGs are connected rostro-caudally, and the connections are modulated by excitatory sensory signals from anterior segments and by local inhibitory ones from the same segment. It was found that, in order to obtain smooth transitions

between different coordination patterns, a neural connection from the meta-thoracic CPG to the pro-thoracic had to be assumed, making the connections cyclic. We present experimental evidence for this caudo-rostral inter-segmental neural connection in the stick insect. A walking hind leg was found to be able to entrain a pilocarpine-induced rhythm in the ThC-CPG of the pro-thoracic segment. Hence, the related assumption in the model could be confirmed. In addition, we used this model to test whether it could serve as basic module of an inter-segmental neuronal control network of n-legged (n>6) walking animals. To this end, we extended the model by additional segmental CPGs that had the same properties as the existing ones in the original model. We changed phase relations between the CPGs by varying appropriate system variables (e.g. excitatory and inhibitory sensory inputs), or model parameters (e.g. drive to the CPGs). In this way, we could simulate different coordination patterns, as well as transitions between them, that were similar to those observed in 8-legged animals. These results show that the inter-segmental network model, which is supported by experimental data gathered in the stick insect walking system, might serve as a fundamental module to simulate walking in animals with more than six legs.

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Poster

538. Circuit Connectivity

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Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Support: NSERC Discovery Grant 386664

Title: Electrical coupling influences the synchrony and pattern of spiking in identified peptidergic neurons

Authors: *C. C. BEEKHARRY, N. S. MAGOSKI
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Abstract: Electrically-coupled neurons communicate through an assembly of channels called gap junctions, which connect one cell to another and mediate the transfer of metabolites and current. Coinciding with an ability to permit current flow, often in both directions, electrical coupling is involved in the synchronization and rapid transmission of action potentials within

circuits. In turn, this is believed to influence the bursting pattern and firing frequency of coupled neurons. The present study concerns an electrically-coupled two-neuron circuit within the CNS of the gastropod mollusc, Lymnaea stagnalis. The two neurons, designated Visceral Dorsal 1 (VD1) and Right Parietal Dorsal 2 (RPD2) are readily identifiable, peptidergic, strongly-coupled, and innervate the heart. Using dual sharp-electrode current-clamp recording in isolated brain preparations, we investigated how the disruption of electrical coupling influences action potential firing frequency and synchrony within the VD1/RPD2 network. Initially, axotomy was performed by cutting the connective linking VD1 and RPD2. This completely uncoupled the neurons, but only disrupted the firing rate and pattern of RPD2, consistent with prior reports indicating VD1 is the master and RPD2 is the follower in the network. Neurons were also exposed to gap junction blockers, assayed by hyperpolarizing current injection into either VD1 or RPD2 to determine the coupling coefficient before and after drug application. The addition of niflumic acid (NFA) and 5-nitro-2-(3-phenylpropylamino) benzoic acid (NPPB) significantly decreased the coupling coefficient, whereas meclofenamic acid, quinine, α- glycyrrhetinic acid, and carbenoxolone had no effect. However, although NFA and NPPB significantly lowered coupling, the drugs typically did not desynchronize the VD1/RPD2 network. Nevertheless, there were instances where NFA and NPPB resulted in RPD2 inappropriately exciting VD1 and causing it to temporarily fire out of sync. Moreover, in the presence of these uncoupling agents, supressing the activity of VD1 with hyperpolarizing current revealed a distinct firing pattern and frequency in RPD2, with some similarities to the pattern seen following axotomy. This suggests that in a continuously active or bursting network, consisting of a master/follower arrangement, strong electrical coupling appears to be a requirement for the network to maintain both synchronous output and rhythm. Perhaps in Lymnaea, such output influences cardiovascular function and heart rate.

Disclosures: **C.C. Beekharry:** None. **N.S. Magoski:** None.

Poster

538. Circuit Connectivity

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Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Support: ERC starting grant "Optoloco"

Title: Recruitment of spinal neurons recorded with GCaMP6 during different modes of locomotion in the larval zebrafish

Authors: *K. E. SEVERI[1,2,3,4], A. PRENDERGAST[1,2,3,4], K. KAWAKAMI[5], C. WYART[1,2,3,4]
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Abstract: Spinal central pattern generators are a challenge to unravel due to the multitude of cell types which may be playing critically different roles yet operating simultaneously. The zebrafish larva offers the advantage of a small, transparent, genetically tractable organism with a vertebrate organization with which to deconstruct the function of particular cell types during locomotion. Glycinergic interneurons have been shown in mammals to play a critical role in left-right alternation as well as in modulating locomotor speed. Multiple glycinergic spinal interneuron classes in the zebrafish are anatomically characterized, and some have known mammalian homologues, but less is known about their functional roles. We aim to genetically target specific interneuron types by performing gene trap and enhancer trap screens and identifying specific spinal expression in glycinergic cell classes. We have chosen two stimuli to evoke locomotion at different speeds: slow locomotion during the optomotor response, and fast locomotion evoked by escape-inducing stimuli. Utilizing these two stimuli we assess the participation of different spinal cell types in the slow or fast locomotor circuits. Our approach combines two-photon *in vivo* calcium imaging of genetically-encoded GCaMP calcium indicators with fictive locomotion to record neuronal activity while the circuit is active. By combining genetic access to specific cell classes, calcium imaging, and recordings of fictive locomotion, we can investigate the selective recruitment of glycinergic cells for different speeds of locomotion.

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Poster

538. Circuit Connectivity

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Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Support: CIHR 15129

NSERC 217435

FRSQ 5249

GLFC 8400272

Title: Organization of the descending locomotor drive from the mesencephalic locomotor region to reticulospinal neurons in salamanders

Authors: *D. RYCZKO[1,2], F. AUCLAIR[1], J.-M. CABELGUEN[3], R. DUBUC[1,2]
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Abstract: In salamanders as in other vertebrates, the Mesencephalic Locomotor Region (MLR) finely controls forward locomotion (Cabelguen et al 2003 J Neurosci 23:2434-9). However the mechanisms by which the MLR controls locomotion in salamanders are not known. Here we examined the anatomy and physiology of MLR outputs to the reticular formation (RF) in the salamander Notophthalmus viridescens, with special reference to the reticulospinal (RS) system known to receive MLR inputs in other species. Tracer injections in the hindbrain RF coupled with immunofluorescence against choline acetyltransferase (ChAT) revealed double-labelled cells in the laterodorsal tegmental nucleus (LDT) that were located in the MLR. ChAT-negative cells located in close proximity to the LDT were found to project to the RF as well. We then recorded intracellularly the activity of reticular cells in a semi-intact preparation in which the brain is exposed and the body is left intact and free to move. Trains of unilateral MLR electrical stimulation elicited alternating limb movements together with spiking activity in reticular cells. Spiking and stepping frequencies were positively correlated with the intensity of MLR stimulation. Single shocks applied to the MLR evoked excitatory synaptic responses in reticular cells. We confirmed that MLR stimulation activated RS neurons by using calcium (Ca2+) imaging of the RS cells of the middle and inferior reticular nuclei retrogradely labelled from a Ca2+ green dextran injection in the spinal cord. Trains of unilateral MLR stimulation elicited bilateral Ca2+ increase in RS cells. Again, MLR stimulation intensity finely controlled RS Ca2+ responses. We explored the contribution of glutamatergic (Glu) and cholinergic (Ach) neurotransmission in these responses. Bath-applied Glu antagonists (CNQX + AP5) reduced RS responses evoked by trains of MLR stimulation. Local microinjections of glutamate onto RS neurons precisely controlled RS responses. Microinjections of Glu antagonists onto RS neurons decreased RS responses elicited by trains of MLR stimulation. Furthermore, bath-application of the acetylcholinesterase inhibitor eserine increased the RS responses elicited by trains of MLR stimulation and microinjections of Ach onto RS neurons elicited Ca2+ responses, though smaller than those elicited by glutamate. The anatomical and physiological organization of this descending locomotor drive seems to be strikingly similar to that previously reported in lamprey.

We thus suggest that the dual Ach/Glu drive from the MLR to RS cells could constitute a blueprint of the locomotor drive in vertebrates.

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Poster

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Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Support: GLFC 8400272

CIHR 15129

NSERC 217435

FRSQ 5249

Title: The anatomy and physiology of the diffuse chemosensory system in the sea lamprey

Authors: *G. DAGHFOUS[1,2], F. BLUMENTHAL[1], F. AUCLAIR[1], M. MANSOURI[1], B. ZIELINSKI[3], R. DUBUC[1,2]
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Abstract: In addition to the olfactory and gustatory systems, vertebrates possess another chemosensory system, the diffuse chemosensory system. Its receptors, the so-called “solitary chemosensory cells” (SCCs), are specialized epithelial cells that are associated with a nerve fiber and share some features with taste receptor cells. Solitary chemosensory cells have been reported in all vertebrate lineages examined so far, including mammals. It was shown that their localization vary widely from one species to the other. For instance, in fish, SCCs are present in the oropharynx, gills and skin, whereas they seem to be restricted to the airways in mammals. Physiological studies pointed out that their function differs as well. In fish, SSCs serve as food or predator detectors, while in mammals SCCs appear to act as toxins detectors. Consequently, despite a wide phylogenetic distribution, the diffuse chemosensory system is still poorly

understood. Knowledge of its anatomy and physiology in lampreys, the most basal lineage of vertebrates, can shed light on this problem. Putative SCCs, referred to as “oligovillous cells”, have already been reported on the body surface and on cutaneous finger-like extensions named “papillae” of brook lampreys [Whitear and Lane, 1983, J Zool Lond]. Our anatomical investigation, in the sea lamprey, revealed the presence of papillae around the oral hood, on the dorsal fins, and on the posterior rim of the gill pores. For the present report, investigation was limited to the gill papillae. Extracellular recordings from individual gill papillae showed multiunitary action potentials in response to chemical stimulation, confirming that they contain chemosensory cells. Among the substances tested, food-related stimuli (trout washing water and amino acids) produced the most potent responses. Examination of the epidermal surface of the papillae under environmental and conventional SEM showed the presence of tufts of several microvilli protruding from cell apices surrounded by epidermal cells. Their location and morphology support a chemosensory role. Immunofluorescence directed against acetylated tubulin, a reliable marker of nerve fibers in the peripheral nervous system of lampreys, demonstrated that gill papillae are well innervated structures. *In vivo* injections of axonal tracers in the gill papillae labeled glossopharyngeal (IX) and vagal (X) ganglion cells with central projections forming an ascending limb that reached the mesopontine border area and a descending limb that reached the rostral spinal cord. Our study constitutes the first step in the characterization of the diffuse chemosensory system in lampreys.

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Poster

538. Circuit Connectivity

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Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Support: ERC Starter Grant - OPTOLOCO

Title: ChannelRhodopsin mediated mapping of CerebroSpinal Fluid Contacting Neuron connectivity in the zebrafish spinal cord

Authors: *J. HUBBARD, C. STOKES, S. NUNES-FIGUEIREDO, C. WYART
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Abstract: Locomotion in vertebrates relies on central pattern generators (CPGs) located in the ventral spinal cord. While most sensory information is thought to project on the dorsal spinal cord, CerebroSpinal Fluid contacting neurons (CSF-cNs) exhibit the morphology of sensory cells in the ventral spinal cord. CSF-cNs are specialized GABAergic neurons which protrude a ciliated brush into the central canal. Optogenetic activation of CSF-cNs can modulate swimming behavior in larval zebrafish, suggesting these cells signal to elements of the CPGs (Wyart et al., 2009). However, to date the connectivity pattern of CSF-cNs remains a mystery. Here we combine fluorescence-guided patch clamp of identified spinal neurons and optogenetic activation of CSF-cNs *in vivo* to uncover neuronal cell types receiving monosynaptic input from CSF-cNs in the spinal cord. Taking advantage of a library of transgenic lines, we record from spinal neuron subtypes with identified morphology, genetic markers and neurotransmitter phenotype. A short pulse of blue light leading to the ChannelRhodopsin2 (ChR2) mediated activation of CSF-cNs reveals the existence of monosynaptic connectivity from CSF-cNs to the recorded cells. In order to restrain the activation to single cells, we have developed optical setups to spatially pattern the blue light in 2D with Digital Mirror Devices and in 3D with Digital Holography. We show that CSF-cNs exhibit redundant and diverse patterns of neuronal innervation, suggesting that subtypes of CSF-cNs preferentially target distinct classes of spinal neurons. Altogether our work reveals part of the functional connectivity diagram of spinal CSF-cNs and provides critical insight on how these neurons can modulate locomotor activity.

Disclosures: J. Hubbard: None. **C. Stokes:** None. **S. Nunes-Figueiredo:** None. **C. Wyart:** None.

Poster

538. Circuit Connectivity

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Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Title: Effect on intrinsic bursting on restoration of rhythmic activity in respiratory network

Authors: *N. TOPORIKOVA[1], N. MELLEN[2]
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Abstract: Rhythmic inspiratory movement of a diaphragm is initiated by neuronal activity of the pre-Botzinger(pre-BotC) complex in ventrolateral medulla. This rhythm is remarkably stable and capable of restoring itself after physiological or pharmacological perturbations. If excitatory

connections among pre-BotC neurons are blocked, the rhythmic respiratory output ceases. However in this condition of synaptic block a small fraction of neurons still produce rhythmic bursting. It is currently unclear if these intrinsic bursting properties are relevant to generation or maintenance of respiratory rhythm generation. In this computational study we tested the effect of intrinsic neuronal properties on restoration and maintenance of transient disturbance in synaptic connectivity. We conducted a series of numerical experiments with several types of network connectivities and varying fractions of intrinsic bursters to compare how fast such networks can fully recover after brief perturbation. Our simulation shows that networks with larger fractions of pacemakers recovers faster from transient synaptic blockade. These results indicate a possible role of intrinsic bursters in restoring respiratory rhythmicity after brief perturbation.

Disclosures: **N. Toporikova:** None. **N. Mellen:** None.

Poster

538. Circuit Connectivity

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Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Title: Network properties underlying vocal production in the African clawed frog, Xenopus laevis

Authors: K. LAWTON, J. PERRY, *E. ZORNIK
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Abstract: Central pattern generators (CPGs) are ubiquitous self-sustaining circuits crucial for the production of rhythmic motor behaviors. The diversity of cell types and network connections provide both precision and flexibility in behavior. Investigations of many CPGs controlling both vertebrate and invertebrate behaviors have elucidated general functional principles across neuronal circuits. The male advertisement call in the frog *Xenopus laevis*, is a powerful model for understanding vertebrate behaviors because the active CPG can be studied *in vitro* in a "singing brain in a dish". Xenopus calls are produced by a two-part CPG: 1) cranial motor nucleus IX-X (n.IX-X) containing motor neurons that project to the larynx and 2) the dorsal tegmental area of the medulla (DTAM) which contains neurons that generate the fast trills: fast trill neurons (FTNs). Two major lines of evidence suggest that an efferent motor copy from n.IX-X is necessary for normal FTN function. First, transections between n.IX-X and DTAM caused FTNs to fire with a variable pattern and faster frequency. Second, blockade of motor neurons (by

backfilling with the intracellular Na^+ channel blocker QX-314) also disrupted normal FTN spiking rates in the functioning circuit. Together, we conclude that an efferent copy from n.IX-X motor neurons to DTAM regulates FTN firing. No direct anatomical connection from n.IX-X MNs to DTAM has been observed, suggesting the existence of an intervening interneuron. Electrical stimulation of the laryngeal motor nerve produced inhibition in the majority of the recorded FTNs. Because motor neurons are cholinergic, we predicted the connection from n.IX-X to DTAM may be mediated via excitatory acetylcholine receptors. Low concentrations (1 – 10 μM) of the nAChR blocker tubocurarine altered fast trill rates. Higher concentrations blocked song production, but induced fast bursting of FTNs as observed during QX-314 and n.IX-X transections. Together results suggest that n.IX-X motor neurons are providing synchronizing inputs to DTAM neurons via activation of nAChR-expressing n.IX-X interneurons.

Disclosures: **K. Lawton:** None. **E. Zornik:** None. **J. Perry:** None.

Poster

538. Circuit Connectivity

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Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Support: NIH R01NS7323

Title: Computational modeling and qualitative analysis of spinal circuits underlying locomotor pattern generation and frequency-dependent left-right coordination

Authors: ***B. BACAK**[1], Y. MOLKOV[2], I. RYBAK[1]
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Abstract: Coordination between left and right neural activities in the spinal cord during locomotion is controlled by commissural interneurons (CINs). Several CIN types have been genetically identified, including the excitatory V3 and excitatory and inhibitory V0 types. Talpalar et al. (2013) reported that genetic elimination of the V0 CINs caused switching from a normal left-right alternating pattern of motor activity to a left-right synchronized “hopping” pattern. Furthermore, ablation of only the inhibitory V0 neurons ($V0_D$ subtype) resulted in a lack of left-right alternation at low locomotor frequencies and preservation of this alternation at high frequencies, whereas selective ablation of the excitatory V0 neurons ($V0_V$ subtype) maintained

the left-right alternation at low locomotor frequencies and switched the motor output to a left-right synchronized ("hopping") pattern at high frequencies. To explain the above findings we developed a simplified mathematical model of neural circuits consisting of four pacemaker neurons representing left and right flexor and left and right extensor half-centers interacting via the commissural pathways representing V3, $V0_D$, and $V0_V$ CINs. The “locomotor” frequency in the model was controlled by a parameter defining the excitability of neurons (via the leak reversal potentials) and commissural pathways, whose changes represented the corresponding changes induced by changing the concentration of N-methyl-D-aspartate (NMDA) applied in to control the locomotor frequency in the isolated rodent spinal cord preparations. The model demonstrated: (1) a typical left-right alternating pattern under control conditions; (2) switching to a synchronized hopping activity at any frequency after removing commissural connections representing both V0 ($V0_D$ and $V0_V$) neurons; (3) a synchronized pattern at low frequencies with alternation at high frequencies after removing commissural connections representing $V0_D$ neurons; (4) an alternating pattern at low frequencies with synchronized hopping at high frequencies after removing commissural connections representing $V0_V$ neurons. We used bifurcation and fast-slow decomposition methods to analyze the behavior of this network in the above states/regimes and transitions between them. The model reproduced a series of experimental phenomena and generated multiple predictions available to experimental testing.

Disclosures: **B. Bacak:** None. **Y. Molkov:** None. **I. Rybak:** None.

Poster

538. Circuit Connectivity

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 538.12/KK24

Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Support: NIH R01NS7323

ERC advanced grant, Söderberg Foundation

Title: Computational modeling of neural circuits in the mammalian spinal cord involved in left-right coordination of neural activity during locomotion

Authors: ***N. A. SHEVTSOVA**[1], A. E. TALPALAR[2], S. N. MARKIN[1], R. M. HARRIS-WARRICK[3], O. KIEHN[2], I. A. RYBAK[1]
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Abstract: Left-right interactions in the spinal cord are mediated by the commissural interneurons (CINs) whose axons cross the midline and affect neural circuits on the contralateral side of the cord. Several types of CINs have been genetically identified, including the excitatory V3 CINs and the inhibitory ($V0_D$) and excitatory ($V0_V$) V0 CINs. Talpalar et al. (2013) have recently demonstrated that: (a) removal of both V0 CIN types leads to a left-right synchronized, "hopping" pattern at all locomotor frequencies; (b) selective ablation of the excitatory $V0_V$ CINs maintains alternation at low frequencies but switches to synchronized activity at high frequencies; (c) ablation of only the inhibitory $V0_D$ CINs leads to a lack of left–right alternation at low frequencies, but maintains alternation at high frequencies. The ipsilaterally projecting V2a interneurons are recruited with the increasing locomotor speed (Zhong et al. 2011) and are necessary for left-right alternation at high frequencies (Crone et al. 2008, 2009). Our objective was to construct a computational model of bilaterally interacting central pattern generators (CPGs) that could reproduce and explain the above findings. In our model, the CPG on each side of the cord consisted of flexor and extensor half-centers. Each neural population consisted of 50-200 neurons modeled in the Hodgkin-Huxley style. The rhythmic locomotor activity was generated by an intrinsic neural mechanism involving the persistent sodium current present in CPG neurons. The left-right coordination was dependent on the balance between the three CIN pathways: a V3-mediated pathway that supported left-right synchronization, and $V0_D$- and $V0_V$-mediated pathways that supported left-right alternation. The activity of the inhibitory $V0_D$ CINs was driven by the ipsilateral flexor half-center. The activity of $V0_V$ neurons was mediated by the ipsilateral V2a neurons recruited with the increasing locomotor speed. The model demonstrates: (1) a left-right alternating pattern under control conditions; (2) a synchronized hopping pattern at any frequency after removing both V0 populations; (3) a synchronized pattern at low frequencies with alternation at high frequencies after removing the $V0_D$ populations; (4) an alternating pattern at low frequencies with synchronized hopping at high frequencies after removing either $V0_V$ or V2a populations. The model closely reproduces multiple experimental data, suggests the organization of commissural interactions in the spinal cord defining the left-right alternation at different locomotor speeds, and generates predictions for future experimental studies.

Disclosures: **N.A. Shevtsova:** None. **A.E. Talpalar:** None. **S.N. Markin:** None. **R.M. Harris-Warrick:** None. **O. Kiehn:** None. **I.A. Rybak:** None.

Poster

538. Circuit Connectivity

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Support: KAKENHI 25115702 (HN)

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KAKENHI 25870915 (RK)

KAKENHI 22115009 (TI)

Title: Synaptic modulation of spinal motoneurons during locomotor-like rhythmic activity in the alpha-chimaerin knockout mouse *in vitro*

Authors: *H. NISHIMARU[1], R. KOBAYASHI[2,3], S. ITOHARA[5], T. IWASATO[6,4]
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Abstract: In the developing mammalian spinal cord, tyrosine kinase receptor EphA4 is expressed on the tip of the extending axon of a population of ipsilaterally projecting neurons and its ligand ephrinB3 is localized in the midline. The interaction of these two molecules functions as a barrier preventing EphA4+ axons from crossing the midline. Deletion of the EphA4 or its downstream-signaling molecule alpha-chimaerin causes aberrant midline-crossing of these ipsilateral-projecting axons in the spinal cord and leads to a hopping gait in mice (Kullander et al., 2003, Iwasato et al., 2007). We have recently shown that in mutants in which these molecules were selectively deleted from excitatory vesicular-glutamate-tranporter-type-2-positive neurons, a similar hopping gait was observed indicating that the alteration of excitatory connections in the spinal circuit is one of the main causes of the hopping locomotor phenotype (Borgius et al. 2014). In this study we examined the synaptic modulation of lumbar motoneurons (MNs) during drug-induced locomotor-like rhythmic activity in the isolated spinal cord preparation taken from alpha-chimaerin knockout (Chn1-KO) neonates. Similar to wildtype MNs, membrane oscillation of an individual Chn1-KO L2-flexor-related-MN was time-locked with the ipsilateral flexor activity but not with that of the contralateral side indicating that it is unlikely that the firing pattern of Chn1-KO MNs is shaped by direct synaptic inputs from the contralateral network. Furthermore, we estimated the instantaneous frequency of excitatory and inhibitory synaptic inputs during the rhythmic activity from the membrane voltage trace based on the Ornstein−Uhlenbeck model (Kobayashi et. al. 2011). Our estimation showed that MNs were modulated by balanced excitatory and inhibitory synaptic inputs during the locomotor activity. These preliminary results suggest that Chn1-KO MNs are synaptically modulated in a similar way to wildtype MNs during locomotion and alteration of the connections between the bilateral

premotor networks may be responsible for the hopping gait in the EphA4 - alpha-chimaerin signaling mutants.

Disclosures: **H. Nishimaru:** None. **R. Kobayashi:** None. **S. Itohara:** None. **T. Iwasato:** None.

Poster

538. Circuit Connectivity

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Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Support: German science foundation CRC 870

German science foundation GRK 1373

Title: Corollary discharge informs cranial motor systems about locomotor activity

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Abstract: Corollary motor discharges are traditionally considered as predictors of self-generated sensory inputs. By interfering with the central processing, motor efference copies are able to counter unwanted consequences of an animal's own actions. In addition, intrinsic signals, generated during locomotion, serve as prospective substrate for assisting self-motion derived sensory feedback by directly activating image-stabilizing compensatory eye movements. The direct coupling between the spinal locomotor and the extraocular motor system opens the possibility that other brainstem motor systems are also influenced by intrinsic spinal efference copies during locomotion. Here, we studied in Xenopus laevis the impact of locomotor corollary discharge on the trigeminal motor system, which controls a number of cranial muscular elements in the rostral head region, including the motility of mechanosensory appendages. Pre-metamorphic tadpoles were used to monitor the movements of the bilateral pair of mobile rod-like tentacles, located at the corner of the mouth during locomotor activity. Free undulatory swimming caused a tonic lateral deflection of both tentacles that lasted throughout the entire locomotor event. Head-fixed semi-intact preparations of larval Xenopus with intact sensory appendages and isolated brainstem/spinal cord were used for quantitative video analysis of tentacle motion dynamics and trajectory during fictive locomotor episodes. In the absence of swimming-related sensory inputs, the tentacle motion exhibited a stereotyped motion pattern

with a reliability that increased with swimming strength. Tract-tracing experiments demonstrated a trigeminal nerve innervation of the single tentacle muscle and outlined a specific cluster of motoneurons in rhombomere 2 and 3. Multiple-unit recordings of the tentacle nerve close to the muscular insertion during fictive swimming revealed a tonic trigeminal motor discharge that closely coincided with the duration of the entire locomotor event. The clear phase-locking of the activity in some trigeminal motoneurons with the rhythmic locomotor commands, suggests a spinal origin of the corollary discharge. Potential consequences of the tentacle retraction during swimming include prevention of reafferent stimulation of touch-receptive Merkel cells and reduction of the hydrodynamic drag caused by extended appendages. The influence of spinal corollary activity on brainstem motor systems appears to be a general feature that ensures spatio-dynamic appropriate tuning of all motor effectors during locomotion as well as subtractive influences on reafferent sensory signals.

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Poster

538. Circuit Connectivity

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Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Support: NIH Grant R01 NS067299

NIH grant T32 NS041234

Title: Developmental modifications of premotor excitatory drive match changes in motoneuron properties

Authors: ***C. M. VANDUNK**, S. KISHORE, D. L. MCLEAN
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Abstract: The first behavior zebrafish embryos produce is spontaneous ‘coiling’, involving bends of the entire body. This behavior is generated by spinal circuits that ultimately control fast escapes in larvae after they hatch. During this period, the earliest born axial ‘primary’ motoneurons (pMNs) migrate dorsally in the spinal cord and nearly double in size, resulting in substantial decreases in excitability. One question that arises is how the early premotor elements responsible for driving movements adjust to these changes to allow for continued expression of

motor behavior through life. Here, we have focused on changes in a major source of premotor excitatory drive in all vertebrates, which arises from Chx10-positive V2a neurons. Using *in vivo* time-lapse imaging of stochastically-labeled V2a cells in a transgenic line labeling the motoneuron pool, we find that the earliest born V2a cells, which will ultimately occupy the dorsal-most positions in the larval spinal cord (dV2a), are initially ventrally located. At this early embryonic stage, putative connections to pMNs at the same ventral location arise from en passant synapses along the main axon. As dV2as and pMNs migrate dorsally during development, this initial contact is apparently maintained locally by secondary axonal branching from the initial site of contact with the main axon. Next, to examine the potential functional impact of this maintained connection, we performed whole-cell voltage clamp recordings of excitatory drive to pMNs during 'fictive' swimming in 2-day old embryos and 4-day old larvae. At both stages, pMNs received phasic excitation driving the cyclical bursts of motor activity and tonic excitation providing a background source of depolarizing drive. However, there was a substantial increase in both phasic and tonic drive commensurate with a decrease in input resistance (Rin) from day 2 to 4, consistent with the idea that synaptic inputs compensate for decreases in cellular excitability to maintain functional output. To examine the contribution of cellular excitability to this process, we are using the bacterial voltage-gated sodium channel, NaChBac, and the inward-rectifying potassium channel, Kir2.1, to provide genetically-targeted increases and decreases in cell excitability, respectively. Whole-cell patch clamp recordings from individual pMNs expressing these constructs have confirmed their utility. Our work thus suggests there are changes in dV2a morphology and excitatory drive that compensate for changes in pMN location, size and Rin, and we are now in a position to examine the contribution of cell autonomous features, like excitability, to this process.

Disclosures: **C.M. VanDunk:** None. **S. Kishore:** None. **D.L. McLean:** None.

Poster

538. Circuit Connectivity

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Program#/Poster#: 538.16/KK28

Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Support: DFG Bu857/14-1

Title: Investigating weakly coupled oscillators in the stick insect locomotor system

Authors: A. BORGMANN, C. MANTZIARIS, N. ROSJAT, S. GRUHN, *A. BUSCHGES
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Abstract: Walking movements result from a complex interplay of central pattern generating networks (CPGs), local sensory feedback about movements and forces generated in the legs and coordinating signals from neighboring limbs. In the stick insect, the antagonistic muscles of each leg joint are driven by one CPG that can be activated by the muscarinic acetylcholine agonist pilocarpine (Büschges et al. 1995). Sensory information plays a crucial role in coordinating the different CPGs of one leg and appears to play a major role in inter-segmental coordination of the different legs as well. However, hardly anything is known about the role and contribution of intersegmental interactions between CPG networks. Preliminary evidence (Büschges et al. 1995) suggested some intersegmental influence between CPGs. In this study we aimed to investigate in more detail the influences between the Coxa trochanter (CTh-) joint CPGs of the three thoracic segments. We analyzed the coordination of the CTh-joint CPGs in and between the three thoracic segmental ganglia in the completely deafferented nervous system of the stick insect, Carausius morosus. Rhythmic motor activity was induced by bath application of pilocarpine. Activity of ThC-motoneuron pools supplying the levator and depressor trochanteris muscles of a leg was recorded by means of extracellular electrodes from lateral nerves C1 and/or C2 in each thoracic ganglion. We used a new method for analyzing time series of several rhythmic systems in order to (i) identify time intervals of coupling between the MN-output of the different CTh-joint CPGs, (ii) provide a measure for coupling strength and (iii) determine if coupling was present with preferred phase relations between the different CTh-joint CPGs. We first focused on contralateral coordination between intra-segmental CPGs of the same ganglion. Phase analysis revealed a weak intra-segmental coupling for each thoracic ganglion. Interestingly, the intra-segmental coordination was affected by inter-segmental signals. This was revealed by preparations in which rhythmicity in a chain of two or three rhythmically segmental ganglia was studied. Our results clearly indicate for the first time that there exist weak intra- and intersegmental neural coupling between the CTh-CPGs in the stick insect thoracic nerve cord.
Büschges et al. 1995, J Exp Biol 198: 435.

Disclosures: A. Borgmann: None. **A. Buschges:** None. **C. Mantziaris:** None. **N. Rosjat:** None. **S. Gruhn:** None.

Poster

538. Circuit Connectivity

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Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Support: DA1182/1-1

UoCEG CONNECT

Title: Using dynamic causal modeling in the study of the stick insect locomotor system

Authors: *N. ROSJAT, T. TÓTH, C. MANTZIARIS, A. BORGMANN, S. GRUHN
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Abstract: Walking results from a complex interplay of central pattern generating networks (CPGs), local sensory feedback signalizing position, velocity and forces generated in the legs, and coordinating signals from neighboring limbs. In the stick insect, the neural basis of inter-segmental coordination, and the precise effects of sensory information on the central networks in establishing coordinated motor output are largely unknown. The antagonistic muscles of each leg joint are driven by one CPG that can be activated by the muscarinic acetylcholine agonist pilocarpine. Sensory information plays a crucial role in coordinating the different CPGs of one leg and appears to play a major role in inter-segmental coordination of the different legs, too. However, precious little is known about the interactions between the different CPG networks. Büschges et al. 1995 could show that there appear to be episodes when CPGs of different segments tended to be active in phase. Here, we aimed to investigate potential coupling between CPGs at the CTr-joint in the different segments, in more detail. To uncover putative coordination in and between the three thoracic segmental ganglia we analyzed the activity in the completely deafferented nervous system of the stick insect, Carausius Morosus. Rhythmic motor activity was induced by bath application of the muscarinic receptor agonist pilocarpine. The activity of the motoneuron pool supplying the depressor trochanteris muscles of a leg was recorded by means of extracellular electrodes from the lateral nerves C2 in each thoracic ganglion. We used dynamic causal modeling (DCM) to investigate the coupling structure and strength of two coupled ganglions. We first focused on the influence of different pilocarpine concentrations on inter-segmental coupling strengths on the ipsilateral side. The analysis revealed that anterior segments were stronger coupled to posterior segments when the pilocarpine concentration has been increased. Then, we integrated the contralateral side into our model. We set up different coupling schemes for DCM and compared them using Bayesian model selection methods. There was a clear preference to models with lateral connections in each segment and ipsilateral connections on both sides to all other tested models. DCM for preparations in which the connectives between two segmental ganglia were cut showed a clearly decrease in coupling strength between the CPGs of different ganglions. Our results show a high probability for the existence of ipsilateral intra- and lateral inter-segmental coupling between the CPGs controlling the coxa-trochanteral joint musculature in the stick insect thoracic nerve cord.

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Poster

538. Circuit Connectivity

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Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Support: Boehringer Ingelheim Fonds PhD Fellowship

Max Planck Society

Title: Reconstructing the connectivity of the larval zebrafish spinal cord

Authors: ***F. SVARA**, J. KORNFELD, J. BOLLMANN, W. DENK
Max Planck Inst. For Med. Res., Heidelberg, Germany

Abstract: Motoneurons in the vertebrate spinal cord can be activated both by interneurons of spinal central pattern generator (CPG) circuits and by direct inputs from the hindbrain reticulospinal (RS) system. While the spinal cord can show sustained neuronal activity without inputs from the brain, it is the brain that selects which patterns are produced, as appropriate for the animal in a given situation. In the larval zebrafish brain, the dominant pathway that sends commands to the spinal cord is the RS system in the hindbrain. How exactly different patterns are selected is not clear, not least because it is unknown how exactly the brain is wired to its spinal cord targets. With 3D Electron Microscopy, and more specifically Serial Block-Face Electron Microscopy (SBEM), that question can be addressed directly. In the larval zebrafish, different patterns of tail motion can be parametrized by bend amplitude and beat frequency, where low-amplitude, low frequency swims are used when swimming slowly and high-amplitude, high-frequency swims are used when swimming fast. Motoneurons and spinal interneurons have been shown to be activated following different principles: Increasing numbers of motoneurons are activated with increasing speed, progressing from small ventral to large dorsal motoneurons. In the case of spinal interneurons, there is a specificity of different cells for different movement speeds, where a cell's activation depends on the interneuron's dorso-ventral position. To clarify how these activity patterns are selected as a function of descending commands, we reconstructed the connectivity between motoneurons and their presynaptic partners, i.e. RS axons, excitatory (CiD, MCoD) and inhibitory (CiA, CoBL) spinal interneurons.

We asked whether motoneuron recruitment principles can be explained by specific RS axons wiring to specific motoneuron subsets or whether it is mostly governed by motoneuron-intrinsic properties, with unspecific wiring. Further, the observed recruitment principles of excitatory interneurons could either lead to the recruitment of appropriate motoneuron pools through an unspecific interneuron to motoneuron wiring scheme, with specific cell-intrinsic and synaptic properties, or through selective wiring between subsets of interneurons and motoneurons. Our data can help to explain the mechanisms by which the brain activates distinct networks in the spinal cord that generate the motor patterns required for the animal's survival and opens up new questions about the physiology and connectivity of spinal neuronal networks.

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Poster

538. Circuit Connectivity

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Program#/Poster#: 538.19/KK31

Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Title: Investigating the cellular basis of the differential recruitment of motoneurons in the *Drosophila* larval locomotor system

Authors: *M. ZWART[1,2], S. R. PULVER[1], R. D. FETTER[1], A. CARDONA[1], M. LANDGRAF[2]
[1]HHMI Janelia Farm, Ashburn, VA; [2]Dept. of Zoology, Univ. of Cambridge, Cambridge, United Kingdom

Abstract: The precise execution of behaviors requires the generation of the appropriate patterns of motoneuron activity. In the locomotor system of *Drosophila* larvae, the peristaltic waves that underlie crawling are generated by sequential contraction of longitudinal and transverse muscle groups. This sequence of activation is caused by the differential recruitment of the two classes of motoneurons in the CNS that innervate these muscle groups. The mechanisms responsible for such group-specific activity patterns are unknown. There are at least two, not necessarily mutually exclusive, possibilities: first, the two different classes of motoneurons may receive input from different presynaptic interneurons; second, differences in motoneuron recruitment may be due to differences in the intrinsic excitable properties of the two classes of motoneurons.

Here, we describe our progress towards resolving this question. We have taken two approaches: first, EM-based reconstruction of the neural network presynaptic to the class of motoneurons innervating the transverse muscles followed by a comparison to the network presynaptic to motoneurons innervating longitudinal muscles; second, patch clamp recordings of identified motoneurons that are differentially recruited during larval crawling to determine their intrinsic and synaptic properties. Our results delineate the precedence of the connectivity of the neural network ("extrinsic properties") versus the physiology of its constituent neurons ("intrinsic properties") in the generation of patterned neural activity in the *Drosophila* larval locomotor system.

Disclosures: M. Zwart: None. **S.R. Pulver:** None. **R.D. Fetter:** None. **A. Cardona:** None. **M. Landgraf:** None.

Poster

538. Circuit Connectivity

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Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Support: NIH Grant NS080047

Title: The role of glycinergic inhibition in the locomotor rhythm of the lamprey hindbrain

Authors: *J. T. BUCHANAN, A. A. RASMUSSEN, K. M. KENNEY, J. M. SCHEEL
Marquette Univ., Milwaukee, WI

Abstract: Although it is well-established that the central pattern generator (CPG) for locomotion in vertebrates resides within the spinal cord, we have found that the hindbrain of lamprey also has a locomotor CPG. When isolated from the spinal cord, the last segment of hindbrain is capable of generating rhythmic bursts of action potentials in the spino-occipital (S-O) nerves, which are the efferent nerves of a column of somatic motor neurons that innervate several muscles of the head. Like the spinal cord, the S-O nerves burst with left-right alternation. The goals of the present study were 1) to determine whether the alternating bursting of the hindbrain locomotor CPG is dependent upon glycinergic inhibition as it is in the spinal cord and 2) to identify the target muscles of the S-O motor neurons and the distributions of motor neuron cell bodies within the S-O motor column. To determine whether the alternating bursting of the hindbrain locomotor CPG is dependent upon glycinergic inhibition, we used an *in vitro* hindbrain

preparation that was isolated from the spinal cord by a complete spinal transection midway between the caudal S-O nerve and the first spinal ventral root. Motor activity was elicited by bath application of either D-glutamate or NMDA while recording the S-O nerves on each side of the hindbrain with extracellular suction electrodes. When the glycine receptor antagonist strychnine was bath applied, all 8 preparations in which rhythmic activity was present both before and after strychnine (of 10 total preparations) showed a statistically significant switch from left-right alternation (181 ± 7 deg) to synchronous (358 ± 11 deg) bursting. To identify the target muscles of the S-O motor neurons, a combination of intracellular/extracellular electrophysiology and retrograde/anterograde axon tracing was used. Based on electrophysiology, five head muscles were found to be innervated by the S-O motor neurons. Two of these muscles are rostral representatives of segmental body muscles: the epibranchial and hypobranchial muscles. It was found that up to four segments of these two muscle groups received innervation from the S-O motor neurons. With retrograde tracing, the S-O motor column was found to contain about 300 motor neurons on each side. While the motor neurons to each muscle had distinct locations within the motor column, there was considerable overlap with the motor neurons of other muscles. In conclusion, the locomotor CPG of the hindbrain switches to synchronous bursting in the presence of strychnine, indicating that glycinergic inhibition is required for the alternating left-right pattern of locomotion, similar to findings in the spinal cord.

Disclosures: J.T. Buchanan: None. **A.A. Rasmussen:** None. **K.M. Kenney:** None. **J.M. Scheel:** None.

Poster

538. Circuit Connectivity

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Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Support: Canadian Institute of Health Research Grant MOP-110950 to Y.Z.

Natural Sciences and Engineering Research Council of Canada Grant 38620 to Y.Z.

Title: V3 spinal interneurons are crucial in regulating weight-loading movement

Authors: *H. ZHANG, H. HAMODAT, Y. ZHANG
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Abstract: V3 interneurons (INs) are the major excitatory commissural interneurons in the mouse spinal cord. Our previous work has shown that genetic deletion of V3 INs caused the animal to exhibit an unstable and incoherent gait. In order to further understand the functional role of V3 INs in the locomotor circuits, we selectively blocked the synaptic transmission of V3 INs by deleting the expression of vesicular glutamate transporter 2 (VGLUT2). The mutant mice could survive and didn't show obvious behavior defects in the cage. When they were put on a treadmill with various speeds, however, these mutant mice couldn't run faster than 15 cm/s, while wild-type mice could run at a rate of above 60 cm/s. On the other hand, during swimming, the mutant mice could reach the same high speed as wild types. Furthermore, electromyography (EMG) recordings in different hind limb muscles have shown that some extensor muscles, such as gastrocnemius and vastus lateralis muscle, showed little change of activity in mutant mice when they altered from running to climbing or to swimming, while there was a significant increase in the activity of these muscles in control animals under the same condition. These results indicate that V3 INs may be involved in regulating the activity of extensor muscles of the hind limbs in controlling animal's locomotor activity, instead of directly setting the rhythms of limb movement.

Disclosures: **H. Zhang:** None. **H. Hamodat:** None. **Y. Zhang:** None.

Poster

538. Circuit Connectivity

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Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Support: Kavli Institution

UCSD Academic Senate

Title: Unraveling the leech connectome

Authors: ***J. PIPKIN**, W. B. KRISTAN
Univ. of California At San Diego, La Jolla, CA

Abstract: The medicinal leech is a segmented annelid with 21 stereotyped midbody ganglia each responsible for sensing, communicating with the other ganglia, and producing behavior relevant to the segment it innervates. These ganglia all contain the same complement of approximately

400 neurons. Each neuron possesses a large cell body arranged on the exterior of the ganglion, and a central region of neuropil where the synaptic circuitry is established. To begin unraveling this circuitry, we are applying a connectomic approach relying on serial blockface electron microscopy (SBEM). Here we present data collected from an entire ganglion collected from a smaller but still behaviorally-mature juvenile leech. Within this volume of data, we have segmented several motor neurons and identified the connections between them. With this dataset, we show that we can identify known synapses (identified via previous experiments using intracellular electrophysiology) and that we can begin answering questions about the number and spatial distribution of these synapses in the neuropil.

Disclosures: J. Pipkin: None. **W.B. Kristan:** None.

Poster

538. Circuit Connectivity

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Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Support: Swedish Research Council: VR-M-K2013-62X-03026

Swedish Research Council: VR-NT-621-2007-6049

Karolinska Institute Research Funds

Title: Neuronal cerebrospinal fluid-contacting cells in lamprey are sensitive to fluid movement

Authors: E. JALALVAND, B. ROBERTSON, *P. WALLEN, S. GRILLNER
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Abstract: Cerebrospinal fluid-contacting (CSF-c) cells are found in all vertebrates but their function has remained elusive. In the lamprey spinal cord they surround the central canal and have processes passing the gray matter to the lateral margin of the spinal cord. We have recently characterized lamprey CSF-c cells according to their morphology, phenotype, and electrophysiological properties, and identified two distinct types (Jalalvand et al., J Comp Neurol 522:1753-68, 2014). Type 1 cells have a bulb-like ending that protrudes into the central canal and a lateral process that ramifies ventrolaterally and laterally in the gray matter, and that ends in a dense plexus surrounding the mechanosensitive dendrites of edge cells located at the spinal cord margin. Type 1 cells are GABAergic (also somatostatin), have neuronal membrane

properties and express both glutamate and GABA receptors. Type 2 cells, on the other hand, have a flat ending protruding into the central canal and a laterally projecting process that only ramifies at the lateral margin. Type 2 cells do not show active membrane properties, and may represent a type of glia cell. These findings suggest that type 1 CSF-c cells may play a modulatory role by influencing edge cells and thus the locomotor-related sensory feedback (Viana Di Prisco et al., Brain Res 530:161-66, 1990). We here address the question of how type 1 cells are activated. Their bulb-like endings have cilia protruding into the central canal, and it is therefore possible that type 1 cells may sense the movements of the CSF occurring for instance during swimming. Using a longitudinally sliced, isolated preparation of the lamprey spinal cord, we patched CSF-c cells and applied brief pressure pulses via a fluid-filled micropipette close to the cilia. In type 1 cells, a short pressure pulse reliably elicited receptor- or action potentials. The receptor potential amplitude increased with increasing pulse magnitude, and action potentials were evoked with even higher pressure pulse settings. These responses remained after application of GABA and glutamate antagonists, suggesting that they were due to movements of the cilia and not to synaptic input. In contrast, type 2 cells did not show any responses to pressure pulse stimulation. These results suggest that the GABAergic type 1 CSF-c cells in the lamprey spinal cord can be activated by fluid movements in the central canal during body undulations, and thereby in turn regulate the sensitivity of the mechanosensitive dendrites of the edge cells, and thus their movement-related feedback to the locomotor CPG. The type 1 CSF-c cells may also influence the interneuronal network in the gray matter directly.

Disclosures: **E. Jalalvand:** None. **P. Wallen:** None. **S. Grillner:** None. **B. Robertson:** None.

Poster

538. Circuit Connectivity

Location: Halls A-C

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Program#/Poster#: 538.24/LL4

Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

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Title: Membrane resonance influences the frequency of an electrically coupled network

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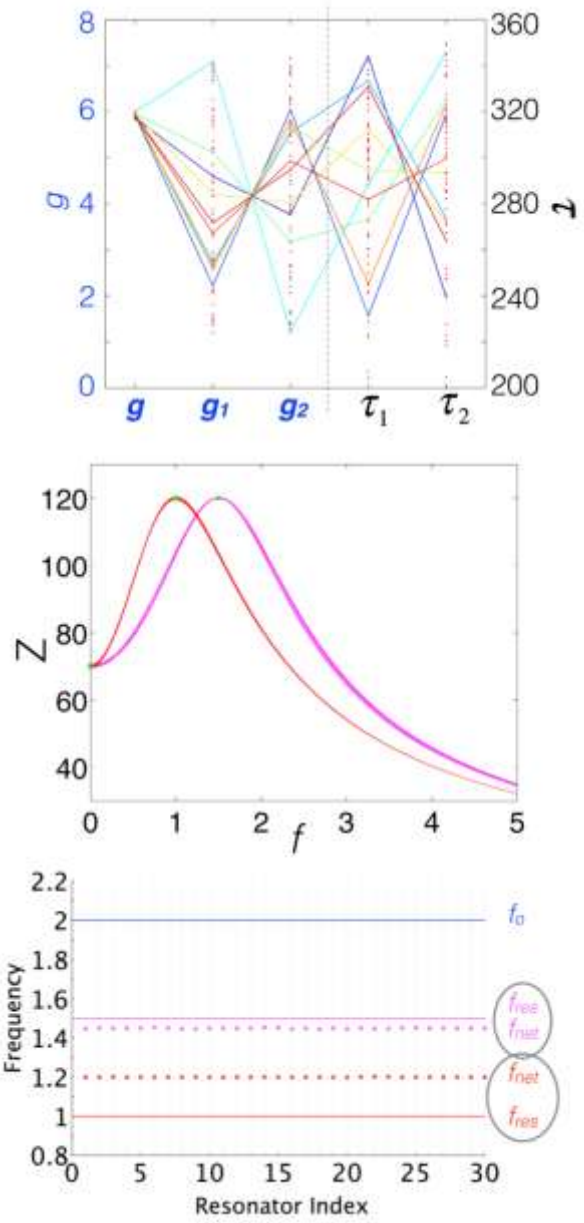
Abstract: Neurons often exhibit membrane potential resonance, a maximal voltage response to sinusoidal current inputs at a non-zero frequency (f_{res}). Resonance in individual cells has been implicated in determining the frequency (f_{net}) of the oscillating networks in which they are embedded. The functionality of resonance is still an open question. We examine the hypothesis that f_{net} monotonically changes with f_{res} in electrically coupled networks. We test this hypothesis in a two-cell model network consisting of an oscillator (with endogenous frequency f_O), electrically coupled to a biophysically-inspired linear resonator, for which different attribute of the impedance profile (*f-Z* curve) can be varied independently (Rotstein & Nadim, *J Comput Neuro*, 2014). We find that shifting the resonant frequency of the resonator positively influences f_{net}, and that this effect is enhanced by increasing the maximal impedance of the resonator. The influence of the resonator on the oscillator is independent of oscillator properties, but is enhanced when f_O is larger than f_{res}. We also find that f_{net} remains constant upon changes in the linear resonator parameter values provided the impedance profile remains unchanged. This result implies that the impedance profile, and not the specific details of the resonator, determines the network frequency. We confirm these results using biophysical nonlinear resonator models. To our knowledge, these results are the first direct demonstration of the influence of membrane resonance of non-oscillating neurons on the frequency of an oscillatory network and show an important role for impedance profile of individual neurons in shaping the output of electrically coupled networks. *Figure:* 30 random resonators with same impedance profile (f_{res}=1), but different parameters, coupled with the same oscillator, show the same f_{net}. Shifting the resonance profile of all resonators to f_{res}=1.5 also increases f_{net} in all cases.



Disclosures: **Y. Chen:** None. **F. Nadim:** None. **H. Rotstein:** None.

Poster

538. Circuit Connectivity

Location: Halls A-C

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Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Support: NSF Grant DMS-0931642

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NIH Grant HD064830

Title: Eupnea, tachypnea, and autoresuscitation in a closed-loop respiratory control model

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Abstract: Incorporation of sensory feedback is essential to guide the timing of rhythmic motor processes. How sensory information influences the dynamics of a central pattern generating circuit varies from system to system, and general principles for understanding this aspect of rhythmic motor control are lacking. It has been realized for sometime, however, that the mechanisms underlying rhythm generation in a central circuit when considered in isolation may be different from the mechanisms underlying rhythmicity in the intact organism (Bässler, Biol Cybern 54:65-69, 1986). Here we investigate the mechanisms of rhythmogenesis in a minimal model incorporating a central pattern generator (CPG) in a respiratory control loop, a preliminary version of which was introduced in Diekman et al. (Conf Proc IEEE Eng Med Biol Soc, 2012:6669-72). We show that the closed-loop system has bistability corresponding to coexistence of a eupneic-like breathing rhythm with normal minute ventilation and blood oxygen level, and a tachypneic-like state with pathologically reduced minute ventilation and critically low blood oxygen, consistent with a similar model developed independently in Ben-Tal and Smith (J Theor Biol, 251:480-497, 2008). We show that an artificially imposed bout of hypoxia can cause the system to leave the basin of attraction for the eupneic-like state and enter the basin of attraction for the tachypneic-like state. In addition, we investigate the relationship between rhythms in the intact (closed-loop) and isolated CPG (open-loop) systems, and find that eupneic-like oscillations with similar time courses nevertheless appear to arise from two distinct mechanisms in the open and closed-loop systems. Finally, we show that conductances endogenous to the Butera-Rinzel-Smith model of the respiratory CPG (Butera et al., J Neurophysiol 82:382-397, 1999) can lead to spontaneous autoresuscitation after short or mild bouts of hypoxia.

Disclosures: C.O. Diekman: None. **C.G. Wilson:** None. **P.J. Thomas:** None.

Poster

539. Motor Neurons and Muscle

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Program#/Poster#: 539.01/LL6

Topic: D.13. Motor Neurons and Muscle

Support: PROAPARC/UNASP-SP

PIBIC/FMJ 27/2010

Title: Sciatic nerve injury affects osseointegration of hydroxyapatite implant in bone defect of rat`s tibia

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Abstract: Motor nervous system interacts with musculoskeletal metabolism. Denervation of peripheral nerves has been associated with skeletal disorders and limited rehabilitation of skeletal system. Preview studies have shown that nerve damage reduces P substance and calcitonin gene-related peptides, also known as neuropeptides that may have a key role on bone healing. In surgical conditions or extensive traumas when a bone defect is critical, implants of synthetic biomaterials have been employed to favour bone growth. Considering that hydroxyapatite implants are widely used in complex injuries associated with peripheral nerve rupture, this study investigated the effects of unilateral sciatic nerve injury on osseointegration after hydroxyapatite implant within a bone defect in rat's tibia. The present study was approved by the ethics committee and all procedures conducted to reduce suffering of animals. Young male wistar rats (n=10) were divided into two groups: the control group having both functioning sciatic nerves (CRT) and an experimental condition for a complete injury of the left sciatic nerve (INJ). All animals were surgically submitted to a bone defect in the left tibia and subsequently filled with hydroxyapatite granules. Housing and handling of animals were standardized for both groups. Two months after the implant, the animals were sacrificed and the samples analyzed by means of histomorphological and radiological analysis. Radiological signs of bone healing were observed in all cases, proving that the hydroxyapatite previously implanted within the bone defect was integrated with the surrounding bone favorably. Histomorphological findings have shown increased bone neoformation adjacent to the hydroxyapatite granules in the CRT group, which

was in minor proportion to the INJ group. The latter have shown predominant connective tissue proliferation in the bone defect, rather than young bone. It may be concluded that osteosynthesis were stimulated by hydroxyapatite within the bone defect and it was further facilitated by peripheral nerve functioning in terms of bone volume and speed of bone formation. These findings suggest that the trophism of peripheral nerves helps bone reconstruction after hydroxyapatite implant within a bone defect. Moreover, a denervation of peripheral nerve may not impede osseointegration or bone growth expected in implants of hydroxyapatite.

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Poster

539. Motor Neurons and Muscle

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 539.02/LL7

Topic: D.13. Motor Neurons and Muscle

Title: Video based behavioural analysis of rodents on the rotarod provides additional parameters to discriminate between drug effects

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Abstract: Impaired motor coordination due to medication can have an important impact on the patient's quality of life. Extrapyramidal motor symptoms, induced by antipsychotic drugs, and muscle relaxant effects of benzodiazepines both disturb coordination of body movements. The rotarod test is widely used to evaluate drug effects on motor coordination in rodents. We have developed an automated version of the rotarod, using image analysis software to identify secondary parameters, such as turnaround behaviour and time on back of the rod. These may help in differentiating side effects of compounds on motor coordination. OBJECTIVE: To compare the effects of the typical antipsychotic haloperidol and the benzodiazepine diazepam on performance in rats and mice on the automated version of the rotarod. METHODS: Male mice (NMRI) and rats (Sprague Dawley) were trained to walk on a rotating rod at constant speed and then tested for baseline performance on the accelerating rotarod. The next day, animals were treated with a dose of haloperidol, diazepam or vehicle, and performance on the rod was determined 30, 60 and 90 min after treatment. RESULTS: In a series of studies in mice and rats, haloperidol showed dose dependent impairment in time on the rod and increased time at the back

of the rod. Time on the rod in mice was restored at 90 min, but time in back was increased. In addition, increased turning behaviour was observed in mice but not in rat. Diazepam impaired time on rod in rats and mice during the 90 min period, however only increased time in back was observed 30 min after treatment. No turning behaviour was seen. CONCLUSION: With automated measurement of rodent behaviour on the rotarod, we are able to define new parameters to assess the performance of rats and mice. Effects of haloperidol differed between rats and mice: i) Time in the back in mice was increased at doses that were without effect on time on rod, wheras both measures were in-line in the rat. ii) In contrast to mice, the turnaround parameter does not appear to be relevant for rats because they are too heavy to turn around. Diazepam revealed no differences between rats and mice. Testing of other pharmacological classes will further evaluate the added value of these additional parameters.

Disclosures: R. Willems: None. **M. Mahieu:** None. **L. Ver Donck:** None.

Poster

539. Motor Neurons and Muscle

Location: Halls A-C

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Topic: D.13. Motor Neurons and Muscle

Support: NIH PPG Grant P01 NS057228

Title: Nerve crush induces a smaller loss of central IA afferent synapses (VGLUT1) on injured motoneurons than complete nerve transection

Authors: *T. M. ROTTERMAN[1], A. DWARAKANATH[2], P. NARDELLI[3], T. C. COPE[3], F. J. ALVAREZ[2]
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Abstract: Peripheral nerve injuries affect nearly one million people annually leaving them with permanent motor deficits, such as a loss in the stretch reflex. After complete nerve transections there is a permanent loss of the central synaptic arbors of IA afferent axons and motor axon recurrent collaterals inside the spinal cord. By difference to the regeneration that occurs peripherally, central changes are permanent. The synaptic connections of Ia afferents with MNs, as defined by vesicular glutamate transporter 1 (VGLUT1), are reduced by 62% on dendrites and 87% on somata of injured MNs after they regenerate and reinnervate muscle. This reduced input explains the loss of stretch-evoked EPSPs in 40% of regenerated MNs and their low amplitude in

the remaining 60%, as well as the reduced connectivity of single IA afferents across the MG motor pool. These morpho-functional changes help to explain the loss of stretch reflexes in rodents and possibly in other species, including humans. In contrast, when the nerve is crushed there is an enhancement in stretch-evoked EPSPs and the stretch reflex is preserved and augmented. We therefore analyzed VGLUT1 inputs on injured MNs after crush and whether GABAergic presynaptic control through terminals expressing the 65 kDa isoform of glutamic acid-decarboxylase (GAD65) was reduced. Adult rats underwent a tibial nerve crush and regenerated from 21 days to 3 months after injury. The medial gastrocnemius (MG) muscle was injected with cholera toxin b (CTb) to retrogradely label the MNs. We did not find significant differences between 21 days and 3 months in coverage of CTb labeled MG MNs by VGLUT1 synapses or the coverage of these synapses by presynaptic GAD65 boutons; therefore data from both ages were pooled together. The results indicate fewer VGLUT1 losses on the cell bodies (41% depletion) and dendrites (10% depletion) of MNs after crush compared to complete nerve transections. Furthermore, the incidence of VGLUT1 synapses covered by GAD65 synapses did not significantly change (range 85-89% of synapses in both control or after nerve crush) although there was a small reduction in the number of GAD65 synapses per VGLUT1 terminal. In control MNs we found on average 2.4±0.4 and 2.8±0.5 GAD65 synapses per VGLUT1 terminal making synaptic contacts respectively on dendrites and cell bodies of MNs. This coverage was reduced by 21-29% after nerve crush. We concluded that there is better preservation of VGLUT1 IA afferents after nerve crush compared to total nerve transection; however the small change in presynaptic inhibition does not seem sufficient to explain the large increase in monosynaptic transmission from stretch sensitive IA afferents.

Disclosures: T.M. Rotterman: None. **A. Dwarakanath:** None. **P. Nardelli:** None. **T.C. Cope:** None. **F.J. Alvarez:** None.

Poster

539. Motor Neurons and Muscle

Location: Halls A-C

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Program#/Poster#: 539.04/LL9

Topic: D.13. Motor Neurons and Muscle

Title: GAL-160, a peripheral chemoreceptor stimulant, selectively increases hypoglossal nerve and genioglossal muscle activities during unobstructed and obstructed breathing in rats

Authors: *S. M. BABY[1], S. I. MARDIROSIAN[2], T. SCHEMM[2], S. PENG[2], F. J. GOLDER[1], D. E. MACINTYRE[1]
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Abstract: Peripheral chemoreceptor stimulation preferentially increases genioglossal muscle (GG) inspiratory activity compared to the diaphragm (DIA) (Bruce et al., 1982, J Appl Physiol). Thus, drugs that stimulate the carotid bodies may improve upper airway patency without causing adverse hyperventilation and be useful in the treatment of obstructive sleep apnea (OSA). GAL-160 is an orally active compound that stimulates the carotid body and decreases the frequency and duration of spontaneous obstructive apneas (OAs) in an anesthetized rat model of OSA. We hypothesized that in this model GAL-160 decreases the frequency of OAs by increasing GG electromyogram (EMG) activity during unobstructed breathing and decreases the duration of OAs by increasing the GG response to airway obstruction. We further hypothesized that GAL-160 does not increase diaphragm activity. To test these hypotheses, we recorded hypoglossal and phrenic neurograms from urethane anesthetized, mechanically ventilated rats before and during biphasic infusions of vehicle (saline) or GAL-160 to achieve GAL-160 plasma concentrations between 60-90 ng/ml. Baseline $PaCO_2$ was set at 3 mmHg above the $PaCO_2$ at apneic threshold for each individual animal and maintained at this level throughout the experiment. GAL-160 dose-dependently increased hypoglossal nerve activity (to 67 ± 10% above baseline) without altering phrenic nerve activity (13 ± 8% above baseline). Vehicle infusion had not effect on either neurogram. Genioglossus and diaphragm EMGs were recorded from urethane anesthetized, spontaneously breathing rats. The peak EMG responses to standardized 10 second airway obstructions were quantified before and during a similar biphasic infusion vehicle or GAL-160. GAL-160 increased the peak GG EMG response to obstruction (before GAL-160: 214 ± 74%; after GAL-160: 589 ± 146%; above baseline) without altering the DIA EMG response (before GAL-160: 60 ± 13%; after GAL-160: 79 ± 19%; above baseline). Vehicle had no effect on the EMG response to obstruction. These data support the hypothesis that GAL-160 selectively increases GG inspiratory activity during unobstructed and obstructed breathing and is the likely mechanism whereby GAL-160 is efficacious in the rat model of spontaneous OAs.

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Poster

539. Motor Neurons and Muscle

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Program#/Poster#: 539.05/LL10

Topic: D.13. Motor Neurons and Muscle

Support: NIH Grant UL1TR000050

Foundation for Physical Therapy

Title: Measuring twitch interpolation and motor evoked potentials in the first dorsal interosseous muscle: Transcranial magnetic stimulation versus peripheral nerve stimulation

Authors: *M. R. RAFFERTY[1], H. E. KIM[1], D. M. CORCOS[3], S. MADHAVAN[1,2]
[1]Grad. Program in Neurosci., [2]Physical Therapy, Univ. of Illinois at Chicago, Chicago, IL; [3]Physical Therapy and Human Movement Sci., Northwestern Univ., Chicago, IL

Abstract: The extent to which neural drive is translated into muscle force is measured with the twitch interpolation technique. The purpose of this study was to investigate the utility of the twitch interpolation technique in the first dorsal interosseous (FDI) muscle, comparing the use of peripheral nerve stimulation (PNS) and transcranial magnetic stimulation (TMS). Fourteen healthy participants (7 male, age 21-33 years) received 28 stimuli with PNS and with TMS while performing isometric finger abduction through a range of contraction strengths. This protocol was repeated 2-14 days later. Superimposed twitch amplitudes were measured, and two muscle activation estimates were compared: central activation ratios (CAR) and voluntary activation (VA). There were no significant differences for any measure between the two testing sessions. Superimposed twitch amplitudes were significantly larger with TMS than with PNS ($p=0.0021$). Thus, muscle activation estimates were larger with PNS compared to TMS ($p<0.0001$). Maximal muscle activation estimates were larger when calculated with the CAR formula than with VA formula, with both stimulation techniques (PNS: $p=0.0005$; TMS: $p=0.0012$). Measuring maximal VA with TMS resulted in low and variable muscle activation estimates ($68.4 \pm 27.5\%$) compared to the other muscle activation estimates (VAPNS = $95.4 \pm 6.5\%$; CARPNS = $99.1 \pm 1.5\%$; CARTMS = $93.4 \pm 6.3\%$). During submaximal contractions at 75% MVC, CARPNS was 96.4% and CARTMS was 87.8%. Motor evoked potentials (MEPS) and M-waves were also analyzed, and shown to be repeatable. In conclusion, the twitch interpolation technique is repeatable in the FDI with both PNS and TMS. The VAPNS, CARPNS, and CARTMS formulas appear to be more appropriate measures of maximal muscle activation than the VATMS formula. However, the collapsed range of activation values above 75% MVC with CARPNS indicate that twitch interpolation with PNS may have limited responsiveness to change in the FDI. Technical, anatomical, and physiological limitations of performing the twitch interpolation technique in the FDI are discussed. Future studies of twitch interpolation in the FDI should explore measurement with TMS rather than PNS and/or focus on EMG outcome measures rather than force.

Disclosures: M.R. Rafferty: None. **H.E. Kim:** None. **D.M. Corcos:** None. **S. Madhavan:** None.

Poster

539. Motor Neurons and Muscle

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Topic: D.13. Motor Neurons and Muscle

Support: NSF IOS-1145796

NSF EEC-1028725

Levinson Award to JJG

Mary Gates Fellowship to JJG

Title: Circadian modulation of neuromotor control

Authors: *H. O. DE LA IGLESIA[1], J. J. GILE[2], O. JOHNSON[2], B. SMARR[3], H. CHIZECK[2]
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Abstract: The circadian system controls daily rhythms of behavior and physiology, including locomotor activity and motor-task performance. The master regulation of these rhythms is achieved by a circadian clock located in the suprachiasmatic nucleus of the hypothalamus. Motor tasks are the result of neuronal programs within the primary motor cortex (PMC) but it is not known whether these programs are modulated by the circadian system. Our hypothesis is that similar motor tasks executed at different times of the day may require different motor programs to account for the predictable daily variance introduced by the circadian system. How and where in the brain this variance in motor control manifests itself has not been established. To identify specific primary motor cortex activity patterns associated with specific motor outputs across the 24-hour day we implant electrocorticographic (ECoG) electrodes onto the motor cortex of mice and record brain wave activity during wheel running and rest at different circadian times. Our results indicate a broad-spectrum power increase in the frequency of PMC electrical signals associated with wheel running. Furthermore, the spectrum frequency associated with wheel running changes predictably throughout the circadian cycle. The decoding of motor cortex signals is at the core of the design of brain-machine interfaces (BMIs). These devices decode signals from the conscious brain to drive the execution of specific tasks by a machine, such as an artificial limb. Our work will directly contribute to the understanding of how the motor cortex

decodes circadian time. This knowledge is essential to create BMIs that can operate effectively throughout the 24-hour day to execute tasks by brain-operated artificial devices.

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Poster

539. Motor Neurons and Muscle

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 539.07/LL12

Topic: D.13. Motor Neurons and Muscle

Title: Functional implications of anterograde tracing in the mouse corticospinal tract

Authors: *H. SCHRODER[1], S. TOCHMAFSCHAN[1], B. DENGLER[1], S. ARNDT[2]
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Abstract: Today there is a plethora of tracing studies available. To the best of our knowledge, however, functional consequences of stereotactic tracer application have not been studied. We here report on an anterograde tracing approach (Miniruby BDA) of the mouse (C57BL/6OlaHsd [Harlan], female, 8-12 weeks old) corticospinal tract by tracer injection into the primary motor cortex. Mice motor functions were assessed by use of Rotarod and Fitness Center before and after the operation (2d / 7d post OP). Animals were perfusion-fixed 14 days post OP and coronal brain sections (50µm) were prepared. The fluorescent microscopic examination showed the typical course of the corticospinal tract from the motor cortex via the internal capsule and the cerebral peduncles to the pyramids of the medulla oblongata and further to the pyramidal decussation and the contralateral dorsal funiculus. The preliminary evaluation of the test data does not show any significant differences in motor function comparing pre- and postoperative testing. The results show that the application of an anterograde tracer into the primary motor cortex does not lead to overt short-term motor impairment.

Disclosures: H. Schroder: None. **S. Tochmafschan:** None. **B. Dengler:** None. **S. Arndt:** None.

Poster

539. Motor Neurons and Muscle

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Program#/Poster#: 539.08/LL13

Topic: D.13. Motor Neurons and Muscle

Support: NCCAM R21 AT-002138-03 (AJB)

Title: Motor-execution network activity following stroke and rehabilitation

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Abstract: Functional neuroimaging studies have demonstrated that even when people are not subjected to explicit stimulation or tasks, several cortical and subcortical areas of the motor system interact in the low frequency range (< 0.1 Hz). It has been found that the interaction among these areas might be functionally disturbed following stroke. Very few studies have discussed the role of rehabilitation to functionally reorganize the network in order to recover the motor ability of stroke patients. Here, we investigated the activity of five core areas in the motor-execution network consisting of the left primary motor area (LM1), the right primary motor area (RM1), the left pre-motor cortex (LPMC), the right pre-motor cortex (RPMC) and the supplementary motor area (SMA) in patients following stroke and rehabilitation. The fMRI data were collected from a total of 30 participants (17 able-bodied participants and 13 stroke participants). Stroke participants had either mental practice or combined mental practice and physical therapy within 14-51 days. Using directed functional connectivity approach, we found that (i) the network activity dominated in the frequency range 0.06 Hz - 0.08 Hz for all the regions and for both able-bodied and stroke participants (ii) causal flow between the regions (LM1 and SMA, RPMC and SMA, RPMC and LM1, SMA and RM1, SMA and LPMC) was reduced following stroke (iii) causal flow did not change significantly after mental practice alone and (iv) the causal flow among the regions during combination of mental and physical practice increased tending towards approaching the values for able-bodied people. In conclusion, this study helps us to understand the reorganization of motor-execution networks in stroke survivors. These findings suggest that a combination of mental practice (MP) and physical therapy (PT) can be an effective treatment for stroke survivors to recover the functionality of motor networks.

Disclosures: **S. Bajaj:** None. **D. Drake:** None. **A. Butler:** None. **M. Dhamala:** None.

Poster

539. Motor Neurons and Muscle

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Program#/Poster#: 539.09/LL14

Topic: D.13. Motor Neurons and Muscle

Title: Sensory deficits in mouse models of ALS

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Abstract: Amyotrophic Lateral Sclerosis (ALS) is a progressive neurodegenerative disease known to target a variety of cells, in particular neurons and glial cells in the spinal cord. In this study, we sought to determine if sensory neurons are also affected by ALS causing mutations. We have found that sensory neurons are compromised in the SOD1-G93A and TDP43-A315T mouse models for the disease. In animals harboring SOD1-G93A, there is an early and significant delay in the response to cold and hot stimuli. Along with these sensory deficits, fewer sensory neurons take up fluorescently labeled cholera toxin (fCTB), a retrograde tracer, from hind limbs. The inability to take up the retrograde tracer is due to loss of peripheral sensory axons. Proprioceptive nerve endings, marked with YFP, undergo early and significant structural alterations in SOD1-G93A mice, and follow a similar degenerative pattern and time course as motor axons. We then asked if sensory projections to the spinal cord were similarly affected. We found a significant decrease in the number of vesicular glutamate transporter 1 (VGLUT1) positive synapses in the ventral horn of presymptomatic and symptomatic SOD1-G93A and TDP43-A315T animals. Surprisingly, degeneration of central and peripheral nerve endings did not culminate in loss of sensory soma. Importantly, these alterations are likely due to intrinsic changes in sensory neurons. Consistent with their degeneration *in vivo*, the axons of cultured sensory neurons expressing SOD1-G93A grow slower and are more susceptible to a microtubule-destabilizing drug than neurons from control animals. These results demonstrate that proprioceptive and likely other sensory neurons are vulnerable to ALS causing mutations.

Disclosures: **S. Vaughan:** None. **S. Zhang:** None. **Z. Kemp:** None. **T. Hatzipetros:** None. **F. Vieira:** None. **G. Valdez:** None.

Poster

539. Motor Neurons and Muscle

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 539.10/LL15

Topic: D.13. Motor Neurons and Muscle

Support: The Health and Medical Research Fund (HMRF), Food and Health Bureau, Hong Kong Special Administrative Region Government (01122016).

Title: Pacific Ciguatoxin-1 modulates motor function and EEG activity in mice

Authors: *G. KUMAR[1], Y. WANG[2], N. P. B. AU[1], Y. L. MAK[3], L. L. CHAN[3], P. K. S. LAM[3], L. L. H. CHAN[2], C. H. E. MA[1,3]
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Abstract: Ciguatera fish poisoning (CFP) is a common foodborne illness causing chronic and persistent neurological effects on peripheral nervous system. Pacific ciguatoxin-1 (P-CTX-1), the most potent CTX isolated thus far, acts on the sodium channel in mouse peripheral neurons directly (i.e. dorsal root ganglion). The modification of sodium channel gating activity provides strong evidence for the cause of sensory disturbances observed in patient; however, the effect of CTX on motor function is largely unknown. In the present study, we investigate the effect of P-CTX-1 on electroencephalogram (EEG) and the correlation with motor function after acute and repeated exposure to P-CTX-1. Male adult C57BL/6 mice were used for neurobehavioral and EEG study. Motor function was assessed by grip strength and rotarod test. EEG electrodes were implanted on right parietal cortex and reference electrode over cerebellum and fixed with dental cement. Sub-lethal dose of P-CTX-1 (0.26ng/g) was administered intraperitoneally twice on day 0 and day 3. EEG signals were amplified, digitally filtered, sampled at 256 Hz, and stored. EEG recording was done at baseline, day 0, 7, 14 and 21 after the second injection. The power spectral analysis for each spectral band was calculated and compared with baseline. Grip strength and rotarod test showed statistically significant decreases as compared with vehicle control after first exposure to P-CTX-1 and returned to normal after 72 hours. However, second exposure to P-CTX-1 showed significant decreases and then returned to the normal in about two weeks. EEG power spectral analysis showed reduction of theta power (4-7 Hz) after one hour of P-CTX-1 (first exposure) and returned to normal after 3 hours. However, second exposure to P-CTX-1 (1 hour post injection) caused significant power reduction of delta (0.1-3 Hz) & theta (4-7 Hz) as

compared with base line and remains decreased till day 7 and returned to normal on day 14. In summary, P-CTX-1 reduces motor function and EEG activity in mice after repeated exposure to P-CTX-1. We show, for the first time, that P-CTX-1 affects not only the motor function but also the temporal dynamic of EEG activity in mouse brain.

Disclosures: G. Kumar: None. **Y. Wang:** None. **N.P.B. Au:** None. **Y.L. Mak:** None. **L.L. Chan:** None. **P.K.S. Lam:** None. **L.L.H. Chan:** None. **C.H.E. Ma:** None.

Poster

539. Motor Neurons and Muscle

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National Science Council, Taiwan 102-2221-E-182 -022 -

Chang Gung Medical Research Program

Healthy Aging Research Center, Chang Gung University Grant EMRPD1D0291

Title: Age-related strength loss is due to peripheral muscle weakness

Authors: *Y.-J. CHANG, N.-J. HUANG, F.-Y. CHANG, Y.-F. CHUANG
Dept. of Physical Therapy and Grad. Inst. of Rehabil. Sci., Chang Gung Univ., Tao-Yuan, Taiwan

Abstract: Muscle weakness is a major cause of frailty in old people. The muscle weakness might start to progress from pre-old age. Investigating the mechanism of muscle weakness in mid-age people is important for early intervention on preventing sever weakness in old age. Twenty six young and 16 mid-age people with a sedentary lifestyle were recruited. Maximal voluntary isometric contraction (MVC), electrical stimulation elicited twitch force of quadriceps, and interpolated twitch technique (ITT) were used to measure the total strength, activation level, and the peripheral component of strength, respectively. The cortical excitability was measured by transcranial magnetic stimulation (TMS). The mid-age group had significant lower MVC and twitch forces than young group ($p<0.05$). No difference was found in activation level, MEP amplitude, intracortical facilitation, or inhibition, In conclusion, the age-related weakness is

significant from mid-age. The cause of age-related weakness is mainly caused by the peripheral muscle weakness.

Disclosures: **Y. Chang:** None. **N. Huang:** None. **F. Chang:** None. **Y. Chuang:** None.

Poster

539. Motor Neurons and Muscle

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Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 539.12/LL17

Topic: D.13. Motor Neurons and Muscle

Title: An investigation of the mirror neuron system activation in expert dancers and their performance on a novel task

Authors: *L. PACKARD[1], C. J. KETCHAM[2]
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Abstract: Recent research on the Mirror Neuron System (MNS) has indicated that experts have increased mirror neuron activity compared to non-experts when observing people perform their skill of expertise (Kim et al., 2011; Orgs et al., 2008; Calvo-Merino et al., 2006; Cross et al., 2006). Little research has been done to determine if this activation transfers to learning of novel skills. The present study investigates mirror neuron activation differences between experts and novice individuals in observation of known and novel skills. Additionally, performance on a novel task was assessed to determine if experts have better transference of motor skills. In this study, EEG activity at the Mu frequency was used to record MNS activity in expert dancers and non-experts and compare the MNS activity to the individual's ability to perform a novel movement. 20 participants (10 expert dancers and 10 novice individuals) were recruited from the local community. The participants watched a randomized series of video clips of upper body movement, either ballet phrases or American Sign Language (ASL) phrases during EEG recording. Participants were then asked to perform a novel movement (a new ASL phrase) where performance was measured in speed and accuracy. Preliminary results showed a positive correlation between MNS activity level during observation and ability to correctly perform a novel ASL phrase. These results support evidence that MNS activity is related to transference of motor skills, which may be a mechanism for observational learning.

Disclosures: **L. Packard:** None. **C.J. Ketcham:** None.

Poster

539. Motor Neurons and Muscle

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Support: Grant-in-aid for Scientific Research (C) from Japan Society for the Promotion of Science

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Grant for Core Research for Evolutional Science and Technology (CREST) from the Japan Science and Technology Agency (JST)

Title: Nerve injury-induced mitochondrial fission is an essential adaptive response to maintain neuronal survival and promote axonal regeneration

Authors: *S. KIRYU-SEO[1], H. TAMADA[1], Y. KATO[1], N. ISHIHARA[2], M. NOMURA[3], K. MIHARA[4], H. KIYAMA[1]
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Abstract: Successful nerve recovery from injury requires huge energy supply by mitochondrial response. Our previous studies have demonstrated that mitochondria could play a critical role to determine the fate of injured motor neurons by losing or maintaining the mitochondrial integrity. Recently, multiple findings have suggested that mitochondrial dynamics including morphology and motility is strictly controlled for neuronal maintenance and axonal integrity. In line with this, the models for neurodegenerative diseases observe the irregular shape, accumulation and altered transport of mitochondria in damaged neurons and axons prior to clinical onset. However, most of evidences about mitochondrial dynamics have been derived from *in vitro* studies, and there remains controversy in functional consequences of mitochondrial dynamics under physiological and pathological conditions *in vivo*. To address this issue, particularly the functional significance of mitochondrial dynamics in injured neurons, here we have successfully created a unique transgenic mice using bacterial artificial chromosome (BAC) technology in which we designed to label mitochondria by GFP and simultaneously express cre recombinase in response to nerve injury. To ensure the nerve injury responsiveness, we employed the BAC clone containing entire

genome region of the activating transcription factor 3 (ATF3), which is known as a marker for axonal injury in nervous system. Using this newly obtained BAC Tg mice, we found that the length of axonal mitochondria became shorter and the turnover was accelerated in regenerative injured motor nerve, suggesting the increased fission activity of mitochondria. To evaluate the functional relevance of the increased fission activity in response to nerve injury, we crossed the BAC Tg mice with the floxed mice of dynamin-related protein (Drp1), which is responsible for mitochondrial fission, and succeeded in the specific ablation of mitochondrial fission in nerve-injured motor neurons. The injured motor neurons lacking mitochondrial fission showed round and gigantic mitochondria in soma and longer tubular mitochondria in axon. The quality and axonal transportation of mitochondria became apparently worse, and eventually leading to neuronal death and axonal degeneration. These findings suggest that mitochondrial fission is an essential adaptive response for injured neurons to maintain mitochondrial integrity and trafficking and consequently to accomplish successful regeneration.

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Poster

539. Motor Neurons and Muscle

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Program#/Poster#: 539.14/LL19

Topic: D.13. Motor Neurons and Muscle

Title: Altered overlapping pattern of hand movement representation by electromyogram triggered neuromuscular stimulation in humans

Authors: *W. TSUCHIHASHI[1], T. ONO[3], S. KASUGA[2], J. USHIBA[2]
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Abstract: Repetition of a simple movement can produce changes in the motor response evoked by transcranial magnetic stimulation (TMS), leading to a transient reorganization in motor connectivity (Classen et al. J Neurophysiol 1998). Moreover, active involvement in task performance leads to a substantial increase in cortical excitability, yet remains unchanged after non-skilled or passive training (Perez et al. Exp Brain Res 2004). It is, however, an open question how repetition of corporative muscle movement in multiple muscles changes motor

representation of each muscle. Furthermore, it is unclear whether cortical plasticity can be induced after repetition of passive movement evoked by electrical stimulation, corresponding with voluntary activation of another muscle. To address these questions, we examined whether artificial corporative movement of two muscles can reorganize motor representation of these muscles in short-time scale, with using Electromyogram Triggered Neuromuscular Stimulation (ETMS), a technique to apply a certain intensity of electrical stimulation to a muscle depending linearly on the amplitude of electromyogram (EMG) recorded in another muscle. Fourteen healthy adults participated this study. The participants received electrical stimulation to right flexor pollicis brevis muscle (FPB) when they performed a voluntary right wrist-extension with contraction of the extensor carpi radials muscle, ECR) repeatedly in 10 minutes. In other words, ECR was electrically activated contingent to FPB contraction. Before and after this motor task, we evaluated the TMS-based motor maps of FPB and ECR to see its alteration (7 x 7 cm grid configuration was used; Each grid point were 1 cm apart; the stimulus intensity of 110% of the lowest resting motor threshold at the target muscle was employed; 6 times of stimulation at each grid point). The averaged MEPs were then summed for each muscle to represent the net excitability. Overlapping area, defined as the number of the grid points where MEPs were elicited in both ECR and FPB, was also calculated as a representative value of the shared motor representation. The results showed that the excitability of ECR motor area included in overlapping area significantly increased after ETMS ($P < .01$). Moreover, overlapping ratio between ECR and FPB motor areas is also significantly increased ($P < .05$). Our results suggest that ETMS altered cortical motor representation of ECR and FPB through the reorganization of neural networks controlling these muscle activities.

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Poster

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Topic: D.13. Motor Neurons and Muscle

Support: NIH grant R01NS080954

Stanford BioX

Title: Optogenetic inhibition of peripheral nervous system circuitry

Authors: *C. GORINI[1], S. M. IYER[1], K. L. MONTGOMERY[1], S. YOUNG[1], H. SCUTT[1], A. CHRISTENSEN[1], D. J. CLARK[2], K. DEISSEROTH[1], S. L. DELP[1]
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Abstract: Many neural circuits in the peripheral nervous system are amenable to optogenetic modulation. These include primary motor neurons in disorders such as spasticity, as well as primary afferent nociceptors in chronic pain. One of the primary challenges involved in achieving optogenetic inhibition is establishing sufficiently strong expression of the opsin in the desired neuronal population. This is particularly relevant if opsin transduction is to be conducted using viral means, as different virus types can produce dramatically different levels of opsin expression and trafficking. We first examine the question of virally mediated optogenetic inhibition of motor neurons, which we have previously achieved in transgenic NpHR-expressing mouse lines (Liske, et al. 2014). We describe a broad scan of AAV serotypes and injection routes, as well as different opsins, and discuss the degree of transduction observed. Preliminary results indicate anterograde transduction of AAV2-eGFP to the mouse sciatic nerve following intraspinal injection of L4/L5. Additionally, we demonstrate significant retrograde trafficking of AAV8 following intrasciatic nerve injections. More work is necessary to characterize different AAV serotypes for potential inhibitory opsin trafficking. We then turn to optogenetic inhibition of nociceptors. We reported previously (Iyer, Montgomery, et al. 2014) that intrasciatic injections of AAV6-hSyn-eNpHR-eYFP resulted in specific expression of halorhodopsin in primary afferent nociceptors, and that transdermal illumination of injected mice could raise mechanical and thermal pain thresholds, both in uninjured mice, and in mice with the chronic constriction injury model of neuropathic pain. We examine whether this inhibitory capacity extends to other models of chronic pain, and in particular, examine a model of incisional pain. Preliminary results indicate that transdermally delivered yellow light may also reverse mechanical allodynia induced by an incisional pain model, indicating the generalizability of the optogenetic inhibition approach used.

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Poster

539. Motor Neurons and Muscle

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 539.16/LL21

Topic: D.13. Motor Neurons and Muscle

Support: NHMRC Australia

Title: Effect of acute upside down body posture on human respiratory control

Authors: *J. E. BUTLER[1,2], A. L. HUDSON[1,2], F. JOULIA[3], A. A. BUTLER[1,2], R. C. FITZPATRICK[1,2], S. C. GANDEVIA[1,2]
[1]Neurosci. Res. Australia, Randwick, NSW, Australia; [2]Univ. of New South Wales, Sydney, Australia; [3]Univ. of Toulon, Toulon, France

Abstract: Introduction: During quiet breathing, there is coordinated and efficient contraction of the obligatory inspiratory muscles. The neural drive to these muscles is distributed in a way that is coupled to their relative inspiratory mechanical advantage. This has been termed 'neuromechanical matching' (1,2). However, it is not known whether the neural drive to the inspiratory muscles adapts with alterations in the mechanics of the muscles or respiratory system. Here, we aimed to alter inspiratory muscle mechanics by altering body posture. We hypothesised that in the upside down posture the inspiratory mechanical advantage of the scalene muscles and diaphragm may be altered by gravitational effects on the rib cage and this would bring about a redistribution of neural drive to the inspiratory muscles. Methods: In 14 healthy human subjects, we determined the effect of the upside down posture on ventilation, thoracoabdominal expansion, inspiratory pressures and inspiratory muscle activity during quiet breathing, compared with standing and lying supine. Subjects breathed with a mouthpiece through a pneumotachometer with calibrated inductance bands around the chest wall and abdomen. Electromyographic activity (EMG) was recorded from scalene muscles with surface electrodes. Crural diaphragm EMG and oesophageal and gastric pressures were also measured with a purpose-built catheter (in a subset of 6 subjects). Thirty seconds of quiet breathing, maximal manoeuvres and standard lung function manoeuvres were performed in each posture. Results: The effect of the upside down posture was to increase inspiratory capacity and reduce end-expiratory lung volume. However, during quiet breathing, tidal volume, mean inspiratory airflow and inspiratory time were similar across all postures. While upside down, the ribcage contribution to tidal volume was less and the abdominal contribution was greater than when standing. Transdiaphragmatic pressure increased by ~ 230% in the upside down posture. Despite this, crural diaphragm EMG was unchanged, whereas scalene muscle EMG was significantly reduced ($p<0.05$). Conclusion: We have shown that the mechanical effects of the acute upside down posture modifies the activation of the inspiratory muscles. This could reflect an acute adaptation of neural drive to the changes in respiratory muscle mechanics; such has been demonstrated for a muscle in the hand (3). 1. Butler et al. Prog Brain Res 209: 295-308, 2014. 2. De Troyer et al. Physiol Rev 85: 717-756, 2005. 3. Hudson et al. J Physiol 587: 917-925, 2009.

Disclosures: **J.E. Butler:** None. **A.L. Hudson:** None. **A.A. Butler:** None. **R.C. Fitzpatrick:** None. **S.C. Gandevia:** None. **F. Joulia:** None.

Poster

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Topic: D.13. Motor Neurons and Muscle

Support: NRF-2013R1A2A2A01067890

Institute of Medical System Enginerring (iMSE) of GIST, Korea

Title: Sensory-parietal cortex stimulation for enhancing motor recovery in chronic subcortical capsular infarct model

Authors: *R. KIM[1], J. CHO[1], D. KIM[1], J.-Y. PARK[1], M.-C. LEE[2], H.-I. KIM[1]
[1]Gwangju Inst. of Sci. and Technol., Gwangju, Korea, Republic of; [2]Chonnam Natl. Univ., Gwangju, Korea, Republic of

Abstract: Introduction: Subcortical capsular stroke leaves severe long-term motor disability, which demands alternative treatment other than traditional treatment. We stimulated sensory-parietal cortex (SPC), where diaschisis was markedly observed in our preliminary studies, to test whether cortical stimulation may contribute to restoring the motor fundtion. Using microPET, behavioral study and analysis of c-Fos protein, we aimed to identify the dynamic changes of the neural substrates coupled with SPC stimulation leading to motor recovery and to elucidate the better strategy of rehabilitation in the subcortical capsular infarction (SCI) model. Material &Methods: Adult male Sprague Dawley rats (n=33) underwent the unilateral photothrombotic subcortical lesioning in the posterior limb of internal capsule (PLIC) and were divided into continuous-stimulation group (CSG: n=20), intermittent stimulation group (ISG: n = 10) depending on the mode of stimulation and non-stimulation group (NSG: n=13). Stimulation groups received cortical stimulation for 2 weeks concurrently with daily single pellet reaching tests, and was subjected to [18F-FDG] microPET static images were obtained longitudinally at 4 day, 1 week and 2 weeks of stimulation. Reconstructed PET images analyzed in voxel-wise fashion. The distribution of c-Fos immunopositivity following stimulation was measured. Results: The rats that received continuous SPC stimulation showed significant improvement of single pellet reaching scores compared to NSG from 3rd day of stimulation ($p<0.01$). On the

other hand, ISG showed motor recovery from 6th day of stimulation but degree of motor recovery was much less than CSG ($p<0.05$). The c-Fos was expressed bilaterally in significantly recovered CSG whereas only in ipsi-lateral cortex in ISG. MicroPET images shows the disappearance of diaschisis in SPC, activation of ipsilateral SPC and hippocampus, contralateral SPC and striatum. Conclusions: This study suggests that SPC stimulation is likely to reverse the diaschisis of the bilateral sensory-parietal cortex, leading to motor recovery. Further coactivation of cortical subcortical network is considered to contribute to the functional reorganization in chronic SCI models.

Disclosures: R. Kim: None. **J. Cho:** None. **D. Kim:** None. **J. Park:** None. **M. Lee:** None. **H. Kim:** None.

Poster

539. Motor Neurons and Muscle

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Topic: D.13. Motor Neurons and Muscle

Support: 5P01-NS057228

Title: Group Ia reciprocal excitation between ankle antagonists in a plantigrade animal

Authors: *A. S. DEARDORFF[1], R. E. W. FYFFE[2], T. C. COPE[3]
[2]VP for Res., [3]Chair, Dept of Neuroscience, Cell Biology, & Physiol., [1]Wright State Univ., DAYTON, OH

Abstract: Rodents are increasingly used for morphologic and genetic analysis of spinal motor circuitry, yet data on rodent segmental circuit function are not well established. For example, a wealth of information exists on the ontogeny and synaptic connectivity of rodent 1a inhibitory interneurons, but physiologic analyses of reciprocal antagonist connections, which are so well documented in the cat, are scarce in the adult rat. Digitigrade operation of the ankle joint in cats requires plantar and dorsiflexors adopt a role in ankle stabilization distinct from plantigrade posturing of the rat or human. We speculate these obvious differences in hindlimb movements to manifest species difference(s) in their segmental circuit function, including the 'classic' 1a reciprocal inhibitory pathway. Nichols & Koffler-Smulevitz (1991) assign an important task in stabilizing the feline ankle joint to reciprocal Ia inhibition; Hongo et al (1984) propose species differences in this segmental circuit may, in part, relate to plantigrade vs digitigrade limb use.

We tested these notions by studying the Ia reciprocal pathways, which are so well documented in cat, for the first time in the adult rat. Intracellular records of synaptic potentials were obtained *in vivo* from motoneurons of acutely decerebrated or ketamine/xylazine anesthetized adult rats. Following selective physiological activation of tibialis anterior Ia afferents, we observed few instances of reciprocal inhibition in rat medial gastrocnemius motoneurons. Rather, we frequently observed marked reciprocal excitation at latencies consistent with a tri-synaptic pathway. Our results demonstrate a pathway for Ia excitation of antagonist muscles which may represent a useful adaptation for species (eg: human) exhibiting plantigrade gait.

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Poster

539. Motor Neurons and Muscle

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Topic: D.13. Motor Neurons and Muscle

Support: NIH/NICHD grant HD32571

Title: Effects of 60-min sciatic nerve stimulation immediately after cut and repair of feline soleus and lateral gastrocnemius nerves on locomotor EMG activity of ankle muscles

Authors: *A. PANTALL[1], R. J. GREGOR[2], R. MEHTA[3], B. I. PRILUTSKY[3]
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Abstract: Patients with peripheral nerve injury do not fully recover motor function after surgical nerve repair (Taylor et al. 2010). The major contributory factor to poor functional recovery is the slow regeneration of nerves (1-3 mm/day; Brushart et al. 2002). Therapeutic interventions being investigated to mitigate this factor include electrical stimulation to the proximal stump of the repaired nerve. Animal studies show this markedly enhances regeneration of severed axons (Gordon et al. 2009). However, it has also been reported that such stimulation increases reinnervation of inappropriate targets (English 2005). Little information is available on functional consequences of this intervention. The aim of this study was to determine the effect of electrical stimulation of axons of soleus (SO) and lateral gastrocnemius (LG) nerves on the locomotor EMG intensity and mean frequency of ankle muscles following reinnervation of SO and LG. EMG fine wire electrodes were implanted into four or more muscles of the hindlimb

including SO, LG, medial gastrocnemius (MG) and tibialis anterior (TA) in several cats. After recording baseline EMG activity and mechanics of overground walking, the nerve innervating SO and LG was cut and repaired using fibrin glue (English 2005). Immediately after LG-SO nerve repair, the sciatic nerve was stimulated through an implanted cuff electrode during four 15-min bouts of stimulation (2T, 7 Hz) with 5-min rest period between each bout. We recorded hindlimb mechanics and EMG during overground walking prior to surgery and weekly for 3 to 9 months following surgery. Mean EMG intensities and frequencies pre-surgery, determined by non-linear wavelet analysis, were similar to our previous findings (Hodson-Tole et al 2012; Pantall et al. 2014). For example, the mean EMG frequency of slow-twitch SO was lower than that of fast-twitch LG, MG and TA ($p<0.05$). However, post-reinnervation, there was a greater EMG intensity increase and reduction in frequency in reinnervated SO and LG and in intact MG compared to non-stimulated cats (Pantall et al 2014). EMG of non-reinnervated antagonist TA (not reported previously) decreased in intensity but increased in frequency. The greater increase in EMG intensity after nerve stimulation could result from greater enlargement of motor units (Krarup et al. 2002) and enhanced reduction in collaterals and inhibitory synapses from Renshaw cells on motor neurons of self-reinnervated and intact synergists (Havton and Kellerth 1990). The greater frequency decrease may be caused by smaller nerve fiber diameter and slower conduction velocity after nerve repair (Krarup et al. 2002).

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Poster

539. Motor Neurons and Muscle

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Topic: D.12. Kinematics and EMG

Support: Jeffress Foundation

Title: Dependence of the nociceptive withdrawal response of the tail on initial angle in intact, unanesthetized rats

Authors: *J. KIM, S. B. DONAIRE, R. E. MULLINS, J. J. YANG, C. L. CLELAND
Biol., James Madison Univ., Harrisonburg, VA

Abstract: Heat stimuli delivered to the tail of the intact, unanesthetized rat evokes a nociceptive withdrawal response (NWR) of the tail (Cleland & Bauer 2002). Previous studies from our

laboratory have shown that the response depends on the circumferential and rostral-caudal location of the stimulus (Bence & Cleland 2012). Although there is some evidence that the human NWR varies with initial posture, such as hip angle, weighting of the leg or ongoing movements, there is little information in animal models. The specific aim of this study was to determine if the NWR of the rat tail in intact, unanesthetized rats depends on the initial angle of the tail in the rostral-caudal/left-right plane. Adult male rats were restrained in a darkened acrylic tube from which only their tail emerged. The tails were marked with 13 black bands distributed evenly from the base to the tip of the tail. The angle of the tail at its base was varied between 0 degrees (parallel to the long axis of the body) through 30 degree intervals to 90 degrees (perpendicular to the long axis of the body). Heat stimuli were delivered with a laser diode (980nm; BWTek) to each side of a band roughly 30% caudal to the base of the tail. The resulting NWR of the tail was recorded using high speed video (650 fps; Redlake/IDT). The 13 marks were tracked in software (Proanalyst, Xcitex) to describe the movement of the entire tail in the horizontal plane over time. The response to the heat stimulus consisted of several components. First, a local bend moved the site of stimulation away from the stimulus. Next, muscles acting on the base of the tail moved the entire tail away from the stimulus. When the tail was bent at the base to the most extreme position (90 degrees) and the stimulus was directed from a caudal position, the local bend away persisted; however, the response of the base tail was now directed toward the stimulus. These results suggest that the NWR of the tail depends on the initial angle at the base of the tail, allowing the rat to adapt movements that might be blocked by the body of rat.

Disclosures: J. Kim: None. **S.B. Donaire:** None. **R.E. Mullins:** None. **J.J. Yang:** None. **C.L. Cleland:** None.

Poster

539. Motor Neurons and Muscle

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 539.21/LL26

Topic: D.12. Kinematics and EMG

Support: Jeffress Foundation

Taliaferro Scholarship, James Madison University

Title: Rat hind limb nociceptive withdrawal response to heat stimuli depends on initial paw posture but not stimulus location

Authors: *K. SEAMON, M. HARTMANN, C. A. CHRZAN, M. N. KABORE, K. A. MOORE, C. L. CLELAND
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Abstract: Rats rapidly withdraw their hind limb in response to a noxious heat stimulus applied to the plantar surface of their paw, which is an example of the Nociceptive Withdrawal Response (NWR). Previous studies in spinalized or lightly anesthetized non-human mammals have shown that the direction of response depends on stimulus location. The goal of this study was to determine if stimulus location, or other factors such as initial posture or response latency, determines the direction of withdrawal in the intact, unanesthetized rat. Rats were placed on a glass plate through which an infrared laser beam (980 nm) was directed to heat a small (1mm) localized portion of the plantar surface of the foot. The resulting withdrawal response was recorded with three conventional camcorders (60 fps @ 1080p), one on the left, one on the right, and the third underneath the rat. From the video beneath the rat, the initial location and angle of the stimulated paw was recorded. In response to the stimulus, the rat then withdrew and rapidly (~40ms) replaced its paw on the glass, at which point the final location and angle of the paw were recorded. Rats withdrew and then replaced their paw on the glass in all possible directions. To determine if the location of the stimulus influenced response direction, the rat's paw was stimulated in five locations (three aligned rostral-caudal and three aligned left-right). Unexpectedly, we found no dependence on stimulus location. However, we did notice the initial position of paw varied in both location and angle. Consequently we explored if initial position (left-right and rostra-caudal) and paw angle influenced final location and angle. Correlation between initial and final locations and angles did reveal a highly significant linear relationship ($p<0.001$), regardless of response latency. These results demonstrate, in contrast to studies in spinalized or anesthetized non-human animals, that initial posture plays a greater role in the programming of the NWR than stimulus location.

Disclosures: K. Seamon: None. **M. Hartmann:** None. **C.A. Chrzan:** None. **M.N. Kabore:** None. **K.A. Moore:** None. **C.L. Cleland:** None.

Poster

539. Motor Neurons and Muscle

Location: Halls A-C

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Program#/Poster#: 539.22/LL27

Topic: D.13. Motor Neurons and Muscle

Support: DoD STTR W81XWH-13-C-0157

NSF 0748001

Title: Combination electrical stimulation and pulsatile acetylcholine release therapy to preserve neuromuscular juntion health in peripheral nerve injury

Authors: *J. R. ELES[1,3], C. L. KOLARCIK[2], K. A. CATT[2], X. T. CUI[2,3,4]
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Abstract: Despite advances in regenerative medicine and scaffolding therapies for peripheral nerve repair and regrowth, functional recovery of large nerve injuries is still limited. Of of the contributing factors to this is the atrophy of neuromuscular junction (NMJ) targets distal to damaged peripheral nerves. We present a novel solution to preserving downstream NMJ targets that involves 1) chronic electrical stimulation to distal NMJs and 2) pulsatile acetylcholine (Ach) release from poly(3 4-ethylenedioxythiophene)/ graphene oxide-acetylcholine complex (PEDOT/GO-Ach) coatings on NMJ-contacting electrodes. To our knowledge, this is the only therapy system that combines the well-established benefits of chronic electrical stimulation with local, on-demand release of therapeutic agents. To optimize our system, we electropolymerized PEDOT/GO-Ach coatings onto gold electrodes. By allowing GO and Ach to interact prior to electropolymerization, cationic Ach can be readily incorporated into the growing PEDOT coat, which improves upon previously used methods of cationic drug incorporation that rely on loading post-polymerization. We have shown that biphasic, low-frequency stimulation of PEDOT/GO-Ach electrodes yields consistent, physiologically relevant amounts of Ach release (5.3 ± 1.7 ng/mm^2 electrode surface area/10 minute stimulation period) for thousands of stimulation cycles. Further, our PEDOT/GO-Ach coatings improve electrical properties of our electrodes, lowering 1Hz-1KHz band electrical impedance, which can improve the charge injection limit. We found that applying our therapeutic electrical stimulation with 5µM Ach to an *in vitro* innervated muscle co-culture model (NSC34/C2C12 cell lines) led to trending improvements in NMJ clustering as labeled by Ach receptor staining (48.8 ± 8.8 v 90.9 ± 22.5 NMJ/mm^2, control v. stimulation + added Ach). Thus, our therapy system presents as a viable candidate for improving functional nerve repair.

Disclosures: J.R. Eles: None. **C.L. Kolarcik:** None. **X.T. Cui:** None. **K.A. Catt:** None.

Poster

539. Motor Neurons and Muscle

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Program#/Poster#: 539.23/LL28

Topic: D.12. Kinematics and EMG

Support: HSF-G-13-0001624

Title: Time course "dose" of cross-education of strength after handgrip training

Authors: *Y. SUN[1,2,3], T. BARSS[1,2,3], T. KLARNER[1,2,3], E. ZEHR[1,2,3,4]
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Abstract: Cross-education is a neural adaptation defined as the increase in strength or functional performance of the untrained contralateral limb after unilateral training. This phenomenon has been found in both neurologically intact and clinical (e.g. stroke, orthopedic injury) populations. Limitations for clinical translation include knowledge on the minimum time course for emergence of the crossed effects. Currently, little is known about the time-course of strength increase in each limb during training. Therefore, the major purpose of this study was to characterize the time-course of strength changes in both the trained and untrained limbs during handgrip training. An anticipated outcome was the determination of the minimum training weeks required to induce cross-education in the upper limb. Neurologically intact right-handed participants were recruited for a 6 weeks of thrice-weekly training. During each training session, participants performed 5 sets of 5 maximal isometric contractions (MVC) by gripping a dynamometer with their right hand. To evaluate the changes in strength and muscle functions, MVC force during handgrip, pinchgrip force, and MVC wrist extension and flexion torque, were measured in both trained and untrained limbs before and after training. In order to assess neurological adaptations, electromyography (EMG) of extensor carpi radialis (ECR), flexor carpi radialis (FCR), biceps brachii (BB) and triceps brachii (TB) muscles were recorded along with H-reflex and M-wave recruitment curves and cutaneous (superficial radial nerve)-conditioned recruitment curves of ECR muscle were also measured during pre and post-training. To track the time course of force changes the trained limbs, handgrip MVC force of all training repetitions were recorded and EMG of ECR and FCR were measured during one session each week. For the untrained limb, MVC of handgrip force and EMG of ECR and FCR were measured once a week from a single contraction. After six weeks of training, handgrip and pinchgrip force and wrist extension and flexion torque increased in both untrained and trained limbs. Changes in the maximal muscle activation and amplitude of the H-reflex were observed. This results of this study emphasize the importance of establishing effective "dose" for time course of functional and neurological adaptations in strength for effective translation to rehabilitative interventions.

Disclosures: **Y. Sun:** None. **T. Barss:** None. **T. Klarner:** None. **E. Zehr:** None.

Poster

539. Motor Neurons and Muscle

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Program#/Poster#: 539.24/MM1

Topic: D.12. Kinematics and EMG

Support: KAKENHI, Grant-in-Aid for Challenging Exploratory Research

KAKENHI, Grants-in-Aid for Scientific Research C

Natural Sciences and Engineering Research Council of Canada

Title: Phase-dependent cutaneous reflex reversal during walking emerges from reflex signs produced by afferents in discrete foot sole regions

Authors: *T. KOMIYAMA[1], T. NAKAJIMA[2], E. P. ZEHR[3,4,5], S. SUZUKI[1], R. A. MEZZARANE[6], H. OHTSUKA[7], G. FUTATSUBASHI[1], T. KLARNER[3], T. S. BARSS[3]
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Abstract: During human locomotion, cutaneous reflexes in ankle flexor muscles following distal tibial nerve (TIB) stimulation are predominantly facilitatory at the end of stance but reverse to suppression at heel strike. Explanatory mechanisms underlying this phase-dependent reflex reversal include the presence of "distinct" facilitatory or inhibitory interneuronal reflex pathways converging on alpha motoneurons. Because the TIB nerve innervates much of the skin of the foot sole, it remains unclear whether specific foot sole regions contribute to the bidirectional reflex actions during walking. Indeed, we previously showed that electrical stimulation of discrete foot sole regions could evoke inhibitory and facilitatory cutaneous reflexes during walking. Therefore, here we investigated possible regional contributions of cutaneous afferents innervating the foot sole on bidirectional reflexes following TIB nerve stimulation during walking.Cutaneous reflexes (MLR: 70-120 ms) in tibialis anterior (TA) muscle were elicited by delivering electrical stimulation to 3 foot sole regions (heel site: HL, fore foot medial site: f-M, fore foot lateral site: f-L) and TIB nerve and recorded during 16 different phases of the step cycle. In addition, we examined summation effect of reflex amplitudes after combined stimulation of each foot sole region (f-M or HL) and TIB nerve. MLRs after TIB stimulation

were strongly facilitatory during the late stance to mid swing phases and reversed to suppression around heel strike. Both f-M and f-L stimulation induced facilitation during the late stance to mid swing phases, but HL stimulation evoked strong suppression during the late stance to end of swing phases. Within each region of foot sole stimulation no evidence of phase-dependent reflex reversal could be found. We also investigated whether there was a summation effect following combined nervetrunk and f-M or HL stimulation. At the stance to swing transition a summation of MLR amplitude occurred during combined f-M (facilitatory) and TIB (facilitatory) stimulation. However, the same was not true for the combined HL (inhibitory sign) & TIB stimulation. At the swing to stance transition, there was a reflex summation during HL (inhibitory) & TIB (inhibitory) stimulation. In contrast, this summation was not observed after the f-M (facilitatory) & TIB (inhibitory) stimulation. Thus, our results suggest that reflex reversal observed during whole nerve stimulation (e.g. TIB) is an emergent property of the distinct effects produced by afferent populations innervating specific regions of the foot sole.

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Poster

539. Motor Neurons and Muscle

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 539.25/MM2

Topic: D.12. Kinematics and EMG

Support: HSF-G-13-0001629

NSERC-217374

Title: Arm and leg cycling training to improve neurological function and walking ability after stroke

Authors: *T. KLARNER[1,2,3], T. S. BARSS[1,2,3], Y. SUN[1,2,3], C. KAUPP[1,2,3], P. LOADMAN[1], E. ZEHR[1,2,3]
[1]Rehabil. Neurosci. Laboratory, Univ. Victoria, Victoria, BC, Canada; [2]Ctr. for Biomed. Res., Victoria, BC, Canada; [3]ICORD, Vancouver, BC, Canada

Abstract: Rhythmic arm and leg (A&L) movements found in walking, cycling, and stepping share elements of central neural control. While it is reasonable to expect some transfer between locomotor tasks, the extent to which training in rhythmic A&L cycling can transfer to improved neurological function and enhanced ability to perform walking remains untested. The purpose of this study was to test the efficacy of A&L cycling training as a modality to improve locomotor function after stroke. Chronic stroke participants (i.e. greater than six months post infarct) were recruited and performed A&L cycling training (Sci-Fit Pro 2 ergometer) three times a week, with 30 minutes of aggregate activity time per session, for a total duration of 5 weeks. To evaluate the physiological cost of activity heart rate and rating of perceived exertion were collected. The progressive element of this training included increasing the resistance of the ergometer over the five weeks in order to maintain the same relative perceived exertion. To assess the strength of arm and leg coupling, interlimb cutaneous reflexes were evoked by simultaneous electrical stimulation (5x1.0ms trains @ 300Hz) to the cutaneous nerves in the hand (superficial radial) and foot (superficial peroneal) during A&L cycling and treadmill walking. Responses recorded in all four limbs were evaluated across all phases of the walking cycle. Changes in walking function were gauged via a customized analysis of muscle activity patterns and lower limb joint kinematics. Functional and clinical walking ability were assessed with the Ambulation Index, Functional Walking Scale, the timed up and go test, and the ten meter and six minute walking tests. Also, to assess possible suppression of exaggerated reflex excitability associated with spasticity, stretch reflexes in the soleus muscle were evoked and the modified Ashworth test and the Tardieu scale were used. Multiple pre-test sessions were used to establish a meaningful baseline of all outcome measures prior to the intervention. Our preliminary data show that after a stroke A&L cycling improves neurological function leading to improved walking ability seen in reflex function, gait analysis, and in an increased distance walked in the six minute walk test. These results suggest that arm and leg cycling could be used as an additional modality to improve interlimb coupling, walking ability, and clinical presentation of spasticity after stroke.

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Poster

539. Motor Neurons and Muscle

Location: Halls A-C

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Topic: D.12. Kinematics and EMG

Support: FAPESP (2010/15522-4)

Title: Effects of presynaptic inhibition on bilateral fluctuations of H- and T-reflexes of the soleus muscle

Authors: *R. A. MEZZARANE[1], F. MAGALHÃES[2], A. KOHN[3]
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Abstract: The stretch reflex of the soleus muscle is a useful tool employed in spinal cord neurophysiology studies. Its amplitude presents an intrinsic trial to trial variability associated with both pre and post-synaptic mechanisms. Previous studies showed that this modulation occurs for both legs and the correlation of bilateral H-reflex fluctuations can be changed according to the task. However, to date, the influences of a higher level presynaptic inhibition (PSI) on bilateral H-reflex fluctuations have not yet been explicitly inspected. Moreover, no study examined bilateral T-reflexes. The present work aimed at investigating bilateral fluctuations of both H and T-reflexes under different conditions: 1) at rest; 2) during soleus voluntary contraction (10% MVC); 3) with PSI conditioning; 4) contraction + PSI. Fifteen subjects, aged 32.2 ± 6.2 years (mean ± STD), were seated in an armchair with the legs immobilized. The mechanical stimulus was 1 cycle of a sine wave with 10 ms duration applied to the Achilles tendon through a vibratory transducer. The electrical stimulus was a rectangular pulse (1 ms duration) applied to the posterior tibial nerve. Five hundred electrical or mechanical test stimuli were applied to both legs simultaneously at 1Hz to elicit H or T-reflexes (20%Mmax) in conditions 1-4. A 1 ms conditioning electrical pulse was applied to the neck of the fibula 100 ms before either the mechanical or electrical test stimulus (conditions 3 and 4). Sequences of cross-covariance (CCV) were calculated in each condition to detect bilateral fluctuations in the peak-to-peak amplitudes of the reflexes obtained from both legs. To avoid the occurrence of false positives, both reflex sequences were whitened by means of an inverse auto-regressive filter. This procedure allows specifying the critical limit for the CCV peaks (at $p<0.05$). Two way ANOVA detected significant effect of voluntary contraction (increased amplitude) (condition 2) and PSI conditioning (decreased amplitude) (condition 3) on both T and H-reflex as compared to condition 1 (rest) ($p<0.05$). There were no differences in the frequency of occurrence of significant CCV peak across conditions for T-reflex. However, H-reflex showed lower number of significant CCV peaks in condition 4 as compared to other conditions (chi-squared test; $p<0.05$). These results seem to reinforce the hypothesis of the existence of an interaction between post and presynaptic mechanisms acting on H-reflex bilateral fluctuations as previously suggested. Further, it is currently suggested that the fusimotor drive can counteract the disruption in bilateral fluctuations caused by this interaction.

Disclosures: **R.A. Mezzarane:** None. **F. Magalhães:** None. **A. Kohn:** None.

Poster

539. Motor Neurons and Muscle

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 539.27/MM4

Topic: D.12. Kinematics and EMG

Support: HSF-G-13-0001629

NSERC-217374

Title: Effects of enhanced sensory feedback on cross-education of strength in the wrist extensors

Authors: *T. S. BARSS[1,2,3], T. KLARNER[1,2,3], K. INOUYE[1], E. ZEHR[1,2,3,4]
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Abstract: An increase in strength of the untrained limb after training of the opposite homologous limb is commonly referred to as "cross-education". Strength training involves forceful contractions that activate cutaneous receptors in the skin, producing widespread and powerful effects between limbs. Providing "enhanced" cutaneous stimulation during unilateral contractions may alter excitability of interlimb reflex pathways, modifying the contralateral increase in strength. To date, no study has directly assessed the possible contribution of afferent pathways to cross-education. Therefore, the purpose of this study was to determine the contribution of cutaneous input in cross-education. This was assessed by determining if unilateral cutaneous stimulation altered ipsilateral and contralateral strength gains. Right handed participants were randomly assigned to either a voluntary contraction (VOL), cutaneous stimulation (CUT), or cutaneous stimulation during voluntary contraction with cutaneous stimulation (VOL+CUT) training group. Each participant completed 6 sets of 8 reps 3x/week for 5 weeks. VOL training included unilateral maximal voluntary isometric contractions (MVICs) of the wrist extensors. CUT training included cutaneous stimulation (2xRT for 3sec @ 50Hz) of the superficial radial (SR) nerve at the wrist only. Training for VOL+CUT included MVICs of the wrist extensors with simultaneous SR stimulation. Two pre-training and 1 post-training session assessed the relative increase in force output during MVICs for wrist flexion, wrist extension and handgrip strength. Simultaneous maximal voluntary muscle activation (EMGmax) was measured from the flexor and extensor carpi radialis (normalized to maximal evoked M-waves), as well as the biceps and triceps brachii muscles. Changes in efficacy in cutaneous reflex pathways were evaluated through stimulation of the SR and median (MED) nerves (3xRT for 5x1ms @ 300Hz)

during graded ipsilateral contractions of 5, 10, 25, and 50 % of EMGmax. Somatosensory evoked potentials were recorded at rest (3xRT for 1ms @ 300 Hz) via single pulse stimulation of the SR and MED nerves at the wrist. If unilateral cutaneous stimulation alters the strength gains in either the trained or untrained limb it will provide the first evidence of a cutaneous afferent contribution to the cross-education effect. This study will help refine a unifying model of unilateral strength training to include contributions from central motor output as well as afferent feedback.

Disclosures: T.S. Barss: None. **T. Klarner:** None. **K. Inouye:** None. **E. Zehr:** None.

Poster

539. Motor Neurons and Muscle

Location: Halls A-C

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Program#/Poster#: 539.28/MM5

Topic: D.12. Kinematics and EMG

Support: BEPE program from FAPESP (proc. no. 2012/05304-5)

Title: Limb-specific control of interlimb reflex transmission during walking in humans

Authors: *S. SUZUKI[1,2,3], T. NAKAJIMA[2], G. FUTATSUBASHI[1,3,4], R. A. MEZZARANE[3,5], H. OHTSUKA[6], T. KOMIYAMA[1,3]
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Abstract: Cutaneous mechanoreceptors located in the limb can give rise to wide spread reflex actions even in the distant limb muscles. We previously reported that the cutaneous inputs from the contralateral foot could differentially modify the soleus H-reflex depending on the executed motor task. To date, however, it remains unclear how cutaneous inputs from discrete and remote limbs modulate the excitability of the spinal reflex during walking. The present study aimed to investigate how cutaneous inputs from the remote hand and foot could modify the H-reflex excitability in the ankle extensor muscle during standing and walking. Healthy volunteers were asked to walk on a treadmill at 4 km/h or to perform a weak tonic voluntary contraction of the

right soleus muscle during standing. The soleus H-reflex was elicited by electrical stimulation of the posterior tibial nerve at the right popliteal fossa. Conditioning non-noxious cutaneous nerve stimulation was applied to the superficial peroneal nerve at the left ankle (cSP), the superficial radial nerve at the right wrist (iSR), or the superficial radial nerve at the left wrist (cSR). The conditioning stimulation preceded the test stimulation to evoke the H-reflex by approximately 100–120 msec. The test stimulation, conditioning stimulation or a combination of the two was delivered during the early stance phase of the ipsilateral limb (~10% of gait cycle). During standing, the amplitude of the conditioned H-reflex increased in a non-specific manner (i.e., the conditioning effect was not dependent on the stimulated nerve). In contrast, a significant decrease in the amplitude of the conditioned H-reflex was observed during walking. Interestingly, the amount of decrease in the amplitude of the conditioned H-reflex was larger for cSP stimulation than for iSR or cSR stimulation. Conditioning cutaneous stimulation never changed the amplitude of the pre-stimulus background or ongoing EMG activity at a given latency (approximately 120-150 ms after the conditioning stimulation). These observations indicate that limb-dependent organization of the interlimb reflex transmission is specific to walking, but not to standing. Moreover, our findings strongly suggest that presynaptic mechanisms would play a crucial role in regulating the interlimb conditioning effect on the H-reflex.

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Poster

539. Motor Neurons and Muscle

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 539.29/MM6

Topic: D.12. Kinematics and EMG

Support: Foundation for Physical Therapy PODS I Scholarship

GK12 Transforming Experiences Project (NSF DGE-0742434)

Title: Changes in acoustic startle reflex in the upper trapezius during periods of low and high psychosocial stress

Authors: *R. J. MARKER, K. S. MALUF
Univ. of Colorado Anschutz Med. Campus, Aurora, CO

Abstract: Introduction: An increase in upper trapezius muscle activity is common during periods of increased psychosocial stress, and may contribute to the development or persistence of chronic neck pain. The mechanisms of this increased activity, however, are relatively unknown. The reticular formation is associated with arousal levels of an individual, indicating that reticulospinal input to the upper trapezius may contribute to stress-induced muscle activity. The purpose of this study was to assess changes in reticulospinal input to the upper trapezius during periods of low and high psychosocial stress, as assessed with the acoustic startle reflex (ASR). Methods: Surface electromyography (EMG) was used to record the ASR from the non-dominate upper trapezius of healthy individuals. Participants were seated and instructed to perform a low-level shrug to provide a constant level of background muscle activity. A startling acoustic stimulus (SAS, 124 dB, 50 ms) was provided via headphones over a constant background sound intensity of 60 dB. In order to prevent habituation of the ASR, participants performed a simple reaction time task, with a non-startling tone as the imperative cue. In 30% of trials, the non-startling tone was replaced with the SAS. Increased psychosocial stress was achieved by providing participants with time demands and a monetary incentive, setting up camera surveillance, and introducing an unfamiliar, authoritative investigator. Percent of SAS trials producing an upper trapezius ASR, percent of ASRs resulting in inhibition of muscle activity, and length of silent period in inhibitory ASRs were calculated in low and high stress conditions. Results: The percent of SAS trials producing an ASR decreased from 70% to 48% during low and high stress, respectively. The percent of ASRs resulting in an inhibition of muscle activity, however, increased from 77% to 96%. Finally, the length of the silent periods present in inhibitory ASRs displayed minimal change (54 ms vs 46 ms, in low and high stress, respectively). Discussion: Reticulospinal input to the upper trapezius, assessed by ASR, appears to be primarily inhibitory. The reduction in ASRs produced during increased psychosocial stress may be one mechanism contributing to stress-induced muscle activity, while the increase in percent of inhibitory ASRs may be a compensatory mechanism, attempting to decrease excess muscle activity. Understanding these mechanism may assist in the treatment of individuals with chronic neck pain.

Disclosures: R.J. Marker: None. **K.S. Maluf:** None.

Poster

539. Motor Neurons and Muscle

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 539.30/MM7

Topic: D.12. Kinematics and EMG

Title: Quantification of patellar tendon reflex response using an iPod wireless gyroscope application with experimentation conducted in Lhasa, Tibet and post-processing conducted in Flagstaff, Arizona through wireless Internet connectivity

Authors: *R. C. LEMOYNE[1,3], T. MASTROIANNI[2]
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Abstract: The patellar tendon reflex provides a foundational role in the standard neurological examination. The attributes of the reflex response can provide preliminary insight regarding the health of both the central and peripheral nervous systems. Traditional approaches to classifying the reflex response involve the application of an ordinal scale. Smartphones and portable media devices have been successfully tested and evaluated as wireless accelerometer platforms for reflex response quantification. The iPod is equipped with a gyroscope sensor capable of measuring rate of angular rotation. With a software application the iPod is capable of functioning as a wireless gyroscope platform. Trial data can be conveyed by wireless connectivity to the Internet through email enabling the experimentation and post-processing resources to be remotely located. The experimentation site was situated in Lhasa, Tibet; and the post-processing resources were located in Flagstaff, Arizona. The iPod wireless gyroscope application successfully demonstrates the capacity to quantify the features of the patellar tendon reflex response.

Disclosures: R.C. LeMoyne: None. **T. Mastroianni:** None.

Poster

540. Cortex and Nuclei: Anatomy and In Vitro Studies

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 540.01/MM8

Topic: D.14. Cerebellum: Central Physiology

Support: Seattle Children's Research Institute

Title: Diversity of Purkinje cell morphology and physiology in the cerebellum of the adult zebrafish

Authors: *V. Z. HAN, J. P. WELSH
Ctr. for Integrative Brain Res., Seattle Children's, Seattle, WA

Abstract: The goal of this study was to explore the electrophysiological and morphological properties of Purkinje cells (PCs) in the zebrafish cerebellum. Sagittal cerebellar slices (~200 μm thick) were prepared from adult wild-type zebrafish (≥6 months old) of either sex. Whole-cell somatic patch recordings were performed in either voltage- or current-clamp mode under visual control, and recording pipettes were loaded with Neurobiotin to label the cells following physiological characterization. PCs were identified by having a soma in the PC layer and an axonal arbor that was restricted to the lower PC/upper granule cell layers where their synaptic targets, the eurydendroid cells, are located. An unexpected diversity in PC dendritic morphology was encountered that segregated into 3 groups. Group 1 (n=5) had dendritic arbors restricted to the inner molecular layer (ML); Group 2 (n=10) had a primary dendrite whose length ascended up to 30 μm through the inner ML before branching in the outer ML; Group 3 (n=11) had dendritic arbors that branched throughout the thickness of the ML, similar in that way to mammalian PCs. The spontaneous firing of zebrafish PCs was characterized by narrow (< 2 ms width) and broad (5-10 ms width) spikes, presumably of axonal and dendritic origin that were blocked by TTX and Cd^{2+}, respectively. The two spike types were co-expressed in some - but not all - PCs. Groups 1 and 3, sharing the trait of branched dendritic arbors near the soma, primarily showed both narrow and broad spikes (13 of 16 PCs) while Group 2, having only a single dendrite close to the soma, primarily showed only narrow spikes (9 of 10 PCs). Focal stimulation in the ML activated graded responses similar to parallel fiber responses (n=7), whereas stimulation in the upper granule cell layer evoked large all-or-none responses similar to climbing fiber responses (n=5). Both responses remained unchanged under the NMDA receptor blocker AP5, but disappeared under the AMPA receptor blocker CNQX. The study revealed that zebrafish PCs are morphologically and physiologically heterogeneous to an extent not seen in mammals, indicating that the functional circuitry of the zebrafish cerebellum may have unique integrative properties. **Support:** Seattle Children’s Research Institute

Disclosures: V.Z. Han: None. **J.P. Welsh:** None.

Poster

540. Cortex and Nuclei: Anatomy and In Vitro Studies

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 540.02/MM9

Topic: D.14. Cerebellum: Central Physiology

Support: National Center for Research Resources Grant 5P20RR016469

NIGMS Grant 8P20GM103427

Creighton University

Title: Dicer knockout mice suggest critical role of micro-rna in cerebellar cell proliferation, organization, and migration

Authors: *E. ARNESON[1], T. MIGHELL[1], M. BOSCH[1], G. SOUKUP[2], C. BRADFIELD[2], A. SHIBATA[1]
[1]Biol., [2]Biomed. Sci., Creighton Univ., Omaha, NE

Abstract: MicroRNAs (miRNAs) are involved in a variety of essential cell mechanisms such as proliferation, differentiation, and apoptosis. Both *in vitro* and *in vivo* studies show that miRNAs are involved in essential processes controlling neural development. We hypothesize that miRNA expression is required for the proper postnatal differentiation, migration, and survival of granule cells in the cerebellum. To investigate miRNA function in cerebellar granule precursor cells (CGPCs), conditional Dicer knock-out (CKO) mice using the expression of Cre recombinase under the control of the cerebellar granule cell specific Atoh1 gene promoter were developed (Soukup et al., 2009). Characterization of the motor behaviors and cerebellar development in Atoh-1 CRE conditional Dicer knockout mice reveals a critical role for miRNAs in the differentiation, migration, survival, and function of CGPCs. Immunohistochemical studies show that the vermis, Crus I, and Crus II regions of the cerebellum are malformed in CKO mice. CGPC differentiation determined by Zic 2 and NeuN staining and Purkinje cell differentiation determined by Calbindin staining are disrupted in CKO mice as compared to wild type. Cell proliferation as determined by Ki-67 staining is reduced and aberrant and also reveals abnormal migration of CGPCs as compared to wild type. Disrupted proliferation of CGPCs may be linked to abnormal sonic hedgehog (SHH) signaling from Purkinje cells. SHH immunofluorescence suggests that this signaling pathway is disrupted in CKO mice. CKO mice also show elevated levels of GFAP and activated Caspase-3 suggesting increased astrogliogenesis and apoptosis following miRNA dysfunction. Western blot analysis confirmed decreased expression of NeuN (-87%) and Calbindin (-71%) and increased expression of GFAP (+146%) and activated Caspase-3 (+256%). Synaptic development may also be significantly affected and western blot analysis demonstrates that PSD-95 expression is decreased by 42% in CKO mice. MiRNA microarray analyses of CKO and wild type mice show significantly elevated levels of miR-293, miR-500, and miR-483, and significantly decreased levels of miR-208a, miR-125b, miR-214, miR-540, miR-33, and miR-878 ($p<0.05$). MiR-878 is of particular interest since it has not been implicated previously in cerebellar granule cell development. Further analysis is focused on investigating the relevance of the differential expression of these miRNAs. These findings support critical role for miRNA expression in CGPCs and provides a model system for investigating the mechanisms of miRNA regulation of cerebellar development and function.

Disclosures: **E. Arneson:** None. **T. Mighell:** None. **M. Bosch:** None. **G. Soukup:** None. **C. Bradfield:** None. **A. Shibata:** None.

Poster

540. Cortex and Nuclei: Anatomy and In Vitro Studies

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 540.03/MM10

Topic: D.14. Cerebellum: Central Physiology

Support: NIAAA F31AA022267

NIAAA T32 AA007468

NINDS R01 NS051561

NIAAA R01 AA12439

OHSU Research Scholars Award

Title: Ethanol disrupts transmissoin through the cerebellar cortex via GABAA and non-GABAA receptor-mediated mechanisms in C57BL/6J and DBA/2J mice

Authors: *J. KAPLAN[1], D. J. ROSSI[2]
[1]Behavioral Neurosci., Oregon Hlth. and Sci. Univ., Portland, OR; [2]Integrated Physiol. and Neurosci., Washington State Univ., Pullman, WA

Abstract: Purkinje cells are the sole integrators of afferent sensory information from the periphery to the cerebellum. Peripheral signals are conveyed to Purkinje cells by mossy fibers via granule cells. One mechanism by which ethanol is thought to impair cerebellar-dependent behavior is by enhancing $GABA_A$ receptor-mediated transmission to granule cells. However, we recently demonstrated that acute application of ethanol to cerebellar slices, on average, increased $GABA_A$ receptor-mediated transmission to granule cells in low ethanol consuming DBA/2J mice that are behaviorally sensitive to ethanol-induced ataxia, but reduced $GABA_A$-mediated transmission to granule cells of high ethanol consuming C57BL/6J mice that are insensitive to ethanol-induced ataxia. The impact of opposite ethanol responses on $GABA_A$ receptor-mediated transmission to granule cells on signal transmission through the cerebellar cortex is unknown, but it may represent a molecular mechanism that confers differential sensitivity to ethanol-induced cerebellar disruption. Using acutely prepared cerebellar slices and whole-cell current-

clamp electrophysiology, we examined the impact of ethanol on mossy-fiber evoked Purkinje cell excitatory post-synaptic potentials (eEPSPs) in these two strains of mice. Ethanol (31mM and 52mM) attenuated the amplitude of evoked EPSPs and Purkinje cell action potential output in both strains of mice, which persisted, but to a lesser extent, in the presence of the broad-spectrum $GABA_A$ antagonist, GABAzine. However, using this method, complex network activity made it impossible to isolate ethanol's impacts on evoked excitatory and inhibitory input to Purkinje cells, independently. Therefore, we used voltage-clamp procedures to isolate these effects. Our results indicate that both $GABA_A$ receptor-mediated and non-$GABA_A$ receptor-mediated mechanisms play important roles in ethanol's disruption of cerebellar processing and highlight the importance of multiple molecular interactions in mediating the overall impact of ethanol on Purkinje cell integration of afferent information.

Disclosures: J. Kaplan: None. **D.J. Rossi:** None.

Poster

540. Cortex and Nuclei: Anatomy and In Vitro Studies

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 540.04/MM11

Topic: D.14. Cerebellum: Central Physiology

Support: SFB-f44 Cell Signaling in chronic CNS disorders

TWF-2013 Tirolerwissenschaftsfond

FWF-F4406

Title: Loss of β_4 reduces the pacemaker frequency of cerebellar Purkinje neurons in an ataxic mouse model

Authors: *B. BENEDETTI, B. E. FLUCHER
Innsbruck Med. Univ., Innsbruck, Austria

Abstract: Mutations of all the major subunit components of the PQ-type calcium channel in cerebellum CaV2.1, β_4 and $\alpha2\delta$-2 cause ataxia and epilepsy in humans and mice. It has been shown that mouse mutants of CaV2.1 and $\alpha2\delta$-2 show reduced synaptic input, altered dendritic morphology and irregular pacemaker activity in the cerebellar Purkinje neurons. However, there is little information on the neuronal phenotype of the ataxic mutant of β_4 *(lethargic).* Although previous work on *lethargic* thalamus and heterologous systems suggests an important role of β_4

in synaptic release, up to date there are no studies on the physiology of β_4 in neuronal circuits controlling motor coordination. Here we addressed this problem by studying the electrical activity of Purkinje cells in acute cerebellar slices. Using cell-attached recordings we measured the pacemaker frequency in *lethargic* neurons. Compared to wildtype controls, a large number of *lethargic* Purkinje cells were electrically silent. In the remaining Purkinje cells the firing frequency was 38 ± 7 Hz, significantly sparser than in healthy controls where the firing frequency was 113 ± 18 Hz. Nevertheless, the pacemaker activity of *lethargic* Purkinje cells was fairly regular. To assess the relevance of β4 for synaptic release we measured the frequency and amplitude of excitatory and inhibitory synaptic currents in Purkinje neurons. Whole-cell recordings in healthy neurons revealed an age-related increase in the frequency of spontaneous excitatory and inhibitory synaptic input. This was equally observed in *lethargic* neurons. Furthermore, tonic GABAergic inhibition of the glutamatergic input was observed in both healthy and ataxic mice after postnatal day 12. Measurements of cell capacitance predicted no difference in the size of healthy and ataxic neurons. Accordingly, morphological analysis of reconstructed Purkinje cells revealed only small differences between the dendritic arborization of *lethargic* neurons and controls. In conclusion, the synaptic input and dendritic morphology of Purkinje cells are not altered in *lethargic* cerebellum, but the output is severely depressed. Thus, the loss of the β_4 subunit may be fully compensated in parallel fiber - Purkinje cell synapses but β_4 appears necessary for Purkinje cell intrinsic mechanisms of action potential generation.

Disclosures: **B. Benedetti:** A. Employment/Salary (full or part-time):; Innsbruck Medical University, Department of Physiology and medical Physics. **B.E. Flucher:** None.

Poster

540. Cortex and Nuclei: Anatomy and In Vitro Studies

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 540.05/MM12

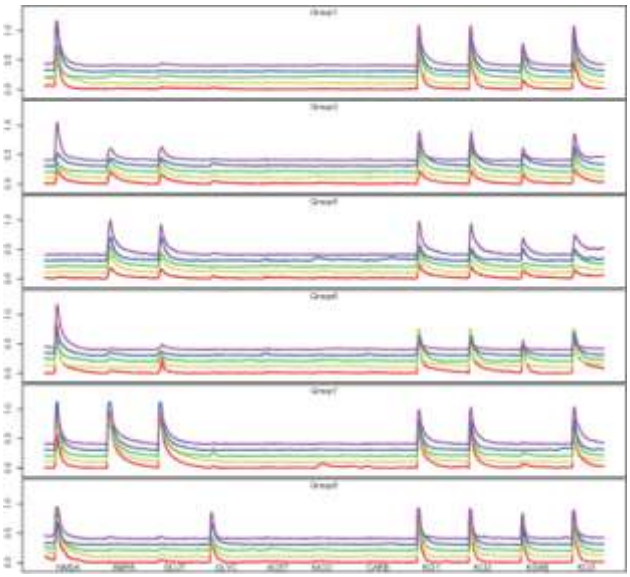
Topic: D.14. Cerebellum: Central Physiology

Support: NIGMS GM48677

Title: The molecular profile of ion channels in cultured cerebellar neurons from mouse

Authors: ***K. J. CURTICE**[1], L. S. LEAVITT[1], T. R. HARRIS[1], K. CHASE[2], R. W. TEICHERT[1], M. P. HORVATH[1], B. M. OLIVERA[1]
[1]Biol., [2]Mathematics, Biol., Univ. of Utah, Salt Lake City, UT

Abstract: The cerebellum is a region of the brain responsible for controlling the coordination and timing of motor activity. Neurons of the cerebellum have classically been defined using cell morphology, neural connectivity, and electrophysiological recordings. These studies have led to identification of distinct cell types within the cerebellum that include Purkinje, Golgi, granule, stellate, basket, and unipolar brush cells. Here we utilized calcium imaging to observe a mixed population of dissociated cerebellar neurons and, through the use of selective pharmacological tools, we defined the ion channel composition in individual neurons. We evaluated glutamate receptors, acetylcholine receptors, GABA receptors, and a variety of voltage gated ion channels. The resulting response profiles were then analyzed with a cluster analysis that revealed distinct neuronal classes and provided the frequency of each cell type. Cultured neurons from mouse cerebellum were dominated by small glutamatergic neurons responsive to N-methyl-D-aspartate (NMDA) that were consistent with granule cells. Additionally, larger cholinergic neurons were observed in lower frequency. Some of the large, cholinergic neurons were also responsive to α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and putatively corresponded to Purkinje cells. The spectrum of response phenotypes observed in our experiments suggests that neuronal diversity exists beyond the classic cell types of the cerebellum. Furthermore, this work elucidates novel cell-specific receptor and ion channel combinations that will be useful for defining the physiological role of individual cell-types of the cerebellum.



Disclosures: **K.J. Curtice:** None. **L.S. Leavitt:** None. **T.R. Harris:** None. **R.W. Teichert:** None. **M.P. Horvath:** None. **B.M. Olivera:** None. **K. Chase:** None.

Poster

540. Cortex and Nuclei: Anatomy and In Vitro Studies

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 540.06/MM13

Topic: D.14. Cerebellum: Central Physiology

Support: R01-NS39395 (IMR)

Title: Intrinsic and synaptic properties of identified nucleo-olivary cells of the deep cerebellar nuclei

Authors: *M. NAJAC, I. M. RAMAN
Dept. of Neurobio., Northwestern Univ., Evanston, IL

Abstract: Neurons in the inferior olive (IO) give rise to climbing fibers that make excitatory synapses onto Purkinje cells and modulate their parallel fiber inputs. Purkinje cells in turn inhibit diverse cell types in the cerebellar nuclei, including small GABAergic nucleo-olivary cells that project to the IO, thus making a tri-synaptic loop critical for motor learning. We retrogradely labeled nucleo-olivary cells by injecting choleratoxin B coupled to Alexa fluor 488 in the IO of P21 mice. Most labeled nucleo-olivary cells were found in the contralateral interpositus and lateral nuclei. Using patch-clamp recordings in acute slices from P25-P30 mice, we compared the intrinsic and synaptic properties of identified nucleo-olivary cells and large neurons, which project to premotor areas. Nucleo-olivary cells had input resistances of 1.43 ± 1.12 GΩ (n=40), higher than large cells (118 ± 109 MΩ, n=24), and capacitances of 6.3 ± 1.9 pF (n=40), smaller than large cells (34.3 ± 10.0, n=24). In response to depolarizing current steps, they fired spikes over a relatively narrow dynamic range, entering depolarization block at rates above 74 ± 11 Hz (n=7). In contrast, large cells fired at rates up to 400 Hz (n=7). GABAA R-mediated IPSCs in nucleo-olivary cells were evoked at 0 mV with local electrical stimulation, with NMDARs and AMPARs blocked. These IPSCs were small and slow (peak, 43 ± 23 pA, decay time constant 32.6 ± 19.1 ms, n=7) relative to large cells (502 ± 209 pA, 2.0 ± 0.6 ms, n=6). To test whether Purkinje cells contribute to evoked IPSCs, we repeated the experiment in mice expressing ChR2 in Purkinje cells. Light-evoked IPSCs in nucleo-olivary neurons had a similarly slow decay time constant (23.1 ± 16.9 pA, n=8) consistent with a dominant contribution of functional synapses from Purkinje cells. Previous work shows that a fast decay of IPSCs allows large cells to entrain to rapid synchronous inhibitory inputs. As nucleo-olivary cells IPSCs had slow kinetics, we tested how they responded to trains of electrical stimulation of increasing frequencies. Whereas in large cells, IPSCs decay fully between stimuli even at 100 Hz, in nucleo-olivary cells, stimuli from 20-100 Hz evoked large tonic currents resulting in summation of IPSCs (e.g. at 100 Hz, total IPSC11/peak IPSC1 = 1.68 ± 0.56 and tonic IPSC11/peak IPSC1 = 1.19 ± 0.59, n=7). These results suggest that nucleo-olivary cells will be sensitive to the frequency of their inhibitory inputs, unlike large cells, which are also sensitive to timing. These two types of

projection cells from the cerebellar nuclei may therefore process different parameters of Purkinje cell activity in ways that are related to their different roles in the circuit.

Disclosures: M. Najac: None. **I.M. Raman:** None.

Poster

540. Cortex and Nuclei: Anatomy and In Vitro Studies

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 540.07/MM14

Topic: D.14. Cerebellum: Central Physiology

Support: CIN - Systems Neurophysiology

Title: Ionic mechanisms shaping DCN responses to pauses in PC background firing

Authors: *C. M. PEDROARENA
Hertie-Inst EKU-Tübingen, Tubingen, Germany

Abstract: Pauses or reductions in Purkinje cells background firing rate are observed during diverse movements and some forms of learned motor acts. Synchronization of these events are postulated as one of the signals that cerebellar cortex uses to modulate the activity of their target neurons in the deep cebellar nuclei (DCNs. The resulting excitation of DCNs is likely to reflect the interaction between the des-inhibition and the activation/deactivation of diverse membrane conductances. In this study we investigated the ionic mechanisms shaping the response of DCNs to pauses in PC background firing. For this purpose WCP recordings from DCNs in rodent cerebellar slices were performed. PC axons were activated using tungsten electrodes in the white matter and different temporal patterns of stimulation. Pharmacological tools were used to manipulate diverse membrane conductances. Our previous studies showed that T-type calcium channels are functional and mediate rebound responses in DCNs (Boehme & Pedroarena, 2011). Since there is evidence that tonic T-type currents may be active at usual membrane potentials, in a first series of experiments we tested how DCN responses to desinhibition were modified by the specific T-type channel blocker,TTA-P2. Our results showed that although TTA-P2 blocked rebound responses to hyperpolarizing pulses and short trains of IPSPs, the responses evoked by suppression of PC background firing were not affected the same procedures.

Disclosures: C.M. Pedroarena: None.

Poster

540. Cortex and Nuclei: Anatomy and In Vitro Studies

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 540.08/MM15

Topic: D.14. Cerebellum: Central Physiology

Support: Simons Foundation Autism Research Initiative

NIH R01-NS39395

Title: Upregulation of mGluR1/5-modulated current in the deep cerebellar nuclei of male Gabrb3 mutant mice and its effect on cerebellar behavior

Authors: *A. TURNOWCHYK, K. J. PALARZ, I. M. RAMAN
Neurobio., Northwestern Univ., Evanston, IL

Abstract: The maternal allele of *Gabrb3*, which encodes 3 subunits of $GABA_A$Rs, is absent in Angelman syndrome, an autism spectrum disorder. Because *Gabrb3* is expressed in the cerebellar cortex, where it is expected to prolong IPSCs, we investigated the effect of loss of maternal *Gabrb3* (m-/p+) on cerebellar output in P17-24 mice. We made whole cell recordings from large projection neurons of the cerebellar nuclei (CbN) in brain slices from male mice and stimulated mossy fibers (MFs) and Purkinje (Pkj) axons (100 Hz, 200 ms, 36-37.5°C). With AMPARs and NMDARs blocked, PSCs consisted of $GABA_A$R-mediated IPSCs and a small tonic excitation mediated by mGluR1/5-modulated currents. The net outward PSC decayed incompletely between stimuli, producing a phasic (evoked) current that was unaltered in m-/p+ cells vs. +/+ controls, and a tonic (residual) current that was reduced in m-/p+ cells by ~50%. $GABA_A$R kinetics, however, were unchanged (single IPSC decay τ: +/+ 2.3 ± 0.1 ms; m-/p+ 2.5 ± 0.2 ms). The mGluR1/5 antagonist CPCCOEt did not affect +/+ PSCs but restored m-/p+ tonic current to +/+ levels. Thus, mGluR1/5-modulated current is upregulated in m-/p+ cells, counteracting tonic IPSCs. In current-clamped CbN cells, stimulation (100 Hz, 500 ms) suppressed spontaneous firing via IPSPs and elicited prolonged rebound firing, which is known to be facilitated by mGluR1/5 activation. In male mice, +/+ cells increased their firing rates by 59 ± 19% (post-stim rate / pre-stim rate), while in m-/p+ cells the increase almost doubled to 103 ± 19%. Consistent with enhanced mGluR1/5-modulated current in the mutants, CPCCOEt removed this difference (+/+ 53 ± 14%; m-/p+ 51 ± 10%). Unlike in males, rebound firing in +/+ and m-/p+ females did not differ (+/+ 79 ± 12%; m-/p+ 70 ± 24%). Pilot Western blots of mGluR1 normalized to actin in CbN from age-matched mice confirmed a sex difference in mGluR1 expression: +/+ females expressed relatively more mGluR1 than +/+ males, and only

males upregulated mGluR1 in m-/p+ mice (n=2, each group). To assay changes in motor learning, mice were tested on the accelerating rotarod. Male P22 +/+ mice remained on the rod for 46 ± 17 sec longer (days 5-7 vs. day 1). Similarly, m-/p+ male mice improved by 43 ± 12 sec. In contrast, while female +/+ mice improved by 71 ± 13 sec, female m-/p+ mice failed to learn, remaining on the rod for 8 ± 11 sec *less* at the end of training. These data support the idea that loss of *Gabrb3* reduces inhibition of Pkj cells, yielding more inhibition of the CbN. Increased mGluR1/5 activity in CbN cells of m-/p+ males, but not females, may compensate for the elevated inhibition, preventing deficits in rotarod learning.

Disclosures: A. Turnowchyk: None. **K.J. Palarz:** None. **I.M. Raman:** None.

Poster

540. Cortex and Nuclei: Anatomy and In Vitro Studies

Location: Halls A-C

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Program#/Poster#: 540.09/MM16

Topic: D.14. Cerebellum: Central Physiology

Support: HHMI International Student Fellowship

NIH P50 MH068830.

Title: *In vivo* two-photon imaging of cortico-cerebellar mossy fiber synapses

Authors: *D. RYLKOVA, Z.-Y. YE, A. CRANK, D. L. LINDEN
Johns Hopkins Univ., BALTIMORE, MD

Abstract: Using a combination of two-photon imaging *in vivo* and in brain slices, we are characterizing the morphological and functional dynamics of basal pontine mossy fibers (mfs), which form the cortico-cerebellar circuit's input stage. To this end, we are labeling pontine mfs with dsRed-express, in conjunction with the genetically-encoded calcium indicators GCaMP3 or GCaMP6 by injecting AAVs into the basal pons. We then perform chronic *in vivo* two-photon imaging in unanesthetized adult rats through a cranial window over the cerebellar hemisphere. Dual labeling allows us to reconstruct multiple axons within a volume and subsequently characterize calcium signals in populations of terminals with reference to their parent axon. During *in vivo* calcium imaging, animals' behavior is recorded using a high speed camera. Spontaneous movements such as grooming are then analyzed offline. In addition to scoring movement onsets and offsets, we are also tracking the position of body regions, which allows us

to examine the relationship between activity and movement parameters in fine detail. Furthermore, sensory-evoked activity is tested in response to tactile, proprioceptive, auditory and visual stimuli. These experiments have allowed us to characterize cerebellar sensorimotor representations at the level of individual presynaptic terminals. While the activity of the majority of imaged axons was correlated with movement, these were interspersed with axons specifically active in response to tactile sensory stimuli. The activity of a third class of inputs was not correlated to either movement or the sensory stimuli tested. Surprisingly, we have observed that calcium transients are not simultaneously detectable at all terminals on an "active" axon. This does not appear to be a result of branch point failure because calcium transients can be detected in terminals distal to an inactive terminal. In general, the activity of terminals on the same axon is more highly correlated than those on different axons, and there is an inverse relationship between distance and correlated activity for all terminals. We have also reproduced this finding in brain slices, where we have observed that, for a given stimulation protocol, terminals sharing a parent axon do not all show Ca responses.

Disclosures: D. Rylkova: None. **Z. Ye:** None. **A. Crank:** None. **D.L. Linden:** None.

Poster

540. Cortex and Nuclei: Anatomy and In Vitro Studies

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 540.10/MM17

Topic: D.14. Cerebellum: Central Physiology

Support: NIH 2T32-MH067564

NIH R01-NS39395 (IMR)

Title: Mossy fiber excitation of cerebellar nuclear cells preserves phase-locked spiking resulting from synchrony of Purkinje cells

Authors: *Y. WU, I. M. RAMAN
Dept. Neurobio., Northwestern Univ., Evanston, IL

Abstract: Neurons of the cerebellar nuclei (CbN) integrate inhibition from Purkinje (Pkj) cells of the cerebellar cortex and excitation from mossy fibers. Recent work showed that synchrony of Pkj simple spikes inhibits spontaneous firing by CbN cells during brief IPSPs, but permits short-latency spiking after IPSPs; this periodic inhibition/disinhibition lets CbN cells phase-lock their

firing to that of synchronized Pkj cells. Here, we investigated how synaptic excitation modulates CbN cell responses to inhibition. Whole-cell recordings were made from large projection CbN cells in P17-P23 mouse cerebellar slices in SR95531 to block endogenous inhibition at 34-37°C. EPSPs were electrically evoked by 200-ms trains of mossy fiber stimulation (90, 133, and 160 Hz) and IPSPs were generated by dynamic clamp, which mimicked either 40 Purkinje inputs each firing at 50 spikes/sec ("asynchronous") or 20 asynchronous inputs with 20 synchronized at 50 or 100 Hz ("50% synchrony"). Voltage-clamp recordings at -70 mV showed that the mean evoked mossy fiber EPSC was 367 ± 91 pA (decay constant 1.6 ± 0.3 ms) and depolarized the membrane potential by 5.1 ± 1.2 mV (n=8). During trains of stimuli, EPSCs depressed by 74.8% at 90 Hz and 80.6% at 160 Hz. In current clamp, CbN cells fired spontaneously at 116 ± 34 Hz (n = 5) or rested in depolarization block (n = 3). During asynchronous IPSPs, CbN firing was greatly reduced (4.6 ± 1.8 spikes/sec, measured in a 200-ms period). The number of spikes did not significantly increase when EPSP trains at 90 or 133 Hz were applied, but rose by 10.9 ± 4 spikes/sec when EPSPs were evoked at 160 Hz. Thus, CbN cells could not detect excitation even up to 133 Hz with a background of asynchronous IPSPs, indicative of effective shunting and a narrow dynamic range for encoding excitatory input. In contrast, with 50% synchronous IPSPs at 50 Hz (no EPSPs), the rate was 11.3 ± 2.2 spikes/sec. Consistent with phase-locking, plots of interspike interval histograms confirmed that most of these spikes fell in 4-ms bins around multiples of the interval between synchronized IPSPs. Against this background of 50% inhibitory synchrony, all frequencies of EPSPs significantly increased the total number of spikes (90 Hz: by 5.1 ± 2.1; 133 Hz: by 10.0 ± 2.4; 160 Hz: by 12.3 ± 4.3 spikes/sec). The fraction of ISIs that fell into bins around multiples of 20 ms (for synchrony at 50 Hz) or 10 ms (for synchrony at 100 Hz) was unchanged by excitation. The data suggest that excitation < ~160 Hz may be insufficient to drive CbN spiking against asychronous inhibition but may increase the spike probability upon disinhibition after synchronous IPSPs, while leaving the jitter on phase-locked spiking relatively unchanged.

Disclosures: Y. Wu: None. **I.M. Raman:** None.

Poster

540. Cortex and Nuclei: Anatomy and In Vitro Studies

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 540.11/MM18

Topic: D.14. Cerebellum: Central Physiology

Support: the National Research Foundation (Singapore)

Title: Optogenetic mapping of inhibitory circuit between Purkinje cells and cerebellar molecular layer interneurons

Authors: *J. KIM[1], G. J. AUGUSTINE[1,2]
[1]LKC Med. School-NTU, Singapore, Singapore; [2]Ctr. for Functional Connectomics, Korea Inst. of Sci. and Technol., Seoul, Korea, Republic of

Abstract: Although anatomical studies have suggested that Purkinje cell axon collaterals form synaptic contacts with cerebellar molecular layer interneurons (MLI; J Neurosci. 9: 2141), the function of these putative connections has not been determined. Here, we investigated the functional properties and spatial organization of this circuit by using a high-speed optogenetic circuit mapping technique (PNAS 104: 8143). Using transgenic mice that selectively express channelrhodopsin-2 in Purkinje cells, we could photostimulate Purkinje cells via small spots of blue light (405 nm), while simultaneously recording resulting postsynaptic responses in MLI. By correlating the location of photostimulation with the presence of postsynaptic responses, we could map the spatial organization of the circuit between Purkinje cells and MLI. Photostimulation of Purkinje cells evoked monosynaptic, GABAA-mediated IPSPs that inhibited the firing of basket cells located in the inner third of the molecular layer. This connection was never observed in stellate cells and was relatively sparse, being observed in only 20% of MLI recordings even when many Purkinje cells were photostimulated. Unlike Purkinje cell-Purkinje cell connections, which are developmentally transient (Nature Neurosci. 12:463), Purkinje cell-MLI connections are functional in adults. The area of the presynaptic input field was similar in size to the optical footprint of individual Purkinje cells, suggesting 1:1 convergence between Purkinje cells and MLI. However, in a few cases, multiple Purkinje cell inputs were observed. The inhibitory inputs from Purkinje cells to MLI were up to 200 μm in width in the sagittal plane, except for rare cases of multiple connections whose range extended up to 300 μm. In coronal slices, Purkinje cells provided inhibitory inputs to MLI within 100 μm of the postsynaptic cell soma. Paired recordings were used to determine whether there are reciprocal inhibitory connections between Purkinje cells and MLI. In no case were mutually inhibitory pairs detected, suggesting that the inhibitory circuit between Purkinje cells and MLI is non-reciprocal. Therefore, inhibition of MLI by Purkinje cells will disinhibit neighboring Purkinje cells, which may help synchronize Purkinje cell activity. These novel features of the Purkinje cell-MLI inhibitory network are likely to be important for information processing within the cerebellum.

Disclosures: J. Kim: None. **G.J. Augustine:** None.

Poster

540. Cortex and Nuclei: Anatomy and In Vitro Studies

Location: Halls A-C

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Program#/Poster#: 540.12/MM19

Topic: D.14. Cerebellum: Central Physiology

Support: NIH 1R01HL093134

NIH P40 OD010996

Title: A tale of two circuits - parallel processing in the rat cerebellum

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Abstract: Efferent projections from the cerebellar nuclei (CN) originate from two separate populations of projection neurons. Glutamatergic projection neurons send their axons to the red nucleus and thalamus (nucleo-premotor; NPM) while GABAergic neurons project to the inferior olive (nucleo-olivary; NO), completing the olivo-cortico -nucleo-olivary loop (OCNO). The OCNO circuit has been proposed to form a cell-by-cell-by-cell closed loop architecture, yet there is a lack of definitive anatomical data to this end. Furthermore, the degree to which Purkinje cell (PC) input is segregated to projection-specific populations of CN neurons has not been firmly established. We addressed these questions in adult male rats using combined viral transneuronal and classical tracing. Injection of a cocktail of the retrograde transneuronal tracer pseudorabies virus (PRV) and the beta fragment of cholera toxin (CTβ; a bidirectional monosynaptic tracer) into the inferior olive (IO) confirmed that OCNO loops are in fact closed, with CTβ labeled IO climbing fibers innervating the same PRV+ PCs from which they receive disynaptic input through the CN. Next, we tested the hypothesis that both pathways receive synaptic input from a common set of local interneurons and the same PCs. To test this hypothesis we injected two isogenic recombinants of PRV that express unique reporters (EGFP or mRFP) into the red nucleus (RN) and the IO, respectively. Retrograde transneuronal spread of virus to the CN and their presynaptic PC afferents was analyzed 48 hours later. We found contrary to our hypothesis that the majority of PCs innervate either the NPM or NO pathways; i.e., most of the PCs expressed only EGFP or mRFP. NPM and NO projecting PCs did not fall along zebrin II parasagittal bands and were intimately interdigitated with neighboring cells, producing alternating PCs expressing only one reporter of infection. Notably, retrograde spread of PRV from the RN and IO differentially infected neurons in distinct CN subfields. CN neurons synaptically linked to the two pathways were also observed. These results question the presumed equivalence of PC influence on NPM and NO projection neurons and place the CN at the core of cerebellar processing, integrating dynamic regulatory control of the IO via OCNO loops with excitatory output of the NPM projection.

Disclosures: G.J. Wojaczynski: None. **J.P. Card:** None.

Poster

541. Transmitters and Neuromodulation

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Program#/Poster#: 541.01/MM20

Topic: D.15. Basal Ganglia

Support: CIHR MOP-115008

Title: Striatal interneurons expressing calretinin in 6-OHDA-lesioned mice

Authors: *S. PETRYSZYN, D. GAGNON, J.-M. BEAULIEU, A. PARENT, M. PARENT
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Abstract: The striatum is the largest and major integrative component of the basal ganglia. It is composed of a large population of morphologically homogeneous projection neurons and a relatively small group of highly heterogeneous interneurons. The functional organization of the striatum is severely affected by the progressive degeneration of its dopaminergic afferent projections that characterizes Parkinson's disease. Despite their small number, interneurons are believed to play a crucial role in the integrative activity of the striatum. This study was designed to describe in details the regional distribution and morphological features of striatal interneurons expressing the calcium binding protein calretinin (CR) in normal condition and to investigate if these interneurons are involved in the reorganization of the striatal microcircuitry that occurs in Parkinson's disease. In order to do so, we examined and compared data gathered from normal mice and from mice that received a unilateral injection of 6-hydroxydopamine (6-OHDA). Immunostaining for CR revealed the presence of two morphological distinct types of CR striatal interneurons, which were designed as CR1 and CR2 and whose distribution and density were determined stereologically in normal, sham and 6-OHDA-injected mice. The CR1 cells abound in the rostral striatum. They had a small (9-12 μm), round and intensely fluorescent cell body that gave rise to one or two typically beaded processes. The CR2 cells were more uniformly distributed than the CR1 cells. They had a larger (15-20 μm) and less intensely fluorescent cell body that yielded 2-4 poorly branched dendrites. In 6-OHDA-lesioned mice, no major difference in the density of CR1 cells was detected between intact and 6-OHDA-lesioned striatum, or between sham and 6-OHDA-lesioned animals. However, the density of CR2 cells was decreased in 6-OHDA-injected mice compared to sham animals, and this diminution was significant on the lesioned side (43 %). Immunostaining for CR in BAC double transgenic mice expressing tdTomato protein under the control of dopamine D1 receptor promoter and GFP under the

control of D2 promoter showed that CR1 and CR2 cells were devoid of D1 or D2 receptors. The latter findings suggest that dopamine exerts its influence upon CR2 striatal interneurons indirectly, either by modulating the glutamatergic excitatory striatal projections of cortical or thalamic origin, or by stimulating the large cholinergic interneurons, which express dopamine receptors.

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Poster

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Topic: D.15. Basal Ganglia

Support: CIHR MOP-115008

Title: Serotonin innervation of the striatum in a primate model of Parkinson's disease

Authors: ***D. GAGNON**[1], T. DIPAOLO[2], M. PARENT[1]
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Abstract: Parkinson's disease is characterized by the progressive loss of midbrain dopaminergic neurons that innervate the striatum. The administration of L-Dopa is the most effective pharmacotherapy for alleviating the motor symptoms of this neurodegenerative disease. However, the chronic use of this compound often produces adverse side effects, such as L-Dopa-induced dyskinesia. Unregulated release of dopamine by serotonin (5-HT) axons following L-Dopa administration is believed to be a major presynaptic determinant of dyskinesia expression. The present study was designed to characterize the reorganization of 5-HT striatal afferent projections following dopaminergic denervation in a primate model of Parkinson's disease. Our sample comprised eight cynomolgus monkeys (*Macaca fascicularis*): four that were rendered parkinsonian following systemic injections of the neurotoxin MPTP, and four others that served as controls. The state of the striatal 5-HT and dopamine innervation was evaluated by means of immunohistochemistry with antibodies raised against the 5-HT transporter (SERT) and tyrosine hydroxylase (TH), respectively. A detailed stereological investigation revealed a 51.2% increase in the number of 5-HT axon varicosities in the dorsolateral sector of the striatum of MPTP-intoxicated monkeys (0.62 ± 0.74 SERT+ axon varicosities/mm^3) compared to controls (0.41 ±

0.88 SERT+ varicosities/mm^3). This significant increase of the 5-HT striatal innervation was particularly obvious in the so-called sensorimotor territory of the striatum, where the dopamine denervation (TH-poor sector) was the most severe. A similar stereological approach applied to transverse sections of the dorsal raphe nucleus immunostained for tryptophan hydroxylase (TPH) indicated no significant difference in the density of TPH+ cell bodies between MPTP-injected monkeys and controls. Electron microscopic examination of the dorsolateral sector of the putamen showed that the SERT+ axon varicosities established about twice as many synaptic contacts in MPTP-injected monkeys (synaptic incidence of 46 ± 3 %) than in controls (20 ± 8 %). These findings demonstrate the highly plastic nature of the 5-HT striatal afferent projections, a feature that becomes particularly obvious in the absence of striatal dopamine, that is, in the parkinsonian state. Although the number of dorsal raphe 5-HT neurons remains relatively constant in parkinsonian monkeys, their ascending axonal projections undergo marked proliferative and synaptic adaptive changes that might play a significant role in the expression of L-Dopa-induced dyskinesia.

Disclosures: D. Gagnon: None. **T. DiPaolo:** None. **M. Parent:** None.

Poster

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Human Brain Project

Karolinska Institutet's Research Funds

Title: The contribution of the dopamine system to selection of motor programs

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Abstract: In the lamprey, as in mammals, dopamine plays a key role in movement control by modulating the excitability of projection neurons in the striatum. The direct "go" pathway

neurons express the dopamine D1 receptor and mediate a net facilitation of motor actions through an inhibition of the pallidal GABAergic output neurons (disinhibition), whereas the indirect "no go" pathway, acting through the D2 subtype, mediates motor suppression (Gerfen and Surmeier, 2011; Ericsson et al., 2013). The dopaminergic innervation of striatum derives from the nucleus of the posterior tuberculum, a region that shows striking similarities with the mammalian substantia nigra pars compacta (SNc). It receives pallial (cortex in mammals) input and shows the same connectivity with the other basal ganglia subnuclei observed in mammals. The SNc also receives input from the optic tectum as the evolutionary basis for salience/novelty detection (Pérez-Fernández et al., submitted to J. Comp. Neurol.). The importance of the dopaminergic innervation from the SNc is reflected in the fact that, when depleted, it gives rise to a marked hypokinesia, as in Parkinson's disease (Thompson et al., 2008). One striking feature is that the SNc in lamprey sends direct dopaminergic projections to different motor command centres, including the diencephalic and mesencephalic motor regions, and the output layer of the optic tectum. The SNc dopaminergic control of motor responses is thus likely to be more complex than generally assumed, and involve additional pathways to the widely studied striatal projection. Here, we explored how dopamine modulates motor responses in the optic tectum, the homologous region of the mammalian superior colliculus. In all vertebrates, including the lamprey, this region has similar features, with a laminated structure, controlling eye, orienting and evasive trunk movements. The dopamine fibers from the SNc innervate the inner motor output layer and *in situ* hybridization shows that tectal premotor cells express D1 and D2 receptors. Patch-clamp recordings of premotor cells show that dopamine differentially modulates their excitability, increasing the excitability of D1-expressing neurons and decreasing excitability of those expressing the D2 receptor. Our results indicate that dopamine directly modulates motor responses in premotor regions and, given the high degree of conservation of the basal ganglia, this previously unexplored mechanism is likely to also be present in higher vertebrates including mammals.

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Poster

541. Transmitters and Neuromodulation

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Topic: D.15. Basal Ganglia

Support: NSERC Grant 401848-2011

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Title: The pedunculopontine nucleus: A precise anatomical and neurochemical description in human and non-human primates

Authors: *L. GOETZ[1,2], M.-J. WALLMAN[1], A. PARENT[1,2], M. PARENT[1,2]
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Abstract: Since the first identification of the pedunculopontine nucleus (PPN) in 1909 by Jacobsohn and its more detailed description in 1954 by Olszewski and Baxter, the delineation of tegmental structures and their terminologies, based on cytoarchitectural, connectivity and functional considerations, have been a source of confusion. It was then unsurprising that controversies regarding PPN anatomical localization were raised in the neurosurgical community when this nucleus became a new promising target for the treatment of freezing of gait in Parkinson disease by deep brain stimulation (DBS). Particularly worth noting is the marked variation encountered in the literature regarding the site of DBS electrode placement in the brainstem, indicating that a detailed anatomical description of the PPN is deeply needed. We used immunofluorescence with antibody against choline acetyltransferase (ChAT), combined to antibody against a vesicular glutamate transporter (Vglut2) and histochemistry for NADPH diaphorase, luxol fast blue and cresyl violet in order to provide a precise anatomical and neurochemical description of the PPN and its surrounding structures. Staining were made on post-mortem brain sections of human, cynomolgus and squirrel monkeys. Examination of axial and sagittal sections of brainstem revealed the presence of a dense cluster of ChAT+ neurons that corresponds to the PPN pars compacta lying at the pontomesencephalic junction along the rostral surface of the superior cerebellar peduncle. This neuronal cluster was surrounded by a more diffuse ChAT+ neuronal population corresponding to the PPN pars dissipata. Different types of ChAT+ neurons were described according to their morphology. Fusiform neurons had a long axis ranging from 36.5 to 54.2 μm (mean 44.3 μm). Triangular neurons were usually endowed with 3 primary dendrites and a cell body ranging from 16.5 to 63.8 μm in diameter (mean 31.7 μm). Ovoid neurons had a diameter ranging from 16.6 to 44.8 μm (mean 28.2 μm). Examination of doubly immunostained sections for ChAT and Vglut2 revealed that a large proportion of PPN neurons expressing ChAT, also contain the VGluT2, indicating a potential co-release of glutamate with acetylcholine by these neurons. Taking into account the different stereotaxic approaches used in DBS literature, we provided a set of coordinates corresponding to this brainstem structure in human. Our data obtained from post-mortem brain sections provide relevant anatomical basis to precisely localize and characterize the neurochemical content of PPN neurons and its surrounding structures in the perspective of anatomo-clinical study of the effect of PPN DBS.

Disclosures: **L. Goetz:** None. **M. Wallman:** None. **A. Parent:** None. **M. Parent:** None.

Poster

541. Transmitters and Neuromodulation

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Topic: D.15. Basal Ganglia

Support: CIHR Grant MOP-115008

Title: Serotonin innervation of the internal and external pallidum in a primate model of Parkinson's disease

Authors: *L. EID[1], D. GAGNON[1], C. WHISSEL[1], T. DIPAOLO[2], A. PARENT[1], M. PARENT[1]
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Abstract: The primate pallidum is divided into an internal (GPi) and an external (GPe) segment, both receiving a highly heterogeneous serotonin (5-HT) innervation arising mainly from the dorsal raphe nucleus. The main purpose of this light and electron microscopic study was to characterize neuroadaptive changes of 5-HT axons in the GPi and GPe following dopaminergic denervation in a primate model of Parkinson's disease. Eight cynomolgus monkeys (*Macaca fascicularis*) were used: 4 were rendered parkinsonian by systemic injections of the neurotoxin MPTP and 4 served as controls. The state of the pallidal 5-HT innervation was evaluated by means of immunohistochemistry with an antibody raised against the 5-HT transporter (SERT). In MPTP-intoxicated monkeys, unbiased quantification at the light microscopic level revealed a two-fold increase in the density of SERT+ axon varicosities in the GPi ($0.83 \pm 0.12 \times 10^6$ SERT+ axon varicosities/mm^3 vs. 0.43 ± 0.05) and the GPe (0.58 ± 0.10 vs. 0.32 ± 0.03). Overall, the GPi appeared more densely innervated by SERT+ axon varicosities than the GPe. This difference between GPi and GPe was exacerbated in MPTP monkeys. Electron microscopic analysis of both pallidal segments indicated that the morphological features of SERT+ axon varicosities were similar between MPTP and control monkeys. Compared to unlabeled profiles, only few SERT+ axon varicosities were seen to establish a synaptic contact in the GPi (synaptic incidence of $23 \pm 3\%$) and the GPe ($35 \pm 10\%$) of control monkeys. A slight decrease of the synaptic incidence for SERT+ axon varicosities was observed in the GPi ($15 \pm 4\%$ vs. $23 \pm 3\%$) and the GPe ($21 \pm 7\%$ vs. $35 \pm 10\%$) of MPTP monkeys, though such difference was not statistically significant when compared to controls. The few synaptic contacts established by SERT+ axon varicosities observed in the pallidum were of the symmetrical and asymmetrical types in equal proportions and targeted exclusively dendritic profiles. Our data indicate that dopaminergic lesion leads to an increase of the number of 5-HT axon varicosities in both pallidal segments. This reorganization

of 5-HT afferent projections may be involved in deregulated neuronal firing pattern of pallidal neurons noted in Parkinson's disease as well as in the expression of motor symptoms and L-Dopa-induced dyskinesia.

Disclosures: L. Eid: None. **D. Gagnon:** None. **C. Whissel:** None. **T. DiPaolo:** None. **A. Parent:** None. **M. Parent:** None.

Poster

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Topic: D.15. Basal Ganglia

Support: NSERC Grant 401848-2011

Title: Cholinergic neurons intrinsic to primate external pallidum

Authors: *A. PARENT, L. EID, M. PARENT
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Abstract: The relationship between cholinergic neurons of the nucleus basalis of Meynert (NB) and neurons of the globus pallidus (GP) was studied in squirrel (*Saimiri sciureus*) and cynomolgus (*Macaca fascicularis*) monkeys by means of choline acetyltransferase (ChAT) immunohistochemistry. In both species, the large (40-50 μm) and multipolar ChAT+ cells of NB, which project to the cerebral cortex, form a densely packed neuronal population lying just beneath the GP. At various points along the rostrocaudal extent of NB, some ChAT+ neurons encroach significantly upon the GP dorsally. At anterior commissure levels, ChAT+ neurons morphologically similar to, and topographically continuous with those of NB surround the rostral pole of the internal segment of the GP (GPi). As the GPi enlarges caudally, the ChAT+ cells become gradually confined to the internal and external medullary laminae. These NB-like ChAT+ neurons remain largely segregated from pallidal neurons throughout the rostrocaudal extent of the GP. However, we noted, in both primate species, the presence of a distinct population of ChAT+ neurons closely intermingled with pallidal neurons in the external segment of the GP (GPe), but not in the GPi. Morphologically, the ChAT+ neurons that occur in GPe display numerous highly branched dendrites emerging from cell bodies that are smaller (25-30 μm) than those of neurons present in the NB and in its dorsal extensions in the medullary laminae. A quantitative stereological analysis of the GPe in squirrel monkeys yields an overall

density of 55 ± 6 ChAT+ neurons/mm^3 compared to 3 000 ± 127 pallidal neurons/mm^3, indicating that the cholinergic elements represent about 1.8% of the total GPe neuronal population. These ChAT+ GPe neurons are uniformly distributed along the rostrocaudal and mediolateral planes, but display a marked dorsoventral increasing gradient, with a density of 38 ± 3 neurons/mm^3 dorsally compared to 86 ± 6 neurons/mm^3 ventrally. Double immunofluorescence studies in cynomolgus monkeys show that, in contrast to all typical pallidal neurons, the ChAT+ GPe neurons are devoid of GAD. Furthermore, at variance with the corticopetal ChAT+ neurons of NB, which typically express the Nerve Growth Factor receptor (NGFr), none of the ChAT+ GPe neurons stain for NGFr. These findings demonstrate the existence of a unique population of cholinergic pallidal neurons that share more similarities with the striatal cholinergic interneurons than with the NB cholinergic projection neurons. We hypothesize that these elements act as a local source of acetylcholine and, as such, influence the activity of the GPe, as well as that of other closely interconnected basal ganglia nuclei.

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Poster

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R01 NS036362

Title: Similarities and differences in GABA and glutamate-mediated modulation of striatal dopamine release between mouse and guinea pig

Authors: *B. O'NEILL, J. C. PATEL, M. E. RICE
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Abstract: Hydrogen peroxide (H_2O_2) is an intra- and intercellular signaling molecule that can influence processes from embryonic development to cell death. Our previous work in guinea pig brain slices showed that H_2O_2 can also mediate rapid signaling by activation of ATP-sensitive K^+ (K_{ATP}) channels in the nigrostriatal dopamine (DA) pathway – a modulatory system that has critical roles in movement and motor learning. In guinea pig midbrain slices, endogenously

generated H_2O_2 in DA neurons activates channels that inhibit DA neuron firing. In guinea pig striatum, the source of H_2O_2 is not DA axons, but rather striatal medium spiny neurons (MSNs). Generation of H_2O_2 in MSNs requires evoked glutamate release, AMPA receptor (AMPAR) activation, and action potentials in MSNs. Diffusible H_2O_2 generated in MSNs activates K_{ATP} channels on DA axons, which inhibits striatal DA release. Thus, blocking AMPARs during local striatal stimulation decreases H_2O_2 generation and enhances DA release. Conversely, MSN excitability and consequent H_2O_2 generation are decreased by $GABA_A$ receptor ($GABA_AR$) activation, such that blocking $GABA_ARs$ leads to further H_2O_2-dependent suppression of DA release. Here, we report that modulation of striatal DA release by H_2O_2 is minimal in mice, compared to guinea pigs. We used fast scan cyclic voltammetry to monitor locally evoked extracellular DA concentration ($[DA]_o$) in dorsolateral striatum in *ex vivo* slices from adult male guinea pigs and mice. Similar to the results in guinea pigs, AMPAR antagonism enhanced evoked $[DA]_o$, whereas $GABA_AR$ antagonism suppressed evoked $[DA]_o$. In contrast to guinea pig, however, this regulation was not H_2O_2 dependent in the mouse, given that co-application of catalase (an H_2O_2 scavenging enzyme; 500 IU/mL) did not prevent DA release modulation by AMPAR or $GABA_AR$ antagonists. To test whether the lack of H_2O_2 dependence in mice might reflect the absence of axonal DA release regulation by K_{ATP} channels, we compared the effect of a K_{ATP} channel opener, diazoxide, in both species. A comparable suppression of evoked $[DA]_o$ in mouse and guinea pig striatum demonstrated the presence of K_{ATP} channels as potential targets of H_2O_2 in both species. Other factors could include species differences in H_2O_2 generation or metabolism. Surprisingly, despite the minimal role for inhibitory H_2O_2, the pattern of DA release regulation by glutamate and GABA appears to be conserved in mice and guinea pigs. Additionally, the similarity of DA release suppression by diazoxide in these species supports a species-independent role for K_{ATP} channels as regulators of DA neuronal function and DA transmission.

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Poster

541. Transmitters and Neuromodulation

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Klingenstein Foundation

Title: Differential expression of cb1 receptor-dependent long-term depression at corticostriatal and thalamostriatal synapses

Authors: Y.-W. WU[1], J.-I. KIM[2], V. L. TAWFIK[3], G. SCHERRER[3], *J. B. DING[4]
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Abstract: Striatal spiny projection neurons (SPNs) receive two principle excitatory inputs, from the cerebral cortex and the thalamus. A dominant form of synaptic plasticity expressed at these glutamatergic inputs onto SPNs is endocannabinoid-dependent long-term depression (eCB-LTD). However, whether eCB-LTD can be induced in all striatal SPNs is still debatable. Using region-specific Cre mouse lines combined with optogenetic tools, we achieved selective investigation of corticostriatal or thalamostriatal projections. We found that eCB-LTD induced by activation of group 1 metabotropic glutamate receptors (mGluR1s) is absent at thalamostriatal synapses, but is successfully induced at corticostriatal synapses, regardless of the expression of dopamine receptor types in the postsynaptic SPNs. This difference is attributable to differential expression of cannabinoid type-1 (CB1) receptors on corticostriatal and thalamostriatal presynaptic terminals. This study reveals that striatal eCB-LTD is input-specific rather than postsynaptic cell-type-specific and lays the groundwork for understanding cortico-thalamo-striatal synaptic plasticity and its relevance to motor learning.

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Poster

541. Transmitters and Neuromodulation

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Topic: D.15. Basal Ganglia

Support: NIH Grant R01NS082650-01

Title: Essential role of VGLUT3 in striatal signaling and behavior

Authors: *C. B. DIVITO[1], H. ZHANG[3], E. C. HOLMSTRAND[1], S. G. WILLIAMS[1], T. F. SUN[1], M. E. RUBIO[2], D. SULZER[4], R. H. EDWARDS[5], R. P. SEAL[1]

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Abstract: Cholinergic neurons within the striatum and basal forebrain express the vesicular glutamate transporter 3 (VGLUT3). In the striatum, VGLUT3 not only mediates the synaptic release of glutamate but also increases the loading of acetylcholine. VGLUT3 knockout (KO) animals were previously reported to have elevated locomotor activity during their waking cycle. It was hypothesized that loss of VGLUT3 in cholinergic interneurons caused an increase in dopamine signaling, leading to the hyperactivity. Interestingly, deletion of the vesicular acetylcholine transporter in the striatum does not alter locomotor activity, supporting a role for glutamate release in this defect. Here, we assess dopamine-related neurochemical and anatomical changes within the striatum of KO mice and we use our newly generated conditional VGLUT3 knockout mouse to determine whether striatal cholinergic interneurons are indeed the locus of the defect. Consistent with the altered locomotor behavior, we find that dopamine release, measured in striatal slices by fast scan cyclic voltammetry, is greater in the KO than in WT mice at night, but is equal in both genotypes during the day. We also find that the content of dopamine in striatal tissue is also elevated in KO mice at night, suggesting a mechanism of increased synthesis. Finally, we show that mice lacking VGLUT3 specifically in forebrain cholinergic neurons do not show hyperlocomotor activity, but do have behavioral impairments related to exploration and motivation.

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Poster

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Topic: D.15. Basal Ganglia

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1ZIAAG000928

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1ZIAAG000945

Title: Dual recording of real-time dopamine release and presynaptic calcium transients reveals distinct properties of dopaminergic afferents in dorsal and ventral striatum

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Abstract: Calcium triggers dopamine release from presynaptic terminals of midbrain dopaminergic (mDA) neurons in the striatum. However, calcium transients within the mDA axon terminals are difficult to study and little is known about how they are regulated. Here we describe presynaptic calcium transient (PreCaT) measurements in striatal presynaptic elements of mDA neurons using brain slice photometry and expression of the genetically encoded calcium indicator (GECI) GCaMP3 expressed in transgenic mice. We have also combined these measurements with simultaneous fast-scan cyclic voltammetry (FSCV) to measure changes in extracellular DA. Electrical stimulation-induced PreCaTs appear to be less sensitive to activation of dopaminergic autoreceptors (D2Rs) or inhibition of the dopamine transporter (DAT) in comparison to DA transients produced by the same stimulation. This may suggest factors that regulate DA release downstream of effects on presynaptic calcium entry, but the well-known power relationship between intracellular calcium concentration and DA release may also account for these differences. Simultaneous measurement of PreCaTs and DA release induced by the same electrical stimulatus were also compared in dorso-lateral (Caudate-Putamen, DLS) versus ventral (Nucleus Accumbens, VS) striatal slices. Single pulse-induced PreCaTs and [DA] release are smaller in magnitude in NAc than in DLS, but stronger paired-pulse facilitation of PreCaTs was observed in VS in comparison to DLS. This difference in potentiation indicates that factors controlling presynaptic calcium levels and DA release are differentially controlled in the two striatal subregions, in a manner that cannot be simply ascribed to differential expression of proteins involved in negative feedback or uptake (D2R, DAT), or the presence of fewer fibers innervating the VS. Subregional differences in effects of calcium channel toxins, Gi/o receptor antagonists and cholinergic agonists and antagonists are also being examined. We will thus determine if these molecules play any roles in the subregional differences in mDA presynaptic function.

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Poster

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Support: NIDA Grant DA029989-01

Title: Evidence against dopamine as a basal ganglia regulator of behavioral disengagement

Authors: *M. N. TERMINEL, T. MORENO, A. E. HERNANDEZ, J. RAMOS, E. CASTAÑEDA
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Abstract: The Basal Ganglia circuitry, a fundamental motor system, has long been known to be compromised in Parkinson's disease. While it is well established that Parkinson's patients suffer from pronounced motor deficits, recent behavioral studies suggest that the function of the Basal Ganglia is disengagement of behavior, which allows transitions between voluntary and respondent movement. Interestingly, one study suggests that dopamine (DA) in the Basal Ganglia, specifically the nigrostriatal pathway, regulates engagement ("starting") and disengagement ("stopping") of movement in rats. Unfortunately, little is known about how DA modulates locomotion during disengagement. The objective of this study is to provide insight about the role of DA within the basal ganglia to produce locomotor behavior. Our central hypothesis states that DA in the nigrostriatal motor pathway, an intrinsic circuitry of the basal ganglia circuitry, is important for initiating and terminating movement. To test this hypothesis we trained rats to walk on a treadmill in a continuous (for a period of 1 hr) and discontinuous (during 1 hr, 30 sec on/15 sec off) fashion. During treadmill walking, *in vivo* intracerebral microdialysis sampling for DA and its metabolites (DOPAC, HVA) were collected at the neostriatum. Dialysate was subsequently assayed using high pressure liquid chromatography (HPLC) coupled with electrochemical detection. We initially predicted that DA utilization would be greater during discontinuous treadmill walking that involves repetitive engage/disengage behavior. Contrary to our hypothesis, we found that both continuous and discontinuous treadmill walking produced an increase in DA release to the same extent. There were similar significant increases in extracellular DA metabolites when rats engaged in locomotor activity across both conditions. Our discussion will consider potential explanations for the present findings and set the foundation for a number of new studies to investigate changes in functionally relevant DA release, in contrast to pharmacologically induced overflow, in questions related to Parkinson's disease and substance abuse disorder.

Disclosures: M.N. Terminel: None. **T. Moreno:** None. **A.E. Hernandez:** None. **J. Ramos:** None. **E. Castañeda:** None.

Poster

541. Transmitters and Neuromodulation

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 541.12/MM31

Topic: D.15. Basal Ganglia

Support: MRC

Title: Opioidergic control of striatal low threshold spiking interneurons (LTSIs)

Authors: R. ELGHABA[1], *J. BERWICK[3], E. BRACCI[2]
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Abstract: The striatum is the largest nucleus and the main input structure of the basal ganglia. Low-threshold spike interneurons (LTSIs) are the only nitrergic neurons in the striatum and represent one of the tonically active neurons (TANs) in the striatum. While opioid receptors and their ligands encephalin and dynorphin play an important role in the striatum, their action on LTSIs has not been investigated. Using transgenic mice in which the NPY-expressing neurons are marked with green fluorescent protein (GFP), we investigated the effect of DAMGO (a μ receptor agonist), DPDPE (a δ recetor agonist) and (-)-U-50488 hydrochloride (a κ receptor agonist) on LTSIs activity. While DPDPE (1 μM) and (-)-U-50488 hydrochloride (20μM) produced hyperpolarizing effects in all LTSIs tested, DAMGO (1μM) produced hyperpolarizing effects in 80% of LTSIs and depolarizing effects in 20% of LTSIs tested when applied in control solution. We investigated the cause of this DAMGO-induce depolarization in a subpopulation of LTSIs. The dual effects of DAMGO persisted in the presence of tetrodotoxin (TTX), a sodium channel blocker. However, when GABAA, nicotinic & muscarinic receptors were additionally blocked, DAMGO effects on LTSIs were always hyperpolarizing. These finding suggest that μ-receptor agonists affect LTSIs both directly (exerting inhibitory effects) and indirectly, through presynaptic inhibition of GABA and acetylcholine release.

Disclosures: R. ElGhaba: None. **J. Berwick:** None. **E. Bracci:** None.

Poster

541. Transmitters and Neuromodulation

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 541.13/MM32

Topic: D.15. Basal Ganglia

Support: NIH R01AA016022

DICBR

Title: Regionally distinct dopamine release dynamics between striosome and matrix compartments of the striatum

Authors: *A. G. SALINAS[1,2], M. I. DAVIS[1], K. T. BLACKWELL[2], D. M. LOVINGER[1], Y. MATEO[1]
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Abstract: The striatum is a basal ganglia brain region involved in a number of neurological processes including learning and action control. It is comprised of GABAergic medium spiny projection neurons (~90%) and an assortment of interneurons (~10%). The striatum receives glutamatergic inputs from several cortical and thalamic areas and dopaminergic innervation from the substantia nigra and ventral tegmental area. The striatum has also been classically characterized as having two major output pathways: the direct pathway, which consists of D1 dopamine receptor-enriched neurons projecting directly to the substantia nigra and internal segment of the globus pallidus, and the D2 dopamine receptor-enriched, indirect pathway, which projects sequentially to the external segment of the globus pallidus, the subthalamic nucleus, and the midbrain. The striatum has also been classified into striosome and matrix compartments, based on the differential expression of a number of proteins, including the mu opioid receptor and Nr4a1 (nuclear receptor subfamily 4, group A, member 1). A number of functional differences between the striosome and matrix compartments have been noted and are implicated in neurological disorders including Huntington's disease, Parkinson's disease, and drug addiction. Given the importance of dopamine signaling in all of these conditions and the differences between striosome and matrix compartments in models of these conditions, we hypothesized that dopamine release dynamics between striosome and proximal matrix compartments would differ. To address this hypothesis, we used mice expressing eGFP under the Nr4a1 promoter to identify striosomes and fast scan cyclic voltammetry to measure evoked dopamine release from striosome and matrix compartment pairs throughout the striatum. We found that evoked dopamine release in striosomes located in the dorsal striatum was reduced approximately 33% compared to the matrix compartment. We further found that in the ventral striatum, this pattern was reversed, such that, dopamine release in striosomes was approximately 46% greater than in the matrix compartment. We found no differences in dopamine clearance between striosome and matrix compartments or between striatal subregions. Further studies are

underway to determine possible mechanisms gating the altered dopamine release between striosome and matrix compartments and striatal subregions.

Disclosures: **A.G. Salinas:** None. **M.I. Davis:** None. **K.T. Blackwell:** None. **D.M. Lovinger:** None. **Y. Mateo:** None.

Poster

541. Transmitters and Neuromodulation

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Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 541.14/MM33

Topic: D.15. Basal Ganglia

Support: Conacyt 152326

Title: Lisophosphatydilinosytol modulates GABA release in substantia nigra pars reticulata trough GPR55 receptor

Authors: ***R. SANCHEZ**[1], G. LOPEZ-RAMIREZ[1], F. PAZ-BERMUDEZ[1], M. RODRÍGUEZ-SÁNCHEZ[1], M. MUNOZ-ARENAS[2], B. FLORAN[1]
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Abstract: The G protein-coupled receptor 55 (GPR55) has been proposed as a cannabinoid receptor and some authors suggest that Lisophosphatydilinosytol (LPI) can be its endogenous ligand. The mRNA for this receptor is high expressed in the Central Nervous System (CNS) in the cerebral cortex, cerebellum, hippocampus, thalamus and striatum. By immunohistochemistry we found GPR55 receptor expression in the striatum and subthalamic nucleus of rat, since both structures project to the SNr, we asked if GPR55 receptor can modulate GABA release. By western blot we found GPR55 expression in nigral synaptosomal fractions. LPI increases K+ evoked [3H]GABA release in a dose dependent fashion, this effect was prevented by the GPR55 selective antagonist CID16020046 [400 nM]. The increase of K+ stimulated [3H]GABA release is abolished by preincubation with EGTA 3 mM but not by thapsigargin 10 uM. This results suggest GPR55 receptor present in GABAergic terminal in the SNr modulate GABA release trough Ca++ extracellular mobilization, but not from intracellular compartments.

Disclosures: **R. Sanchez:** None. **G. Lopez-Ramirez:** None. **F. Paz-Bermudez:** None. **M. Rodríguez-Sánchez:** None. **B. Floran:** None. **M. Munoz-Arenas:** None.

Poster

541. Transmitters and Neuromodulation

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 541.15/MM34

Topic: D.15. Basal Ganglia

Support: SAIOTEK S-PE12UN068

Title: Cannabinoids disrupt the cortical information transmission through the sensorimotor circuit of the basal ganglia

Authors: *T. MORERA-HERRERAS, I. BUSTINZA, A. GUTIERREZ, L. UGEDO
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Abstract: The CB1 cannabinoid receptor which is densely located in the basal ganglia is known to participate in the regulation of movement activity. The aim of this study was to determine the effect of cannabinoids (Δ^9-tetrahidrocannabinol (Δ^9-THC), and WIN 55,212-2) on spontaneous and cortically evoked activity in the substantia nigra pars reticulata (SNpr) by extracellular recording techniques in anaesthetized animals. Administration of Δ^9-THC (0.5 mg/kg, i.v.) stimulated (by 127 ± 14%) 6 of 11 SNpr recorded neurons, whereas it inhibited (by 73 ± 14%) the remaining 5 neurons. After Δ^9-THC the regularity of neuron activity was increased in all recorded neurons and the firing pattern changed toward a more bursting discharge. On the other hand, administration of WIN 55,212-2 (125-250 μg/kg, i,.v.) increased the firing rate of SNpr neurons (by 125 ± 7%, n=6) and the regularity of neuron activity, whereas did not modify the firing pattern. Previous administration of the cannabinoid receptor antagonist AM 251 (1 mg/kg, i.v.) completely blocked the effects induced by both agonists. Moreover, when AM 251 (1 mg/kg, i.v.) was administered alone also induced an increase (13 of 21 SNpr recorded neurons) or a decrease (remaining 8 neurons) in firing rate. After Δ^9-THC or WIN 55,212-2 administration, the inhibitory component of the cortically evoked response (activation of the direct striatonigral circuit) and the late excitatory response (activation of the indirect striato-pallido-subthalamo-nigral circuit) were decreased or completely lost. However, the early excitatory response (activation of the hyperdirect corticosubthalamic circuit) was not modified by cannabinoids administration. Previous administration of AM 251 (1 mg/kg, i.v.) completely blocked these effects without any modification of the cortically evoked responses. These results suggest that CB1 receptor activation modulates the sensorimotor transmission through the trans-

striatal pathways. This modulation may be relevant in the understanding of involvement of the cannabinoid system in motor control.

Disclosures: **T. Morera-Herreras:** None. **I. Bustinza:** None. **A. Gutierrez:** None. **L. Ugedo:** None.

Poster

541. Transmitters and Neuromodulation

Location: Halls A-C

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Program#/Poster#: 541.16/MM35

Topic: D.15. Basal Ganglia

Support: NIDA (P50 DA-05312 and R01 DA-12964)

Title: Selective D3 dopamine receptor activation reduces the head twitch response and improves cortico-striatal alterations induced by 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI)

Authors: *C. RANGEL BARAJAS[1,2], A. ESTRADA-SÁNCHEZ[1,3], M. MALIK[2], R. H. MACH[4], R. R. LUEDTKE[2], G. V. REBEC[1]
[1]Psychological and Brain Sci., Indiana Univ., Bloomington, IN; [2]Pharmacol. & Neurosci., Univ. of North Texas Hlth. Sci. Ctr., Fort Worth, TX; [3]Intellectual and Developmental Disabilities Res. Center, Semel Institute, Dept. of Psychiat, Univ. of California Los Angeles, Los Angeles, CA; [4]Dept. of Radiology, Univ. of Pennsylvania Sch. of Med., Philadelphia, PA

Abstract: Historically, the development of antipsychotic drugs has been based on the hypothesis that psychosis is related to increased dopaminergic tone. Although D2 dopamine receptor antagonists have been used to manage psychotic episodes, their efficacy is limited by motor side effects. Recent evidence, however, indicates that a D2-D3 partial agonist lacks these side effects, while retaining antipsychotic action (Caccia et al., 2013, Therap Clin Risk Man 9:319-328). We recently reported the ability of D2-like and D2 dopamine receptor selective compounds to attenuate the head twitch response (HTR) in DBA/2J mice (Rangel-Barajas et al., 2014, Neuropharmacology 83:18-27). Here, we studied the effect of the selective D3 dopamine receptor partial agonist WW-III-55 on HTR induced by the serotonin 5HT2A/2C receptor agonist 2,5-dimethoxy-4-iodoamphetamine (DOI), a hallucinogen used to screen for antipsychotic activity. Vehicle or WW-III-55 (6 mg/kg, ip) was administered 5 min before DOI (5 mg/kg ip) to FvB/NJ mice. DOI-induced HTR was monitored every 5 min for 30 min. Neuronal activity was recorded in primary motor cortex (M1) and dorsal striatum. We found that

WW-III-55 significantly inhibited DOI-induced HTR with a maximum efficacy of 50.2%. We also found a reversal of DOI-induced electrophysiological activity. In both M1 and dorsal striatum, DOI increased the rate and pattern of spike bursts and WW-III-55 reversed these effects. Our results suggest that selective activation of D3 dopamine receptors may represent a potential therapeutic target for psychiatric disorders.

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Poster

541. Transmitters and Neuromodulation

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Program#/Poster#: 541.17/MM36

Topic: D.15. Basal Ganglia

Support: NIDA (P50 DA-05312 and R01 DA-12964)

Title: Activation of D3 dopamine receptors by the novel full agonist WC 44 improves dysregulated corticostriatal neuronal processing related to drug-induced head twitching

Authors: *A. N. STRICKHOLM[1], A. M. ESTRADA-SANCHEZ[1,2], C. RANGEL-BARAJAS[1,3], M. MALIK[3], R. MACH[4], R. LUEDTKE[3], G. V. REBEC[1]
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Abstract: Activation of serotonin 5HT2a/2C receptors by 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI) induces involuntary motor responses characterized by spontaneous head twitching. Given that corticostriatal pathway plays a critical role in motor output, we evaluate the activity of cortical pyramidal neurons (CPNs) in primary motor cortex (M1) and medium spiny neurons (MSN) in dorsal striatum for 30 min in freely behaving mice (FvB/N) following injection (ip) of 5mg/kg DOI. Our results show that DOI decreased firing rate in both CPNs and MSNs. Interestingly, although no changes in the burst rate of individual spike trains were recorded, the properties of spike bursts, such as the number of spikes per burst, burst duration, and burst surprise value, were significantly augmented in both CPNs and MSNs. After DOI-

induced corticostriatal neuronal changes were characterized, we evaluated the effect of the novel dopamine D3 receptor agonist, WC 44, on the number of head twitches and the observed changes in CPNs and MSNs activity. Pretreatment with 5 mg/kg WC 44, 5 minutes before DOI, significantly reduced the number of head twitches and reversed the changes in CPN and MSN activity induced by DOI. Collectively, our results implicate the D3 dopamine receptor as a possible target for the control of involuntary movements associated with various neuropathological conditions.

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Poster

541. Transmitters and Neuromodulation

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Topic: D.15. Basal Ganglia

Support: AHFMR

CIHR Grant MOP82846

Title: Cholinergic mechanisms of DBS in entopeduncular nucleus

Authors: *F. LUO, T. CHOMIAK, Z. KISS
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Abstract: Chronic, high frequency (>100 Hz) electrical stimulation, known as deep brain stimulation (DBS), of the internal segment of the globus pallidus (GPi) is a highly effective therapy for Parkinson disease and dystonia. However, how it works remains unknown. Several hypotheses about its mechanisms of action have been proposed, such as depolarization blockade, activation of inhibitory synapses, depletion of neurotransmitters, and/or disruption/alteration of network oscillations. To date, none of these hypotheses have discussed cholinergic mechanisms. Yet, cholinergics are ubiquitous in the basal ganglia and could play an important role in DBS therapeutic benefit. GPi is the major output nucleus of the basal ganglia and receives cholinergic inputs from the pedunculopontine tegmental nucleus in addition to its own local cholinergic interneurons. Here we investigated the cellular mechanisms of simulated DBS (sDBS) in entopeduncular nucleus (EP, rat equivalent of GPi) neurons using whole-cell patch clamp

recordings focusing on cholinergics. We found that sDBS applied inside the EP nucleus induced a prolonged afterdepolarization that was dependent on stimulation frequency, pulse duration, and current amplitude. The high frequencies (>100 Hz) and pulse widths (>0.15 ms) used clinically for dystonia DBS could reliably induce these afterdepolarizations which persisted under blockade of ionotropic glutamate (kynurenic acid, 2 mM), GABA (picrotoxin, 50 μM) and acetylcholine nicotinic (DHβE, 2 μM) receptors. However, this effect was blocked by atropine (2 μM, non-selective muscarinic antagonist) or TTX (0.5 μM). A 10 s intracellular current (100 pA) injection combined with activation of muscarinic receptors with non-selective agonist oxotremorine (10 μM), while under GABA (picrotoxin 100 μM) and glutamate blockade (2 mM kynurenic acid), was also able to mimic the effect of a 10 s sDBS train. Finally, the muscarinic-dependent afterdepolarizations were sensitive to either phospholipase C pathway or calcium-sensitive nonspecific cationic channels (CAN channels) blockade. Hence, these data suggest that muscarinic receptor activation during sDBS can lead to feedforward excitation through the opening of CAN channels. This study for the first time describes a cholinergic mechanism of sDBS in EP neurons that provides new insight into our understanding of the underlying mechanisms of clinical DBS in humans.

Disclosures: F. Luo: None. **T. Chomiak:** None. **Z. Kiss:** None.

Poster

542. Plasticity of Voluntary Movements

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Topic: D.17. Voluntary Movements

Support: BMBF Grant 01GQ0810

cTMS machine was loaned by Rogue Resolutions, Inc.

Title: TMS can selectively activate and condition two different sets of excitatory synaptic inputs to corticospinal neurones in human

Authors: M. SOMMER[1,2], K. D'OSTILIO[1,3], M. CIOCCA[1,4], R. HANNAH[1], P. HAMMOND[1], S. M. GOETZ[5,6], R. CHIEFFO[1,7], J.-C. A. CHEN[8], A. V. PETERCHEV[5], N. NEEF[9], W. PAULUS[2], *J. C. ROTHWELL[1]
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Liege, Belgium; [4]Ctr. Clinico per la Neurostimolazione, le Neurotecnologie ed i Disordini del Movimento, Fondazione IRCCS Ca'Granda, Ospedale Maggiore Policlinico, Milan, Italy; [5]Dept. of Psychiatry and Behavioral Sci., Duke Univ., Durham, NC; [6]Tech. Univ., Munich, Germany; [7]Dept. of Neurol., Scientific Inst. Hosp. San Raffaele, Milan, Italy; [8]Neurosci. Laboratory, Dept. of Neurol., China Med. Univ. Hosp., Taichung, Taiwan; [9]Bernstein Focus Neurotechnology, Göttingen, Germany

Abstract: Background: Current protocols or repetitive transcranial magnetic stimulation (rTMS) induce mixed facilitatory and inhibitory effects. More selective, quasi-monophasic high-frequency stimulators have now become available. We sought to investigate the impact of current direction and pulse width on intermittent theta burst stimulation (iTBS) effects on human motor cortex excitability. Also, we estimated strength-duration time constants from motor threshold and input-output (IO) curves for posterio-anterior (PA) and AP orientations. Methods: We stimulated the dominant hand representation of the motor cortex in 15 healthy subjects, using "unidirectional biphasic" pulses generated by a controllable TMS machine (cTMS-3, Rogue Resolutions Ltd., Cardiff, UK), connected to a standard figure-8 coil. iTBS was applied conventionally, using 20 sequences of 2 seconds iTBS (10 bursts at 5 Hz burst repetition frequency, each burst consisting of 3 pulses of 80 % AMT intensity repeated at 50 Hz frequency). In separate sessions pulses differing in current direction and shape were applied: a) PA dominant current direction in the brain, 75 μs (iTBS_PA75). b) AP current direction, 45 μs (iTBS_AP45). Before and for 30 minutes after iTBS, we monitored the modulation of motor evoked potential (MEP) amplitude from the dominant first dorsal interosseus using conventional, monophasic, suprathreshold pulses generated by a Magstim 2002 stimulator, inducing PA currents in the brain, at 0.2 Hz frequency. In an additional study on ten healthy subjects, we investigated the effect the two coil orientations with three different pulse widths (30, 60 and 120 μs) on the IO curve and the latency of the motor evoked potentials (MEPs). Results: iTBS_AP45 yielded a pronounced and slightly delayed inhibition of MEP amplitude in all but one subjects; the amount of inhibition was unrelated to the MEP latency differences. iTBS_PA75 had a variable and inconsistent effect that was in part related to the AP-LM latency difference, in that long latency differences were correlated with the induction of inhibition rather than facilitation. We found a longer time constant for AP than PA orientation. MEP latencies yielded an interaction between pulse width and orientation, due mainly to longer onset latencies following AP stimuli of short duration. Conclusions: Current direction influences the outcome of iTBS, with a preference for AP currents. PA and AP stimuli activate the axons of neurons with different time constants. Those activated by AP pulses excite corticospinal outputs with a longer latency than those activated by PA pulses. AP pulses of short duration recruit long latency inputs most selectively.

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inventor on patents and patent applications on TMS technology assigned to his current and former employers.. **R. Chieffo:** None. **J.A. Chen:** None. **A.V. Peterchev:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); A. V. Peterchev is inventor on patents, patent applications, and invention disclosures on TMS technology assigned to Columbia University and Duke University; he has received research support, patent, patent royalties, and travel support from Rogue Research for cTMS technology licensed to them, TMS hardware donation from Magstim, and TMS equipment loan from MagVenture.. **N. Neef:** None. **W. Paulus:** None. **J.C. Rothwell:** None.

Poster

542. Plasticity of Voluntary Movements

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 542.02/NN3

Topic: D.17. Voluntary Movements

Support: NSERC

Title: Aerobic exercise modulates the cortical response to motor training

Authors: *A. M. SINGH, J. L. NEVA, W. R. STAINES
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Abstract: Aerobic exercise may modulate cortical excitability in primary motor cortical (M1) regions. Previous work from our lab indicates that acute exercise can decrease intracortical inhibition in M1 and also enhances the induction of early, long-term potentiation (LTP)-like plasticity. However, the effect on motor training has not been studied. In this study, we sought to determine the effect of acute aerobic exercise on an upper limb bimanual training task. It was hypothesized that a session of moderate-intensity aerobic exercise would facilitate cortical adaptations to bimanual training. Thirty young, healthy, right-handed participants were recruited and divided into three groups with the following experimental interventions: a) bimanual motor training alone; b) exercise alone; and c) exercise followed by bimanual motor training. The resting excitability of the extensor carpi radialis (ECR) muscle was measured at baseline and again immediately following the intervention. Single-pulse transcranial magnetic stimulation (TMS) was applied over the left M1 in order to map the boundaries of the cortical right ECR representation. After determining the resting motor threshold (RMT), the cortical map was obtained by sampling from sites in all directions from the motor hotspot in steps of 1 cm. Ten

single pulses at 110% of RMT were delivered at each site with an inter-stimulus interval of 3 s. The sites at which motor-evoked potentials (MEPs) were no longer observed were considered to be the boundaries of the representation. Sites with an average MEP amplitude greater than 30 μV were considered active. Following motor mapping, participants underwent one of the following interventions: a) 160 trials of a bimanual visuomotor training task; b) twenty minutes of stationary biking at 70% of age-predicted maximum heart rate; or c) both tasks, with the exercise bout immediately preceding the bimanual training. Immediately following each intervention, the TMS protocol was repeated to re-map the representation of the ECR muscle. Changes in excitability were quantified as either a change in the size of the ECR map as reflected by the number of active sites, a shift in the center of gravity of the map, or a difference in the average MEP amplitude within and across active sites. Preliminary analysis indicates that when training is preceded by exercise, this combination facilitates expansion of the motor cortical map to a greater extent than performing exercise or training alone. The current findings suggest that exercise may act to prime M1 for experience-dependent plasticity and can improve the effectiveness of subsequent motor training.

Disclosures: **A.M. Singh:** None. **J.L. Neva:** None. **W.R. Staines:** None.

Poster

542. Plasticity of Voluntary Movements

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 542.03/NN4

Topic: D.17. Voluntary Movements

Support: NSERC

Title: Evaluating the role of the premotor cortex in early somatosensory processing using somatosensory evoked potentials (SEPs) and continuous theta burst stimulation (cTBS)

Authors: ***M. J. BROWN**, J. L. NEVA, A. M. SINGH, W. R. STAINES
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Abstract: Frontal N30 somatosensory evoked potentials (SEPs), elicited by median nerve (MN) stimulation, are recorded maximally over the scalp from fronto-central electrodes. These frontal N30 SEPs represent early somatosensory input into non-primary motor areas rather than traditional primary sensory areas. There has been conflicting evidence from intracranial recordings and repetitive transcranial magnetic stimulation (rTMS) as to whether the

supplementary motor area (SMA), dorsal premotor cortex (PMd) or both are involved in the generation of frontal N30 SEPs. The purpose of the current study was to evaluate the role of the PMd in the generation of frontal N30 SEPs using continuous theta burst stimulation (cTBS). Frontal P20 and N30 SEPs were recorded from the FCz electrode position after MN stimulation to both left and right wrists. Changes in N30 peak and P20-N30 peak-to-peak amplitudes were compared at rest before to after (0, 15, 30 and 60 minutes) the application of cTBS to the right PMd (2.5 cm anterior to FDI motor hotspot in M1). Furthermore, previous research has suggested that variability in individual responses to cTBS in M1 may be predicted by the onset latencies of motor-evoked potential (MEP) with different coil orientations. Thus, MEP onset latencies were measured and compared in the current study when applying monophasic single pulse TMS to the M1 motor hotspot representation of the right FDI with anterior-posterior (AP), posterior-anterior (PA), lateral-medial (LM) and medial-lateral (ML) coil orientations. Preliminary group results did not reveal any significant modulation of frontal N30 peak or P20-N30 peak-to-peak amplitudes after cTBS to the right PMd. However, significant correlations were revealed between MEP latency difference (between AP-LM and AP-PA coil orientations) and the amount/direction of P20-N30 peak-to-peak amplitude change (elicited by left MN stimulation) after cTBS. These results demonstrated that a greater latency difference between MEPs elicited with AP-LM and AP-PA coil orientations was associated with increases in P20-N30 peak-to-peak amplitude while decreased latencies were associated with decreases in amplitudes. Based on these findings, individual variability in response (inhibition, excitation or no response) to PMd cTBS may be predicted by MEP onset latency differences with different coil orientations. Furthermore, although there is variability in the response after PMd cTBS, these results support that the PMd may be a contributor to the generation of frontal N30 SEPs.

Disclosures: **M.J. Brown:** None. **J.L. Neva:** None. **A.M. Singh:** None. **W.R. Staines:** None.

Poster

542. Plasticity of Voluntary Movements

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Program#/Poster#: 542.04/NN5

Topic: D.17. Voluntary Movements

Support: Irish Research Council EMBARK postgraduate scholarship

Title: Changes in corticospinal excitability induced by paired associative transcranial alternating current stimulation

Authors: *E. MCNICKLE[1], R. G. CARSON[1,2]
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Abstract: Many types of non-invasive brain stimulation alter corticospinal excitability (CSE). Paired Associative Stimulation (PAS) attracts particular attention as its effects ostensibly adhere to Hebbian principles of neural plasticity. In prototypical form, a single electrical stimulus is directed to a peripheral nerve in close temporal contiguity with transcranial magnetic stimulation (TMS) delivered to the contralateral primary motor cortex (M1). Repeated pairing of the two discrete stimulus events (i.e. association) over an extended period either increases or decreases the excitability of corticospinal projections from M1, contingent on the interstimulus interval (ISI). We studied a novel form of associative stimulation, consisting of 500 ms trains of peripheral afferent stimulation paired with short bursts of high frequency (≥ 80 Hz) transcranial alternating current stimulation (tACS) over contralateral M1. Elevations in the excitability of corticospinal projections to the forearm were observed for a range of tACS frequency (80Hz, 140Hz and 250Hz) and current (1mA, 2mA and 3mA) parameters. tACS bursts of 1000ms duration generated larger increases in CSE than 250ms or 500ms bursts. The effects were more reliable than those brought about by PAS or transcranial direct current stimulation (tDCS). When paired with tACS, muscle tendon vibration induced greater elevations of CSE than electrical nerve stimulation. In demonstrating that associative effects are expressed when the timing of the peripheral and cortical events is not precisely circumscribed, these findings suggest that multiple cellular pathways may contribute to a LTP-type response. Their relative contributions will differ depending on the nature of the induction protocol that is used.

Disclosures: E. McNickle: None. **R.G. Carson:** None.

Poster

542. Plasticity of Voluntary Movements

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 542.05/NN6

Topic: D.17. Voluntary Movements

Support: Wellcome Trust

Title: Changes in neuronal responses induced by paired associative nerve stimulation

Authors: *B. HABEKOST, S. N. BAKER
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Abstract: Non-invasive methods have been developed to induce plastic changes in the sensorimotor cortex. These rely on stimulating pairs of afferent nerves. By associative stimulation of two afferent nerves, excitability changes in the motor cortex occur as indicated by studies reporting changes in motor evoked potentials (MEPs) elicited by transcranial magnetic stimulation (TMS). The repetitive stimulation of those nerves has a potential in rehabilitation and treatment of neurological disorders like stroke or spinal cord injury. Despite promising results and applications in human subjects using these methods, little is understood about the underlying basis for the changes which are seen. In the present study, we record from identified pyramidal tract neurons and unidentified cells in primary motor (M1) and somatosensory cortex (S1), in a single macaque monkey before and after one hour associative stimulation of the median and ulnar nerve. Cell discharge was recorded during selective independent movements of thumb and index finger, as well as in response to electrical stimulation of each nerve independently. Cells in M1 (and S1) showed increased and decreased firing rates in response to nerve stimulation after one hour of associative nerve stimulation. These findings suggest that the sensorimotor cortex undergoes plastic changes in response to associative nerve stimulation.

Disclosures: B. Habekost: None. **S.N. Baker:** None.

Poster

542. Plasticity of Voluntary Movements

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 542.06/NN7

Topic: D.17. Voluntary Movements

Title: Reliability of measures of motor cortical inhibition derived from transcranial magnetic stimulation

Authors: *A. VIRANI[1], D. RADKE[2], T. DAVIDSON[2], F. TREMBLAY[3]
[2]Human Kinetics, [3]Sch. of Rehab Sci., [1]Univ. of Ottawa, Ottawa, ON, Canada

Abstract: Neurophysiological properties of the motor system can be studied non-invasively using transcranial magnetic stimulation (TMS). The cortical silent period (CSP, Werhahn et al., 1999) and short afferent inhibition (SAI, Tokimura et al., 2000) are two common measures derived from TMS to probe the excitability of local inhibitory networks within the primary motor

cortex (M1). There is still relatively little information, however, regarding the reliability of these measures of cortical inhibition when repeated between sessions. In the present study, we examined the inter-session reliability of CSP and SAI measurements, as well as the inter-rater reliability of CSP measurements. The CSP and SAI were measured in two different sessions, 1 week apart, in healthy participants (n=10) using standardized procedures. For the CSP, suprathreshold TMS pulses (130%) were delivered while participants exerted a submaximal force (10-20% MVC) against a pinch dynamometer. For SAI, electrical pulses were delivered to the median nerve at the wrist (200 μs pulse, minimal twitch) at an interval of 20 ms before the TMS pulse (120%). SAI levels were expressed as % $MEP_{conditioned}/MEP_{unconditioned}$. Comparisons of SAI levels and CSP durations revealed good reliability between sessions, with intraclass coefficients of 0.72 and 0.74, respectively. In addition, comparisons of estimates of CSP duration from visual inspection derived from different examiners revealed very high inter-rater reliability (ICC = 0.996). In sum, both CSP and SAI proved to be reliable measures in young healthy adults when repeated between sessions at an interval of one week. In addition, our results concur with other studies (e.g., Kimberley et al. Neurosci Lett 464, 84-87, 2009) and confirm the high inter-rater reliability of CSP duration estimates derived from visual inspection.

Disclosures: **A. Virani:** None. **D. Radke:** None. **T. Davidson:** None. **F. Tremblay:** None.

Poster

542. Plasticity of Voluntary Movements

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 542.07/NN8

Topic: D.17. Voluntary Movements

Title: Hemispheric inhibition between motor cortices: A reliability study of TMS-derived measures of interhemispheric inhibition

Authors: *D. P. RADKE, A. VIRANI, T. DAVIDSON, F. TREMBLAY
Bruyère Res. Inst., Ottawa, ON, Canada

Abstract: The motor transcallosal pathway is thought to be critical both for bimanual motor coordination and for unimanual tasks. One way to assess *in vivo* the transcallosal connections between motor cortices is by means of transcranial magnetic stimulation (TMS). With this technique, two different measures of transcallosal inhibition (TCI) can be derived. The first one is based on double coil stimulation to examine inter-hemispheric inhibition (IHI) elicited either at short- (IHI_{10}) or long- interval (IHI_{40}). The second measure is obtained via supra-threshold

cortical stimulation during active contraction via the ipsilateral silent period (iSP). The present study examined the inter-session reliability of measures of TCI derived from double coil stimulation (IHI_{10}, IHI_{40}) and iSP in 10 healthy young participants. The IHI measures were obtained using two focal coils coupled with Magstim 200 (test stimuli 120% MT) and Rapid^2 (conditioning stimuli 120% MT) stimulators. The iSP was obtained by applying suprathreshold stimulation (130%) to the left motor cortex while participants exerted maximal effort with the left hand and light contraction with the right hand (target muscle: 1st dorsal interosseous). Reliability between sessions (1 wk interval) was assessed via the intraclass correlation coefficient (ICC) and Pearson's moment correlation (r). The inter-rater reliability of iSP analysis was also examined. The inter-session reliability of both IHI_{10} and IHI_{40} was found to be fair (ICC of 0.41 and 0.47, respectively), while the inter-session reliability of indices derived from visual inspection of iSP traces (latency onset and duration of TCI: LTI, DTI) were both found to have excellent reliability (ICC of 0.98 and 0.94, respectively). The inter-rater reliability of LTI and DTI indices from the iSP were also found to be highly reliable between examiners (ICC of 0.98 and 0.97, respectively). Finally, in line with Chen et al (2003), we also found high correlation ($p<0.01$, $r^2= 0.37$) between IHI_{40} and iSP duration (DTI), whereas such association was not found for IHI_{10}. Our results indicate that measures of TCI derived from iSP traces are more reliable than measures derived from variations in MEP amplitude using the double coil stimulation. In addition, our results confirm the close association between IHI seen at 40 ms interval and TCI measured via the iSP, as suggested by Chen et al. (J Neurophysiol 89, 1256-1264, 2003).

Disclosures: D.P. Radke: None. **A. Virani:** None. **T. Davidson:** None. **F. Tremblay:** None.

Poster

542. Plasticity of Voluntary Movements

Location: Halls A-C

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Program#/Poster#: 542.08/NN9

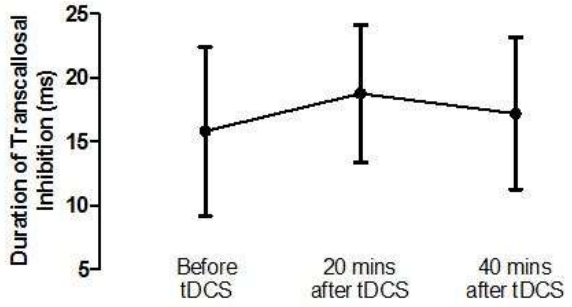
Topic: D.17. Voluntary Movements

Support: Connect Canada #117

Title: Modulation of transcallosal inhibition in the motor cortex by transcranial direct current stimulation (tDCS)

Authors: *T. DAVIDSON[1,4], F. TREMBLAY[2,4], M. BOLIC[3]
[2]Sch. of Rehabil. Sci., [3]Sch. of Electrical Engin. and Computer Sci., [1]Univ. of Ottawa, Ottawa, ON, Canada; [4]Bruyère Res. Inst., Ottawa, ON, Canada

Abstract: Anodal tDCS of the primary motor cortex (M1) is known to lead to a lasting increase in corticospinal excitability, as evaluated using transcranial magnetic stimulation (TMS). However, there is still little information with regards to the effects of tDCS on TMS-derived measures of transcallosal inhibition. Here, we investigated the effects of a single session of anodal tDCS on both cortical excitability, as reflected in the facilitated motor evoked potential (facMEP) amplitude, and motor transcallosal inhibition, as measured with the ipsilateral silent period (iSP). Participants (young adults, n=12) underwent anodal tDCS (2 mA) over the left M1 for 20 minutes. Changes in MEP amplitude and iSP parameters were monitored before and at specific time intervals after tDCS. Following tDCS, a majority of participants exhibited the expected MEP facilitation (7/12, 76% increase), while the remaining tended to show depression (-31% decrease). In parallel, the duration of transcallosal inhibition (DTI) also increased in a majority of participants (7/12, 20% increase) after tDCS. Correlation between changes in MEP amplitude and in DTI post-tDCS revealed a trend for significance between the two (r=0.53, p=0.07). These results are in line with those of Lang et al (2004) and suggest that increase in corticospinal excitability induced by tDCS is accompanied by parallel changes in the excitability of the transcallosal connections from the stimulated M1 to the opposite MI. Our results also highlight the inherent variability of individual responses to plasticity inducing protocols by non-invasive brain stimulation techniques. We are currently investigating this issue using variations in MEP latency, as shown by Wiethoff and colleagues (Brain Stimul, 7, 2014).



Disclosures: **T. Davidson:** 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.; NorDocs Technologies. **F. Tremblay:** None. **M. Bolic:** None.

Poster

542. Plasticity of Voluntary Movements

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 542.09/NN10

Topic: D.17. Voluntary Movements

Title: Hemispheric differences in modulation of corticospinal excitability associated with mental tasks

Authors: *L. FERRON, F. TREMBLAY
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Abstract: Observing others performing actions is a common way to learn new motor skills. Such ability appears to be linked with one's ability to mentally simulate actions. While mental simulation has been widely used in the context of athletic performance, the same approach has also been advocated in rehabilitation settings, where they target clinical populations with limb injuries or affected with chronic pain. Here, we investigated with transcranial magnetic stimulation (TMS) hemispheric differences in modulation of motor evoked potentials (MEPs) elicited in the context of mental tasks involving either speech production or hand rotations. We hypothesized that internal speech would lead to greater MEP facilitation in the left hemisphere (LH) owing to its major role of in language. Conversely, we anticipated that mental rotations involving hand laterality judgments would lead to larger MEP facilitation in the right hemisphere, given evidence of the right hemisphere role in kinesthesia (Goble & Brown, Neurosc & Biobehav Rev 32, 2008). Corticospinal excitability was tested (target muscle: 1st dorsal interosseus) with participants (n=16) seated in front of computer screen while they were asked to perform two mental tasks: 1) backward counting and 2) judging hand laterality. A third task wherein participants just watched an image of a "foot" was added as a "control". In all conditions, TMS (110% resting motor threshold) was delivered at a specific time delay based on prior assessment of performance in the hand laterality task (i.e., delay= half of the mean response time). Comparison of task-related variations in MEP log-amplitude reveals no significant hemispheric main effect or interaction, although MEPs tended to be larger in general in response to left TMS. A "task condition" effect was observed owing to the large MEP facilitation elicited during the mental counting task, which was significantly different ($p<0.003$) from either the control task or the hand rotation task. In fact, the latter task was associated with MEP depression, which almost reached significance ($p=0.056$) when compared to the control task. While no significant hemispheric asymmetry was found, our results point to larger task-related effect in the left vs. right hemisphere, especially in association with internal speech. Finding MEP depression with hand mental rotation suggests an active process which may be related to disengagement of

the distal hand representation in favor of more proximal muscles. We are currently investigating this issue.

Disclosures: L. Ferron: None. **F. Tremblay:** None.

Poster

542. Plasticity of Voluntary Movements

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 542.10/NN11

Topic: D.17. Voluntary Movements

Support: NSERC

CIHR

AIHS

Title: Effects of stress on motor map expression and behaviour

Authors: *K. A. SCULLION, A. KIM, M. GRAY, M. N. HILL, G. C. TESKEY
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Abstract: Acute and chronic stress can have opposing effects within the CNS. Stress effects have been well documented in the limbic system but little attention has been given to the motor system. Previous research has demonstrated that acute stress increases glutamate receptors in the prefrontal cortex while chronic stress has the opposite effect. Consequently, glutamatergic transmission is altered during stress and this could result in changes in motor map expression. Motor maps are the topographical representation of movements and are derived by stimulating pools of pyramidal neurons in layer V of the neocortex using a technique called intracortical microstimulation (ICMS; Young et al., 2011). Motor maps can be considered an assay of brain excitability as the balance between cortical inhibition and excitation determines map expression. The quantity and type of neurotransmitters present in the neocortex also determines map expression. We examined the role of acute and repeated stress on the expression of cortical forelimb motor maps and behaviour. We tested three hypotheses 1) acute stress will decrease movement thresholds and increase motor map size 2) repeated stress will increase movement thresholds and decrease motor map size 3) repeated stress will impair the rat's ability to perform the single pellet skilled reaching task and will result in less motor map reorganization and expansion typically seen after skilled training. In experiments 1 to 3, the rats were stressed by

being placed on an elevated platform in a brightly lit room for 30 minutes each day. Blood samples were collected from the rats pre- and post- stress. All rats underwent high resolution intracortical microstimulation (ICMS) to map forelimb (digit, wrist, elbow, shoulder) and non-forelimb movement representation areas. For experiment 1, the rats were stressed and underwent ICMS on the same day. For experiment 2, the rats were stressed once daily for 5 consecutive days. On the last day of stress testing, ICMS was performed. For experiment 3, rats were trained on the single pellet skilled reaching task for 15 days. On the 16th day, the rats underwent stress testing once daily for the next 5 days. On the 5th day of stress testing, the skilled reaching performance of the rats was assessed and then ICMS was performed. Our preliminary results indicate that only repeated stress alters forelimb motor map expression. These results show that prolonged elevation of glucocorticoids play an inhibitory role on motor map expression. Outcomes of this investigation could lead to further understanding of how stress mediated effects can lead to motor system disorders.

Disclosures: K.A. Scullion: None. **A. Kim:** None. **M. Gray:** None. **M.N. Hill:** None. **G.C. Teskey:** None.

Poster

542. Plasticity of Voluntary Movements

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Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 542.11/NN12

Topic: D.17. Voluntary Movements

Title: Changes of corticospinal tract after wearing prosthesis in serial bilateral below knee amputation

Authors: *S. KANG[1], D. KIM[1], K. SEO[1], J. KIM[2]
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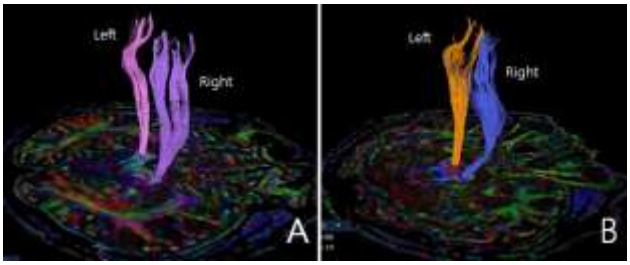
Abstract: After amputation, brain has been known to be reorganized especially in motor primary cortex. We report a case of a patient whose corticospinal tract (CST) showed change after serially performed bilateral below knee (BK) amputation using diffusion tensor imaging (DTI). A 78 years old man visited orthopedics clinic complaining of right big toe's gangrene in 2011. In past medical history, he had diabetes mellitus for 10 years, and has had amputated 2nd toe of right foot since 2005. He also had below knee amputation on his left side since 2008 due to diabetic arterial stenosis. After BK amputation on his left side, he was able to walk

independently with his prosthesis. With his frequent pain and small multiple wounds, we assumed that he walked with the left prosthetic limb for supporting weight, not with right leg. He underwent right BK amputation in 2011, and transferred to rehabilitation department. DTI was performed before beginning gait training with new prosthesis for his right leg, and it showed larger number of fiber lines in right CST than left CST (Figure A). This result reflects that he walked with his left prosthetic limb more than right limb for supporting weight after left BK amputation in 2008. He had intensive rehabilitation program for 2 months, and discharged when he could walk with walker independently. He returned to his walk and he serially visited our clinic for 2 years, and he did not report any medical or surgical problems during this period. He was able to walk with bilateral cane without complication on his bilateral stump. A follow up DTI was performed at this point (2013), and it showed no definite difference in the numbers of fiber lines between both CST (Figure B). This result reflects that he has walked with bilateral prosthetic limb almost equally for supporting weight after right BK amputation in 2011. In conclusion, we found that side-to-side differences in corticospinal tract in unilateral below knee amputation were equalized after proper use of bilateral prosthesis in a patient with diabetic atherosclerosis.



Disclosures: **S. Kang:** None. **D. Kim:** None. **K. Seo:** None. **J. Kim:** None.

Poster

542. Plasticity of Voluntary Movements

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

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Topic: D.17. Voluntary Movements

Support: Canadian Institutes of Health Research

Title: Influence of BDNF polymorphism on neurophysiology in human motor cortex

Authors: *R. CASH, K. UDUPA, R. CHEN
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Abstract: INTRODUCTION: The influence of genetic variation in the brain-derived neurotrophic factor gene (BDNF) on human cortical plasticity has recently attracted much interest, however careful studies of the effects on intracortical circuitry have been lacking. We investigated the influence of a common single nucleotide polymorphism (SNP) BDNF Val66Met on TMS measures of intracortical circuitry using carefully chosen parameters that are likely to tease out differences. METHODS: 18 participants were recruited, 9 Val homozygotes (aged 35 ± 2 years) and 9 Met allele carriers (aged 33 ± 4 years). The following circuits were investigated: GABAA receptor mediated short interval intracortical inhibition (SICI), GABAB receptor mediated long interval intracortical inhibition (LICI) and an excitatory circuit termed short interval intracortical facilitation (SICF). LICI was measured using 3 conditioning stimulus (CS) intensities: 90, 110 and 130% of resting motor threshold (RMT) and an interstimulus interval (ISI) of 150ms to avoid floor effects or saturation. SICI was measured at a CS intensity of 65% RMT & ISI 2ms, thus minimising contamination by excitatory circuitry. Test stimului (TS) evoking 1mV MEP were used for SICI and LICI. A SICF ISI curve was generated at 0.2ms intervals comprising the first I-wave peak and trough (1.1-2.3ms), using stimuli of equal intensity (110% RMT, Tokimura et al. 1996). As SICF peaks and troughs vary between individuals, maximal (max) and minimum (min) SICF was also computed across individuals. RESULTS: SICI was consistently stronger in Val/Val (23±3.5%) compared to Met allele carriers (57±11.3). LICI was stronger in Val/Val subjects at intensities of 110 and 130% RMT with no inhibition in either group at 90% RMT. SICF ISI curves differed strikingly between groups. SICF(max) was greater in Val/Val (1470±364 %) compared to Met carriers (555±133%), while there was little difference at SICF(min): 156±56% vs 100±12% of TS alone in Val/Val and Met groups. SICF TS amplitude (at 110% RMT) was equal between groups (~0.2mV). There were no differences between Val/Val and Met groups in RMT (49±4 and 49±4% MSO respectively) or the intensity required to generate 1mV peak-to-peak MEP amplitude (62±5 and 68±5% MSO respectively). DISCUSSION: We found stronger inhibition in GABAA and GABAB mediated circuitry, and stronger excitation in Val/Val homozygotes compared to Met allele carriers. As BDNF release is greater in Val/Val homozygotes, these results may be attributable to the role of BDNF in the development and maintenance of cortical circuitry, or its role in potentiating synaptic transmission, which contribute to BDNF's role in enhancing plasticity.

Disclosures: **R. Cash:** None. **K. Udupa:** None. **R. Chen:** None.

Poster

542. Plasticity of Voluntary Movements

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Program#/Poster#: 542.13/NN14

Topic: D.17. Voluntary Movements

Support: INBRE 5P20RR016472-12

Title: Brief high intensity cycling enhances BDNF release and locomotor learning

Authors: *E. E. HELM, K. MATT, K. KIRSCHNER, D. REISMAN
Univ. of Delaware, Newark, DE

Abstract: Exercise, when coupled with a motor learning task, is believed to enhance neural plasticity and therefore promote improved learning post-stroke. Animal models have demonstrated exercise-induced neural plasticity through upregulation of proteins involved in synaptic plasticity, neural remodeling and learning particularly Brain Derived Neurotrophic Factor (BDNF). Literature concerning the role of BDNF in enhancement of motor learning in the human population is limited; however intensity of exercise has been shown to moderate the relationship between peripheral BDNF levels and cognitive learning. We theorize that high intensity exercise may also enhance motor learning. We hypothesize that a single 5 minute session of high intensity upper extremity cycling immediately prior to a locomotor learning task will elicit an up-regulation of peripheral BDNF levels and this upregulation will correlate with enhanced locomotor learning. In an ongoing study, 6 neurologically intact subjects have been randomly assigned to an Exercise+Learn or Learn only condition. Subjects participated in 2 sessions of split-belt treadmill walking on 2 consecutive days. On the first day subjects in both conditions walked on the split-belt treadmill at a 1:1 ratio for 2 minutes in order to assess baseline step asymmetry. Subjects then walked at a 3:1 speed ratio for 15 minutes. Both groups performed the identical motor learning task with the only difference between conditions that subjects in the Exercise+Learn condition performed a short bout of high intensity exercise on an upper body ergometer prior to split-belt walking. Cycling consisted of pedaling for 1 minute with high resistance immediately followed by 1 minute with low resistance to achieve 80% of subjects maximum heart rate. Subjects received a 1 minute rest and then repeated the cycling protocol. Subjects in the Learn Only group quietly rested for 5 minutes to account for time differences. On Day 2, longer term learning was assessed by having participants walk on the split belt treadmill in the same configuration as Day 1. Consistent with our hypothesis, initial results demonstrate that 5 minutes of high intensity exercise can increase serum BDNF levels relative to quiet rest. Additionally, the current trend indicates that those within the Exercise+Learn group demonstrate enhanced learning of the split belt learning task as assessed by improved step length symmetry. If the current trend is maintained with increased sample size, these results will directly impact the design and implementation of neurorehabilitation programs targeted toward facilitation of neuroplasticity.

Disclosures: **E.E. Helm:** None. **K. Kirschner:** None. **K. Matt:** None. **D. Reisman:** None.

Poster

542. Plasticity of Voluntary Movements

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NIMH Training Program in Systems and Integrative Neuroscience T32

MH019524 to GW

Title: (In) Activity-induced structural changes of the cerebellar cortex noradrenergic fibers

Authors: ***H. NEDELESCU**[1,2], K. TATEYAMA[3], T. CHOWDHURY[3], G. WABLE[3], G. ARBUTHNOTT[1], C. AOKI[3]
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Abstract: The noradrenergic system plays an important role in arousal, vigilance, response to stress and panic, as well as in motor learning. The cerebellum is an important region involved in the timing and plasticity underlying motor behaviors. Noradrenergic fibers, originating from the locus coeruleus, project to the cerebellum particularly innervating the Purkinje cell layer and the lower portion of molecular layer. Despite its undoubted presence in the cerebellum, the noradrenergic system in this brain region has received little attention. The goal of our study was to determine how the noradrenergic innervation of voluntarily active rats is altered relative to sedentary rats. In addition, we compared mild voluntary exercise on the running wheel (5

km/day) with excessive exercise (15 km/day), which is evoked by the stressful situation of food restriction. Previous studies have shown that excessive exercise on the running wheel exhibited by food restricted rats is maladaptive, in that it causes negative energy balance to the point of death, unless the food restriction is attenuated. To quantify the effect of exercise on the noradrenergic innervation, eight rats were singly housed in the presence of a running wheel starting P36 until P44 (EX group), while eight other rats were excessive exercise-induced (EEX group) by allowing voluntary wheel access and limiting food availability to 1 hr/day. In addition, eight other rats were singly housed without a wheel but with ad libitum food access (CON group) for the matching ages to obtain baseline values. We used the primary antibody dopamine-β-hydroxylase and subsequently visualized noradrenergic fibers and their varicosities with 3,3'-diaminobenzidine (DAB) as the immunolabel. We acquired image stacks of the DAB labeled noradrenergic fibers using a Photo Multiplier Tube (PMT) for digitization of the confocal microscope image. Next, using the Neurolucida Software (MBF Bioscience), we traced each noradrenergic fiber and added a marker demarcating each encountered varicosity. The inter-varicosity distances were 8.51μm for the CON group (N =8) and reduced to 7.79 μm for the EX group (N = 8) and also reduced to 7.45 μm for the EEX group (N =8). This difference across the groups suggests that voluntary wheel running during adolescence increases the amount of norepinephrine available for release in the molecular layer of the cerebellum.

Disclosures: H. Nedelescu: None. **K. Tateyama:** None. **T. Chowdhury:** None. **G. Wable:** None. **G. Arbuthnott:** None. **C. Aoki:** None.

Poster

542. Plasticity of Voluntary Movements

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Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 542.15/NN16

Topic: D.17. Voluntary Movements

Title: Frontal and cerebellar networks interact to determine potential for motor cortex plasticity

Authors: *J. L. MIRDAMADI, L. Y. SUZUKI, S. K. MEEHAN
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Abstract: Motor skill learning is driven by experience-dependent changes in multiple cortical and subcortical networks. During early learning, changes in the motor cortex (M1) are associated with increased attention, mediated by the dorsolateral prefrontal cortex (DLPFC), and feedback-corrections, mediated by the cerebellum. Increasing visual attention load attenuates the

facilitatory after-effect of intermittent theta burst stimulation (iTBS) through gating of somatosensory inputs to cortex, most likely through a DLFPC-thalamic network. However, both DLPFC and cerebellar-mediated networks can influence sensory processing, yet how these two networks interact to optimize learning is uncertain. Here, we used cerebellar TBS to assess whether cerebellar networks influencing sensory processing may interact with the attention sensory gating mechanism to determine potential for M1 plasticity. First, we hypothesized that in the absence of cerebellar iTBS, M1 plasticity would be attenuated by increase attentional demands, as previously shown. Second, we hypothesized that cerebellar TBS would inhibit thalamic projections to M1 and revert the effect of attention loads on subsequent M1 plasticity. M1 plasticity in the first dorsal interosseous was assessed using 2-second trains of TBS (3 pulses at 50 Hz, every 200 msec, 80% AMT) while participants engaged in a visual detection task requiring low or high loads of visual attention. Motor cortical excitability was assessed using single pulses of TMS (120% RMT). Five pulses were delivered 4 seconds apart prior to the TBS. 6 additional pulses were delivered every 4 seconds post-TBS, starting from 2 seconds after cessation of TBS. Trials were performed both before and after iTBS over the right posterior cerebellar cortex (20 two-second trains of TBS, 8 second inter-train interval). 15 trials were completed for each combination of attention load and cerebellar priming. Preliminary results (N=9) suggest that in the absence of cerebellar priming, MEP amplitude was facilitated after M1 TBS under low load, but unchanged after M1 TBS under high load, consistent with previous research. Following cerebellar iTBS, M1 TBS under low load failed to enhance MEP amplitude. In contrast, M1 TBS under high load enhanced MEP amplitude. These results suggest that both attention and error driven networks influence sensory input to the cortex. However, in circumstances where both serve to reduce excitability of M1, via the thalamus, a metaplastic response may be initiated that may be counterproductive to early motor skill learning. These results highlight the importance of controlling for attention when studying motor learning and plasticity.

Disclosures: J.L. Mirdamadi: None. **L.Y. Suzuki:** None. **S.K. Meehan:** None.

Poster

542. Plasticity of Voluntary Movements

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 542.16/NN17

Topic: D.17. Voluntary Movements

Title: Short-term memory requirements decrease potential for plasticity in motor cortex

Authors: L. Y. SUZUKI, J. L. MIRDAMADI, M. SIERANT, *S. K. MEEHAN
Sch. of Kinesiology, Univ. of Michigan, Ann Arbor, MI

Abstract: Cognitive processes, such as attention and working memory are important determinants of motor learning. It has been proposed that increased loads of attention reduce motor cortical excitability through somatosensory gating. However, the impact of working memory upon motor cortical excitability has yet to be determined. The current study sought to determine the impact of increasing short-term memory loads upon recruitment of neuroplastic mechanisms that underlie skilled motor learning. It was hypothesized that increasing demands upon short-term memory would prevent theta burst stimulation (TBS) induced increases in motor evoked potential amplitude (MEP) when TBS was applied during the retention but not the acquisition phase of the Sternberg short-term memory (STM) task. Short-term memory requirements were manipulated by increasing the set size to be encoded and remembered from 2 digits to 6 digits. The memory set was presented for 2 seconds and was followed by a 2 second retention phase prior to the presentation of a single probe digit. TBS was either timed to coincide with the presentation of the digit set (encoding phase) or the two seconds following digit presentation but preceding presentation of the single digit probe (retention phase). TBS was delivered over the first dorsal interosseus (FDI) motor cortical hotspot (3 stimuli at 50 Hz, repeated every 0.2 seconds for 2 seconds total, 80% AMT). Motor cortical excitability was assessed using single pulses of TMS (120% RMT). Five pulses were delivered 2 seconds apart prior to the TBS. 10 additional pulses were delivered every 2 seconds post-TBS, starting from 2 seconds after cessation of TBS. 12 trials were completed for each combination of set size and STM task phase. Preliminary results show that for TBS delivered during the acquisition phase of the STM task the impact of set size differed across time. For a set size of 2 digits there is an initial facilitation for the first 6 seconds post-TBS followed by a return to pre-TBS amplitude. During high load there was no change during the first 10 seconds post-TBS, but a facilitation 12-20 seconds post-TBS. For the retention phase, there was no change in MEP amplitude compared to pre-TBS regardless of STM load. These results suggest that short-term memory may influence motor skill learning, in part, by directly influencing LTP- and/or LTD-like mechanisms in motor cortex. These effects appear to be dependent upon the extent to which information must be encoded and retained over short periods of time. There results may have important implications for developing instructions/feedback that may need to be held in short-term memory during rehabilitation interventions.

Disclosures: L.Y. Suzuki: None. **J.L. Mirdamadi:** None. **M. Sierant:** None. **S.K. Meehan:** None.

Poster

542. Plasticity of Voluntary Movements

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 542.17/NN18

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

Support: NIH K08

Title: Separate representations of the unimpaired and the impaired forelimbs in primary motor cortex following neonatal pyramidotomy in rats

Authors: *T. WEN[1], D. GUPTA[1], J. B. CARMEL[1,2]
[1]Burke Med. Res. Inst., White Plains, NY; [2]Neurol. and Pediatrics, Brain and Mind Res. Inst., New York, NY

Abstract: Background: After significant injury to one hemisphere in early development, the corticospinal tract from the uninjured hemisphere maintains connections to both sides of the spinal cord. During acquisition of motor skill, the uninjured hemisphere assumes control over both sides of the body. This comes at a cost; many movements of the impaired side are mirrored on the unimpaired side. We are interested in using brain stimulation to restore function to the impaired side. To understand how best to sculpt corticospinal circuits with electrical stimulation, we sought to determine how each forelimb is represented in the same motor cortex in the uninjured hemisphere. We hypothesized that each forelimb would have overlapping but distinct representations. Methods: We cut the corticospinal tract from one hemisphere at the level of the medulla oblongata in the postnatal-day 7 rat. Three months after pyramidotomy, we mapped motor responses of ipsilateral impaired and contralateral unimpaired forelimbs using intracortical microstimulation (ICMS) of forelimb motor cortex. Following ICMS, we injected the retrograde neural tracer fast blue into the spinal cord to map spinal connections on the ipsilateral impaired or the contralateral unimpaired side of the cervical spinal cord. Two weeks after injections, the fast blue-labeled corticospinal neurons were counted and the distribution reconstructed by mapping the counted neurons onto a 3-dimensional brain atlas. Results: During ICMS mapping, there were more sites that elicited contralateral than ipsilateral forelimb responses. Sites with low threshold contralateral responses tended to show ipsilateral responses as well. Thus, the representations were coextensive, but the contralateral map was larger and more robust. However, retrograde tracer experiments also showed important differences. Fewer neurons projected to the ipsilateral spinal cord than the contralateral, and these were more widely scattered throughout the motor cortex in the uninjured hemisphere. The contralateral representation, but not the ipsilateral representation, was clearly divided into two areas: the rostral forelimb motor area and the caudal forelimb motor area. Thus, the representation of the ipsilateral forelimb covers a larger area but is sparser that the contralateral. Conclusion: The

differences in the representation of each forelimb suggest a substrate for independent control that may be targeted for therapy.

Disclosures: T. Wen: None. **D. Gupta:** None. **J.B. Carmel:** None.

Poster

542. Plasticity of Voluntary Movements

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 542.18/NN19

Topic: D.17. Voluntary Movements

Support: SPH Faculty Research Grant Program

SPH Student Research Grant

Title: The effect of motor point associative stimulation and tDCS on manual dexterity and neurophysiology of the motor cortex

Authors: *N. HOSEINI, H. J. BLOCK
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Abstract: Manual dexterity, the ability to manipulate objects with the hands, is often impaired after stroke. Proprioception (position sense), which is critical for manual dexterity, is also frequently affected. Associative stimulation of motor points (MPAS) in hand muscles both modulates motor cortex excitability and improves manual dexterity (McDonnell & Ridding 2006). However, it is not known if the effect of this peripheral stimulation can be improved by central stimulation of sensorimotor cortex with transcranial direct current stimulation (tDCS). tDCS is a noninvasive brain stimulation technique in which a small electric current is passed through the head via two sponge electrodes. The current flow modulates cortical excitability in the target area in a polarity-specific way, resulting in measurable effects on behavior and neurophysiology. Here we investigated the effect of MPAS, with and without tDCS over sensorimotor cortex, on motor cortical neurophysiology, manual dexterity, and proprioceptive acuity in healthy adults. These measures were assessed pre and post-intervention with transcranial magnetic stimulation (TMS), the Purdue Pegboard Test, and an adaptive staircase method, respectively. Pilot data from 5 subjects supports the idea that MPAS increases motor cortex excitability. The TMS intensity needed to evoke a muscle twitch of 1mV amplitude pre-MPAS was compared with post-TMS. On average (±SE), muscle twitch amplitude increased by

0.55 ±0.35 mV after real MPAS, compared to only 0.03 ±0.20 mV after sham (asynchronous) MPAS. Preliminary results from 4 subjects who completed two experimental sessions each (MPAS with Sham tDCS vs. MPAS with excitatory (Anodal) tDCS) suggest that anodal tDCS may increase the effect of MPAS on motor cortex. After MPAS/Anodal tDCS, the slope of the TMS input/output curve increased 50 ± 25%, indicating greater recruitment of motor cortical neurons for a given TMS intensity, vs only 34 ± 21% after MPAS/Sham tDCS. Anodal tDCS may also increase the effect of MPAS on manual dexterity: average time to place 20 pegs in the Pegboard task decreased 1.67 ± 0.76 s after MPAS/Anodal tDCS, vs. only 0.78 ± 0.73 s after MPAS/Sham tDCS. While very preliminary, these results suggest the possibility that MPAS in combination with tDCS over sensorimotor cortex may be a stronger intervention than MPAS alone. Since proprioception is an integral part of manual dexterity, interventions that influence sensory as well as motor pathways may be more likely to improve manual dexterity in stroke patients. MPAS does so peripherally, while tDCS applied over sensorimotor cortex does so centrally. The combination may have benefits for sensorimotor function.

Disclosures: N. Hoseini: None. **H.J. Block:** None.

Poster

542. Plasticity of Voluntary Movements

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 542.19/NN20

Topic: D.17. Voluntary Movements

Support: CIHR MOP-130269

Title: Myelin water fraction imaging to evaluate white matter content changes after upper-limb training in a semi-immersive virtual reality environment

Authors: *B. LAKHANI[1], J. N. JACKSON[1], M. R. BORICH[2], A. L. MACKAY[1], L. A. BOYD[1]
[1]Univ. of British Columbia, Vancouver, BC, Canada; [2]Emory Univ., Atlanta, GA

Abstract: Background: It is well accepted that the brain undergoes numerous structural and chemical changes when learning a novel motor skill. Neuroimaging studies that evaluate these changes have primarily focused on changes in grey matter of the central nervous system (CNS). However, data characterizing white matter, which consists of myelinated tracts that transfer signals across the CNS, has been limited. Multi-component T_2 relaxation imaging (MCRI) can

quantify the proportion of water trapped between lipid bilayers of myelin to provide an objective, validated marker of white matter myelin content (myelin water fraction or MWF). To date, there is no research that has attempted to longitudinally quantify changes in myelin content in response to motor skill learning. Therefore, the objective of the current study is to explore the relationship between changes in MWF and acquisition of a novel upper-limb motor task in healthy young adults. **Methods:** Fifteen, right hand dominant, healthy young adults (26 ± 4 years old; 8 female) underwent magnetic resonance imaging (MRI) prior to and within 24 hours of the last of ten sessions of upper-limb training in a semi-immersive virtual reality environment using the Microsoft Kinect© gaming system. The training was conducted over a four-week period and each training session consisted of five blocks of 200 skilled motor movements with the right arm used to control an avatar displayed in a 2-dimensional environment on a monitor. The goal was to intercept and release a moving target. Participants progressed in difficulty levels upon achieving at least an 80% success rate in two consecutive training blocks. Differences in MWF following training were assessed in a priori regions of interest (posterior limb of the internal capsule (PLIC), intraparietal sulcus (IPS), whole-brain normal appearing white matter (NAWM)). **Results:** There was a significant reduction in mean time to target interception (movement time) between the first and last training sessions (**Δ**=698ms). Preliminary results to date indicate that, following training, the % MWF change (post-pre) increased in the left IPS (**Δ**=7.08%;) but not in the right IPS (**Δ**=-1.40%), left PLIC (**Δ**=-1.93%), right PLIC (**Δ**=0.89%), NAWM (**Δ**=0.12%). **Conclusions:** Results from this study indicate that MWF in task-related regions of interest may be modifiable following practice of a skilled motor task in healthy young adults. The outcomes from this work may have important implications for neurological populations (e.g. stroke) that may benefit from therapeutic interventions that can selectively increase white matter myelin content in an effort to improve rehabilitation outcomes.

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Poster

542. Plasticity of Voluntary Movements

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Program#/Poster#: 542.20/NN21

Topic: D.17. Voluntary Movements

Support: NIH Grant 1R43NS084566-01

Title: Vagus nerve stimulation dependent enhancement of cortical plasticity requires cholinergic innervation of the cortex

Authors: *D. HULSEY[1], S. HAYS[1], N. KHODAPARAST[1], R. CASAVANT[2], A. RUIZ[1], P. DAS[1], E. NUTTING[1], X. CARRIER[1], M. IYENGAR[1], I. QUARESHI[1], S. SULTANA[1], R. RENNAKER, II[1], M. KILGARD[1]
[1]The Univ. of Texas At Dallas, Richardson, TX; [2]Microtransponder Inc., Austin, TX

Abstract: Primary motor cortex (M1) transiently reorganizes in response to motor skill learning. Pairing forelimb movements with Vagus Nerve Stimulation (VNS) drives enhanced and robust analogous plasticity within M1. These changes occur outside of the typical period for motor plasticity and are independent of new skill learning. The mechanism by which VNS enhances M1 plasticity is not well understood. Skill learning and the associated cortical plasticity is dependent on cholinergic innervation of the cortex. VNS may enhance plasticity by engaging neuromodulatory systems necessary for plasticity. We hypothesize that cholinergic innervation of M1 is necessary for motor plasticity associated with VNS pairing. To test this hypothesis, we trained female Sprague Dawley rats on a skilled lever pressing task emphasizing use of the proximal forelimb. After task acquisition, one group of rats received a lesion to the cholinergic neurons of the basal forebrain using 192-IgG-Saporin, while another group received a control injection. All subjects also received a VNS cuff implant during the surgery. After one week of recovery, all subjects receive VNS paired to successful task performances for five days. Intracortical microstimulation was performed to derive M1 maps of each group 24 hours after their final VNS paired session. Subjects with an intact cholinergic system show significant expansion of proximal forelimb representation over naïve animals within the cortex. Subjects without cholinergic innervation of the cortex show no difference in M1 organization when compared to naïve animals. We conclude that cholinergic innervation is necessary for the effects of VNS on motor plasticity.

Disclosures: **D. Hulsey:** None. **S. Hays:** None. **N. Khodaparast:** A. Employment/Salary (full or part-time):; Microtransponder Inc. **R. Casavant:** A. Employment/Salary (full or part-time):; Microtransponder Inc.. **A. Ruiz:** None. **P. Das:** None. **E. Nutting:** None. **X. Carrier:** None. **M. Iyengar:** None. **I. Quareshi:** None. **S. Sultana:** None. **R. Rennaker:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Vulintus Inc. **M. Kilgard:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Microtransponder Inc..

Poster

542. Plasticity of Voluntary Movements

Location: Halls A-C

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Program#/Poster#: 542.21/NN22

Topic: D.17. Voluntary Movements

Support: USAMRAA W81XWH-10-1-1020

USAMRAA W81XWH-13-1-0496

Title: Amputation-related changes in inter-hemispheric interactions are reversible through transplantation of the human hand

Authors: *K. F. VALYEAR[1], B. A. PHILIP[1], C. L. KAUFMAN[2], J. E. KUTZ[2], S. H. FREY[1]
[1]Dept. of Psychological Sci., Univ. of Missouri, Columbia, MO; [2]Christine M. Kleinert Inst., Louisville, KY

Abstract: Use of the intact hand by unilateral amputees is associated with increased activity in both the contralateral (intact) and ipsilateral (former) sensorimotor hand territories. Ipsilateral responses are believed to reflect reductions in normal levels of inter-hemispheric inhibition maintained by activity-dependent, transcallosal, GABAergic pathways. Former amputees who have undergone hand transplantation provide a unique opportunity to address whether these activity-dependent reorganizational changes can be reversed. If so, then following regeneration of peripheral nerves, movements of the intact (non-transplanted) hand should exhibit the same pattern as healthy adults, primarily activating contra- but not ipsilateral sensorimotor cortex. Here, we used functional MRI to map cortical sensorimotor representations in patient DR, a right-hand dominant male who suffered traumatic amputation of his left hand proximal to the wrist in 1998 (age 23) and underwent hand transplantation 13 years later (age 36). Patient DR completed the same protocol 15 and 26 months post-transplant. At 15 months post-transplant, DR showed a pattern of activation similar to amputees: specifically, he activated both contra- and ipsilateral sensorimotor cortices when moving his intact hand. However, when tested at 26 months post-transplant, patient DR showed a pattern of activation similar to controls: specifically, he activated only contralateral (but not ipsilateral) sensorimotor cortex when moving his intact hand. Between 15 and 26 months post-transplant, DR exhibited substantial improvements in hand functions that reflect regeneration of peripheral sensory and motor nerves. These results are consistent with the hypothesis that amputation-related changes in the normal inhibitory balance between sensorimotor cortices can be reversed when afferent and efferent activity between hand and brain is restored. The fact that this was observed in a fully mature brain has potentially important implications for understanding the role of experience in recovery from injuries to the central and/or peripheral nervous systems.

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Poster

542. Plasticity of Voluntary Movements

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 542.22/NN23

Topic: D.17. Voluntary Movements

Title: Anodal transcranial direct current stimulation (tDCS) enhances prolonged motor skill learning in chronic stroke patients

Authors: M. HAMOUDI[1], H. M. SCHAMBRA[2], L. G. COHEN[3], A. SCHOECHLIN-MARX[1], B. FRITSCH[1], *J. REIS[1]
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Abstract: Background: Anodal tDCS applied over M1 can enhance motor skill learning in healthy subjects1. However, the effects of tDCS on specific temporal subcomponents of motor skill learning have not been investigated in stroke patients. Here we study the effects of anodal tDCS on acquisition (online effects), consolidation (offline effects) and long term retention of a visuomotor skill as well as general motor function in other tasks in the chronic phase after stroke. Methods: Thirty-six patients with chronic unilateral, first ever ischemic stroke and a mild to moderate hemiparesis (UEFMS ~ 58) participated. They practiced the sequential visual isometric pinch force task (SVIPT)1 for 5 consecutive days with their paretic hand while receiving either anodal tDCS (n=18) or sham tDCS (n=18) for 20 minutes over M1 of the affected hemisphere. Follow ups were performed at day 8, 29, 57, 85 and 113. A skill measure based on movement time and error rate was developed. The Jebsen Taylor hand function test (JTT) and the Grooved Pegboard Test (GPT) were performed to test for general enhancement of motor function. A control group (n=12 to date) performed the JTT and GPT only, without stimulation or training to measure improvements on these tasks due to repeated assessment. Results: In general, patients became more skilled in the task. There were significantly greater total skill gains with anodal tDCS compared to sham, mainly mediated through online effects. The skill advantage of the anodal tDCS group was due to significantly faster movement times compared to sham, achieved at similarly increasing accuracy. First results of the follow ups indicate that anodal tDCS did not

change the retention rate relative to sham, hence skill remained superior with anodal tDCS in the long term. The interim analysis of JTT and GPT data suggests that training also enhanced performance on other motor tasks, with anodal tDCS showing the greatest improvements. Only minor improvements were observed in the control group. Conclusions: Our preliminary results suggest that anodal tDCS combined with 5 days of motor training can facilitate motor skill learning in chronic stroke patients. Training itself was successful to induce general enhancements in motor function, a process that may be further enhanced by anodal tDCS. These findings could be directly transferable to neurorehabilitative treatments of stroke patients as a strategy to break behavioral ceiling effects. 1.Reis et al., PNAS 2009;106(5):1590-5.

Disclosures: **M. Hamoudi:** None. **J. Reis:** None. **B. Fritsch:** None. **A. Schoechlin-Marx:** None. **H.M. Schambra:** None. **L.G. Cohen:** None.

Poster

542. Plasticity of Voluntary Movements

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Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 542.23/NN24

Topic: D.17. Voluntary Movements

Support: DFG RE 2740/3-1

Title: Motor skill learning in the acute phase of stroke: Does training-induced plasticity surpass spontaneous biological recovery?

Authors: P. PAPE, A. SCHÖCHLIN-MARX, *B. FRITSCH, J. REIS
Univ. of Freiburg/ Neurocenter, Freiburg, Germany

Abstract: Objective: Stroke is the leading cause of disability in adults. Here, we aimed to reveal mechanistic information about the pathology of motor skill learning in the acute phase after stroke that will contribute to the understanding of plasticity after brain injury. We characterized both the speed-accuracy-tradeoff (SAF) of a visuomotor task as well as motor skill learning in acute stroke patients with mild to moderate hemiparesis in comparison to age-matched healthy controls. Furthermore, we distinguished learning effects from of spontaneous biological recovery (SBR) in patients. Methods: In patients with acute, unilateral, first-ever ischemic stroke causing a moderate hemiparesis (no neglect, no apraxia, no aphasia) we measured the SAF on day 1 and day 5 after stroke. The change from the initial SAF (day 1) over the course of early spontaneous recovery (clinical treatment as usual, SAF retest on day 5, n=14) was compared to the SAF

change in patients receiving specific motor skill training on days 2-4 (n=10). A patient group tested on the SAF only once on day 5 (n=13) served as a second control for task repetition effects and SBR. Furthermore, SAF shift and motor skill learning were compared between the trained patients and a group of trained, healthy age-matched controls (n=12). Results: All patients showed similar initial impairment (UEFMS 56 ±2, NIHSS 5±1). Compared to the patient group without training (SBR), trained subjects showed a significantly greater shift in the SAF, i.e. lower target error rates at predefined speeds. This shift was distinguishable from SBR and task repetition effects. Compared to healthy controls, the initial SAF was shifted towards higher error rates in patients, but the training induced shift was similar. In accordance, patients had a significantly poorer baseline skill on the training task, but motor skill increased similarly in both groups and was preserved at a 1 month follow up. Nearly all trained patients had returned to maximum UEFMS at the 1 month follow-up. However, there was no further shift, i.e. improvement, in the SAF curve indicating a remaining skill deficit compared to healthy subjects. Conclusions: Our preliminary data suggest that motor learning ability in acute stroke patients is preserved. Motor skill changes as assessed by the shift of the SAF curve in trained patients seem to exceed the shift observed with spontaneous biological recovery. The findings emphasize the effectiveness of specific motor skill training during the first days of admission to a stroke unit to enhance fine motor function. 1.Reis et al., PNAS 2009;106(5):1590-5.

Disclosures: **P. Pape:** None. **B. Fritsch:** None. **J. Reis:** None. **A. Schöchlin-Marx:** None.

Poster

542. Plasticity of Voluntary Movements

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 542.24/NN25

Topic: D.17. Voluntary Movements

Title: Cortical and behavioral correlates of a conditioning protocol that combines action observation with peripheral electrical nerve stimulation

Authors: *A. BISIO[1], L. AVANZINO[1], N. GUEUGNEAU[1], T. POZZO[2], P. RUGGERI[1], M. BOVE[1]
[1]Univ. Genoa, Genova, Italy; [2]Italian Inst. of Technol., Genoa, Italy

Abstract: Action observation (AO) is known to affect the activity of the primary motor cortex (M1). However, the AO effects may vanish if motor practice is not concurrent or immediately follows it. This suggests that a prompt comparison between the visual and the somatosensory

representations of movement could be necessary to induce plasticity in M1. The aim of this study was to test whether action observation combined with a peripheral nerve electrical stimulation was able to evoke cortical plastic changes in the left M1. To do that we proposed a stimulation protocol (AO-PNS) where the observation of a video showing repetitive thumb-index tapping movements was combined with electrical stimuli delivered on the right peripheral median nerve, i.e., the nerve innervating the abductor pollicis brevis (APB) muscle (the target muscle of the video and electrical stimulation). In the first part of this work, left M1 excitability, measured by mean of transcranial magnetic stimulation (TMS-recruitment curve), was compared with that assessed after action observation (AO) and peripheral electrical median nerve stimulation (PNS) alone. Then, we tested the long-term effects of AO-PNS by recording MEP amplitudes in the APB muscle before (PRE), immediately after and 15, 30, and 45 minutes after the stimulation protocol. Finally, the effects of AO-PNS on the MEPs amplitudes of the right APB muscle were compared with those elicited in the adductor digiti minimi (ADM) muscle to assess the topographic specificity of the AO-PNS. Results showed that left M1 excitability increased only after AO-PNS whereas the effects of AO and PNS alone on M1 excitability vanished immediately after video observation. Notably, the increased M1 excitability was still present 45 minutes after the AO-PNS protocol administration and was specific for the stimulated muscle - APB. Thus, we showed that plasticity in M1 can be induced by the activation of the mirror neuron system but only in an associative context (e.g., afferent signals from periphery). Looking at these findings, one might speculate that M1 plasticity induced by AO-PNS could have a behavioral correspondence dealing with changes in motor behavior such as an increased efficiency in finger-tapping movements. For that reason, in the second part of the work, we are testing the motor performance of two groups of participants who performs a finger tapping movement before and after AO and AO-PNS. Following the results of the first part of the experiment, we predict that after AO-PNS the motor efficiency, in term of movement frequency measured 45 minutes after the conditioning protocol, will increased than after AO alone.

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Poster

542. Plasticity of Voluntary Movements

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Topic: D.17. Voluntary Movements

Support: USPHS NIH R01 HD061462

NIDRR H133P100014

AHA 14CRP18150008

Title: Neuroplasticity within repetitive robotic arm training changes with age

Authors: *C. L. MASSIE[1], S. S. KANTAK[3], P. NARAYANAN[2], G. F. WITTENBERG[2]
[1]Physical Therapy and Rehabil. Sci., [2]Neurol., Univ. of Maryland Sch. of Med., Baltimore, MD; [3]Moss Rehabil. Res. Inst., Elkins Park, PA

Abstract: Use-dependent neuroplasticity is an important factor for stroke rehabilitation, yet the potential is considered to decrease with age. The objective of the study was to determine how age influences the degree of neuroplasticity during repetitive robotic arm training. Ten young and 14 older adults participated with mean ages of 26 and 66, respectively. Participants completed 480 movements in a robotic reaching intervention. Transcranial magnetic stimulation (TMS) was used as a neurophysiologic assessment before, during, and after the reaching training. Ten suprathreshold (120% of resting motor threshold) stimulations were delivered and motor evoked potentials (MEP) were recorded from the biceps, triceps, anterior deltoid, and posterior deltoid. The amplitude and direction of evoked movements were also recorded by the robot. Change scores from baseline were calculated and analyzed with an ANOVA (time by age). Biceps and anterior deltoid MEP were significantly different between age groups ($p < 0.05$), but not the triceps or posterior deltoid ($p>0.05$). There was a trend for a significant interaction for the amplitude of TMS evoked movements with the older adults generally having smaller percent changes over time compared to the increases in younger adults. The direction of evoked movement changes were similar across age groups. These results suggest that neuroplasticity is influenced, not limited, by age within an intervention and demonstrates the importance of understanding dosing implications within the potential use of robotic training in older adults.

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Poster

542. Plasticity of Voluntary Movements

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Topic: D.17. Voluntary Movements

Support: NSERC Discovery Grant

Canadian Foundation of Innovation Grant

Title: Short-term plasticity in human motor cortex is associated with changes in cerebral blood flow delivery

Authors: R. HERMOSILLO, K. FJELD, T. BURNETT, D. CHENG, F. COLINO, G. BINSTED, *P. VAN DONKELAAR
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Abstract: Our understanding of the neural correlates of sensorimotor plasticity has increased substantially over the past two decades. Previous studies examining this issue has typically used neural imaging to measure correlates of alterations in neuronal activity. However, it is unclear whether these alterations are associated with changes in cerebral blood flow delivery. Repetitive transcranial magnetic stimulation (rTMS) has been used widely to transiently disrupt function in the brain. Previous studies examining the effect of rTMS on the brain have shown both neuronal and behavioural disruptions. However, it is not currently known how neurons respond to the stimulation, and furthermore if the plastic changes are accompanied by changes in cerebral blood flow. In the present experiment, we probed this relation by quantifying how rTMS alters motor maps in the primary motor cortex, while simultaneously measuring cerebral blood flow in the middle cerebral artery using transcranial Doppler ultrasound. We sought to observe whether these motor changes were accompanied by a corresponding change in cerebral flow. Motor mapping was carried out prior to and after a 30 minute session of either 1Hz real rTMS or sham rTMS. After real TMS, cortical excitability increased compared to sham and this was associated with an alteration in blood flow. Interestingly, some sites within the motor map that were previously unresponsive became responsive after real TMS. This suggests that the short-term cortical changes due to rTMS induce changes in cerebral blood flow delivery.

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Poster

542. Plasticity of Voluntary Movements

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 542.27/NN28

Topic: D.17. Voluntary Movements

Support: ERASMUS Grant

Title: Physical training improves brain connectivity

Authors: *H. E. HULSHOFF POL[1], A. SVÁTKOVÁ[2], R. MANDL[1], T. SCHEEWE[1], R. KAHN[1], W. CAHN[1]
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Abstract: There are indications that keeping your body fit is healthy for your brain. While learning a new skill causes structural changes in the brain, it is unclear whether continuous physical training of learned skills can improve structural brain connectivity. We measured connectivity of the brain before and after a six months interval on diffusion tensor images obtained at 3 Tesla MRI. Changes in connectivity over the interval were measured by the difference in the fractional anisotropy map at time 2 minus the map at time 1, as analyzed using family-wise corrected TBSS and by white matter fiber reconstructions based on FSL. A total of 81 young adult individuals participated, of which 48 were healthy and 33 had a diagnosis of schizophrenia. Individuals were assigned to either one of the two conditions, i.e., physical exercise or life-as-usual, balanced for diagnosis. Physical exercise involved supervised indoor trainings twice weekly, and included biking. With an estimated 1,3 bikes owned per person over four years of age, biking is a highly overlearned skill in the inhabitants of The Netherlands. We report that 6 months of regular physical exercise including bicycling significantly increases the integrity of motor-functioning related brain white matter fibers whereas life-as-usual leads to a decrease in fiber integrity, irrespective of diagnosis. Thus keeping the body fit through continuous physical stimulation improves the brains' wiring in health and disease. Indeed, brain connectivity improves from repeated physical exercise.

Disclosures: H.E. Hulshoff Pol: None. **A. Svátková:** None. **R. Mandl:** None. **T. Scheewe:** None. **R. Kahn:** None. **W. Cahn:** None.

Poster

542. Plasticity of Voluntary Movements

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 542.28/NN29

Topic: D.17. Voluntary Movements

Title: Targeting the right dorsolateral prefrontal cortex with transcranial direct current stimulation during a concurrent implicit and explicit sequence task

Authors: *B. GREELEY[1], R. SEIDLER[1,2]
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Abstract: There is an ongoing debate concerning whether implicit and explicit systems interact during motor learning. One line of behavioral and neuroimaging evidence suggests that implicit learning occurs independently of explicit knowledge (Song et al., 2005; Destrebecqz et al., 2005). However, another line of both behavioral and neuroimaging evidence suggests instead that implicit and explicit learning mechanisms interact (Boyd & Winstein, 2004; Aizenstein et al., 2004). Here, we attempt to elucidate the relationship between implicit and explicit learning by using transcranial direct current stimulation (tDCS), a non-invasive form of brain stimulation, while participants engage in a concurrent implicit and explicit sequence motor learning task. The prefrontal cortex is engaged during the cognitively demanding early stages of motor learning (Doyon & Benali., 2005). Anguera and colleagues (2009) found that the right dorsolateral prefrontal cortex (DLPFC) was involved in early learning, but not in late learning of visuomotor and spatial working memory tasks. The right DLPFC has also been shown to be involved in explicit learning paradigms (Destrebecqz et al., 2005; Fletcher et al., 2005; Hazeltine el al., 1997; Toni et al., 1998). In the current study, all participants will complete a concurrent implicit and explicit motor sequence learning task similar to that used by Aizenstein and colleagues (2007) and be placed in one of three tDCS groups. One group of participants is receiving tDCS targeting the right DLPFC, another group is receiving tDCS targeting the left DLPFC, and the third group is receiving sham tDCS. We anticipate that participants in the right DLPFC tDCS group will demonstrate a faster rate of learning early in the explicit, but not in the implicit sequence learning trials. Preliminary results demonstrate that the concurrent implicit and explicit sequence task results in simultaneous learning of implicit and explicit sequences. This study contributes to a greater understanding of motor learning and will help discriminate implicit and explicit motor learning systems.

Disclosures: B. Greeley: None. **R. Seidler:** None.

Poster

543. Luteinizing Hormone Secretion, Puberty, and Seasonality

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 543.01/NN30

Topic: E.01. Neuroendocrine Processes

Support: NIH DK096541-01A1

NSF REU DBI-0754293

Title: Immunolesions of melanopsin receptive neurons in the adult Pekin drake attenuates the hormonal reproductive axis

Authors: *G. S. FRALEY
Hope Col., HOLLAND, MI

Abstract: Several light sensitive receptors have been described in the avian brain that are thought to regulate the reproductive axis independently from the eyes and pineal gland. Recently, my lab has described the presence of three of these photoneuroendocrine systems in the Pekin duck: opsin, opsin 5, and melanopsin. I set out to test the hypothesis that melanopsin receptive neurons are necessary to maintain seasonal reproductive status in the Pekin drake. To accomplish this, 50-week-old Pekin drakes were housed in the aviary at Hope College under long day length (18 hrs lights on) conditions in floor pens (5 drakes per pen). To specifically lesion melanopsin-receptive neurons, drakes were anethestized (8 mg/kg Propofol, IV), given analgesics (2 mg/kg ketfen, SC) skin incised and a trephine hole drilled 10 mm caudal to bony orbits and 1 mm to the left of midline. A 33 gauge stainless steel needle attached to a Hamilton syringe was lowered stereotactically 3.5 mm ventral to dura into the lateral ventricle. Three microliters of an anti-melanopsin-saporin conjugate (MSAP, 100 ng/ul) was injected into the lateral ventricle (n = 10). Control drakes were injected with 3 ul of equimolar unconjugated anti-melanopsin and saporin (SAP, n = 10). The incision was closed with VetBond, and the drakes returned to the aviary after complete recovery from anesthesia. After 4 weeks, birds were euthanized (400 mg/kg FatalPlus, IP) and body weight measured, and brains, pituitaries, and testes collected and stored for analyses. MSAP-treated drakes had significantly ($p < 0.001$) reduced relative teste weights compared to SAP controls. qRT-PCR analyses (n = 5 per treatment) of anterior pituitary showed a significant reduction ($p < 0.001$) in both LH-beta and FSH mRNA's. Immunoctyochemical analyses (n = 5 per treatment) showed a significant reduction in melanopsin and GnRH-immunoreactivities. These data underscore the importance of the photoneuroendocrine system in maintaining the reproductive axis in seasonally breeding birds.

Disclosures: G.S. Fraley: None.

Poster

543. Luteinizing Hormone Secretion, Puberty, and Seasonality

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 543.02/NN31

Topic: E.01. Neuroendocrine Processes

Support: NIH R01 HD17864 to R.L.G

Title: Season- and steroid-dependent regulation of D2 dopamine receptor, kisspeptin, and neurokinin B in KNDy cells of the ovine arcuate nucleus

Authors: *P. W. WEEMS[1], R. L. GOODMAN[4], L. M. COOLEN[2], M. N. LEHMAN[3]
[1]Neurosci., [2]Physiol. & Biophysics, [3]Neurobio. & Anatom. Sci., Univ. of Mississippi Med. Ctr., Jackson, MS; [4]Dept. of Physiol., West Virginia Univ., Morgantown, WV

Abstract: Seasonal reproduction in sheep is a result of a dramatic increase in the ability of estradiol (E_2) to inhibit the pulsatile secretion of gonadotropin-releasing hormone (GnRH) during the non-breeding season (anestrus). Recent work suggests that a key intermediary in the negative feedback influence of E_2 is a neuronal subpopulation in the arcuate nucleus (ARC) that co-expresses kisspeptin (Kiss), neurokinin B (NKB), and dynorphin (termed KNDy neurons). The pathway responsible for E_2 negative feedback in anestrus includes dopaminergic projections from the retrochiasmatic area to the ARC. Dopamine released from these afferents binds to D2 dopamine receptors (D2R) on KNDy cells to decease Kiss release and thus inhibit GnRH pulses during anestrus. However, while ARC Kiss is reduced by E_2 during anestrus, the effects of E_2 on expression of NKB or receptors in these cells have not been examined. In the present study we examined the influence of season and E_2 on the regulation of D2R, Kiss and NKB in KNDy neurons of the ewe. Adult Suffolk ewes were ovariectomized (OVX) (n=16). Following surgery, half of the ewes (n=8) received a s.c. capsule containing E_2 (OVX+E). Brains were collected from OVX and OVX+E ewes perfused either during anestrus (May) or breeding season (November) (n= 4/season/group). Coronal sections through the middle ARC were processed for triple-label immunocytochemistry for D2R, Kiss and NKB, and the number of single- double- and triple-labeled neurons were counted in each animal. We found that OVX+E ewes had significantly lower numbers of D2R, Kiss, and NKB cells compared to OVX ewes, in both the breeding season and anestrus. Thus, E_2 inhibits D2R, Kiss, and NKB expression at both times of years. In addition, OVX+E animals showed a seasonal decrease in both Kiss and NKB during anestrus, but this did not alter the high degree of colocalization of these two peptides. The percentage of KNDy neurons containing D2R increased in OVX+E anestrous ewes, but this was due to the decrease in Kiss and NKB expression because the number of KNDy neurons containing D2R did not change seasonally. Thus, it appears that changes in D2R may not contribute to the increased ability of E_2 to inhibit GnRH pulses during anestrus. These results are consistent with the hypothesis that increased dopamine release during anestrus, rather than an increased response to dopamine, is responsible for inhibiting Kiss and NKB from KNDy

neurons. Thus, both of these stimulatory neuropeptides could play a role in seasonality in the ewe.

Disclosures: P.W. Weems: None. **R.L. Goodman:** None. **L.M. Coolen:** None. **M.N. Lehman:** None.

Poster

543. Luteinizing Hormone Secretion, Puberty, and Seasonality

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 543.03/NN32

Topic: E.01. Neuroendocrine Processes

Support: NIH Grant RO1 HD039916

Title: The stimulatory effect of Neuromedin U on pulsatile LH secretion: Insights from a seasonal mammal

Authors: *P. GRACHEV, R. B. MCCOSH, J. A. LOPEZ, G. L. NESSELROD, M. VALENT, S. L. HARDY, J. M. CONNORS, S. M. HILEMAN, R. L. GOODMAN
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Abstract: Central signaling mechanisms involving the activation of the neuromedin U receptors type 1 (NMU1R) and type 2 (NMU2R) by their endogenous ligands, neuromedins U (NMU) and S (NMS), have been shown to regulate LH secretion, the timing of puberty, appetite, food intake and energy expenditure. NMU, NMS and NMU1R and NMU2R are highly conserved through evolution; however previous studies have relied almost exclusively on rodent models. Although NMS is predominantly expressed within the suprachiasmatic nucleus of the hypothalamus (SCN), its role in photoperiod regulation of reproduction in seasonal mammals remains yet to be elucidated. As a first step in exploring this question, we treated anestrous ewes with NMU-8 (1 nmol, intracerebroventricularly), a synthetic peptide the sequence of each resembles the receptor-binding portion of both, endogenous sheep NMU and NMS, and subjected them to frequent blood sampling to determine the effect of hypothalamic NMU2R activation on pulsatile LH secretion. NMU-8 administration triggered single LH pulses and increased LH secretion (area under the curve) more than 3.5-fold ($P<0.05$) in the 48-min period following administration compared to vehicle (100 μl saline, intracerebroventricularly). These data demonstrate that acute activation of central NMU1R and/or NMU2R stimulates hypothalamic circuitry involved in GnRH pulse generation and provide the first insight into the roles of NMU/NMS in this species.

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Poster

543. Luteinizing Hormone Secretion, Puberty, and Seasonality

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 543.04/NN33

Topic: E.01. Neuroendocrine Processes

Support: R01 HD039916

Title: Kappa opioid receptor is present within a majority of KNDy neurons in the ewe

Authors: ***C. F. WITTY**[1], P. W. WEEMS[2], R. L. GOODMAN[4], L. M. COOLEN[1,3], M. N. LEHMAN[3]
[1]Physiol. & Biophysics, [2]Grad. Program in Neurosci., [3]Neurobio. & Anatom. Sci., Univ. of Mississippi Med. Ctr., Jackson, MS; [4]Physiol. & Pharmacol., West Virginia Univ., Morgantown, WV

Abstract: Kisspeptin-Neurokinin B-Dynorphin (KNDy) cells of the arcuate nucleus (ARC) of the hypothalamus have been proposed as a critical component regulating pulsatile release of gonadotropin-releasing hormone (GnRH) neurons, including the negative feedback control of GnRH pulses by estradiol and progesterone. Current evidence in sheep and goats suggest that dynorphin released by KNDy cells acts as a “stop” signal for the pulse generator, and terminates each GnRH pulse by acting upon kappa opioid receptors (KOR). However, the precise localization of KOR, and whether they are present in KNDy cells in the sheep, is unknown. We first determined the distribution of KOR in the ovine hypothalamus using a KOR antibody whose specificity we confirmed by Western blot analyses and blocking peptide controls. Tissue sections from gonadal-intact ewes perfused during the luteal phase of the estrous cycle (n = 4) were processed for single-label immunoperoxidase detection and revealed KOR-positive cells in a number of hypothalamic regions, including the preoptic area (POA), the paraventricular nucleus (PVN), and the arcuate nucleus (ARC), areas which also contain KOR mRNA. In a second set of experiments, we determined whether KOR is co-expressed in KNDy cells in the ewe hypothalamus. Tissue sections from the same luteal-phase ewes (n = 4) were processed for dual-label immunofluorescent detection of KOR and neurokinin B (NKB). Confocal microscopic analyses showed a high degree of co-localization, with 94% of NKB cells containing KOR,

while 46% of KOR cells in the ARC were double-labeled for NKB. In conclusion, a large majority of KNDy cells in the sheep ARC co-localize KOR. In addition, there are also a sizable proportion of KOR-positive cells in the ARC which do not contain NKB, and hence represent a separate subpopulation from KNDy cells. We suggest that dynorphin may exert its inhibitory control of GnRH pulse frequency by binding to postsynaptic KOR either within KNDy cells (autoregulatory actions), or by actions upon adjacent non-KNDy ARC neurons, which, in turn, may project onto KNDy and/or GnRH neurons.

Disclosures: C.F. Witty: None. **P.W. Weems:** None. **R.L. Goodman:** None. **L.M. Coolen:** None. **M.N. Lehman:** None.

Poster

543. Luteinizing Hormone Secretion, Puberty, and Seasonality

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 543.05/NN34

Topic: E.01. Neuroendocrine Processes

Support: USDA 2013-00896

Title: Is dynorphin involved in the prepubertal suppression of LH secretion by estradiol in female sheep?

Authors: J. A. LOPEZ[1], L. J. MEADOWS[1], R. B. MCCOSH[1], *R. L. GOODMAN[2], S. M. HILEMAN[1]
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Abstract: Neural mechanisms underlying the onset of puberty are not well understood. In sheep, this process involves an increase in GnRH release due to decreased sensitivity to estradiol (E2)-negative feedback. As GnRH neurons do not express the relevant estrogen receptors, this pathway must involve interneurons. Recent data suggests that neurons of the arcuate nucleus of the hypothalamus that coexpress kisspeptin (kiss), neurokinin B, and dynorphin (DYN), i.e. KNDy neurons, may play a critical role. Kiss and neurokinin B are stimulatory to GnRH, while DYN is inhibitory. Thus, we hypothesized that kiss expression would be positively associated, and DYN expression negatively associated, with LH secretion in prepubertal female sheep. Ewe lambs were randomly assigned to three groups: ovariectomized (OVX), OVX+E2, or left ovary-intact. Jugular blood samples were collected every 12 min for 4h to assess luteinizing hormone (LH) secretion. As expected, mean LH and LH pulse frequency were increased by ovariectomy

compared to either ovary-intact or OVX+E2 lambs. As hypothesized, numbers of kiss-immunopositive cells were increased by OVX compared to either ovary-intact or OVX+E2 lambs. Contrary to our hypothesis, very few DYN-immunopositive cells were found in any treatment group, even though they were readily evident in concurrently immunostained hypothalamic tissue collected from adult ewes in the luteal phase of their estrous cycle. This may be due to either reduced expression of DYN or high rates of DYN secretion during the prepubertal period. Further work will be necessary to distinguish between these two possibilities.

Disclosures: J.A. Lopez: None. **L.J. Meadows:** None. **R.B. McCosh:** None. **R.L. Goodman:** None. **S.M. Hileman:** None.

Poster

543. Luteinizing Hormone Secretion, Puberty, and Seasonality

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 543.06/NN35

Topic: E.01. Neuroendocrine Processes

Support: Health Research Council in New Zealand

Title: Neuronal insulin signaling is not required for reproductive maturation or competency in mice

Authors: *M. C. EVANS, G. M. ANDERSON
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Abstract: Central insulin signaling plays a critical role in the metabolic control of fertility. Mice exhibiting insulin receptor (InsR) deletion from all neural progenitor cells (NIRKO mice) display hypothalamic hypogonadism and reduced fertility. However, the neuronal population(s) critically involved in mediating insulin's central effects on the neuroendocrine reproductive axis remain largely unidentified. To date, the targeted deletion of InsR's from any neuronal population tested has failed to recapitulate the reproductive impairments observed in the NIRKO mice. In an attempt to narrow down the critical insulin-responsive candidates, we used the Cre-loxP system to target the deletion of InsR's to GABAergic (InsR-lox/lox, Vgat-Cre+) or glutamatergic (InsR-lox/lox, Vglut2-Cre+) neurons and then compared multiple reproductive and metabolic parameters between the knockout (KO) and control (InsR-lox/lox) mice. Puberty onset, estrous cyclicity and adult fertility were not impaired in either the male or female GABAergic or glutamatergic InsR KO mice relative to littermate control mice, although female GABAergic

InsR KO mice exhibited increased body weight and adiposity. We then re-evaluated the widespread belief that neuronal insulin signaling is required for fertility by generating forebrain neuron InsR KO mice (InsR-lox/lox, CamKIIα-Cre+). These KO mice showed a complete lack of hypothalamic insulin responsiveness (phospho-AKT activation), and both the males and females displayed increased body weight and other metabolic impairments. Surprisingly, however, the male and female forebrain neuron InsR KO mice were fully fertile, with normal puberty onset, estrous cyclicity, and age of reproductive senescence. We conclude from these experiments that neuronal insulin signaling is not critically involved in the metabolic control of the neuroendocrine reproductive axis, but plays an important role in regulating metabolic function. It remains possible that insulin exerts a critical role through non-neuronal brain cells.

Disclosures: M.C. Evans: None. **G.M. Anderson:** None.

Poster

543. Luteinizing Hormone Secretion, Puberty, and Seasonality

Location: Halls A-C

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R01 HD13254

U54 HD08610

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Title: Evidence for a repressive role of zinc finger genes in the hypothalamic control of primate puberty

Authors: J. M. CASTELLANO[1], V. MATAGNE[1], A. LOMNICZI[1], C. TORO[1], M. TENA-SEMPERE[2], T. M. PLANT[3], *S. R. OJEDA[1]
[1]Oregon Natl. Primate Ctr., BEAVERTON, OR; [2]Univ. of Cordoba, IMIBIC/HURS, Cordoba, Spain; [3]Ctr. for Res. in Reproductive Physiology, and Magee Womens Res. Institute, Univ. of Pittsburgh Sch. of Med., Pittsburgh, PA

Abstract: Puberty in primates is triggered by reactivation of pulsatile hypothalamic GnRH release, considered to result from a concomitant loss of transsynaptic inhibition and increased transsynaptic and glial excitatory inputs to GnRH neurons. Emerging evidence suggests that a balance of permissive and repressive transcriptional controls, including epigenetic regulation, operating in cellular subsets upstream to GnRH play an important role in this process. However, the identity of the various factors involved and the underlying complexity of these regulatory mechanisms remain incompletely characterized. To gain insight into this issue we interrogated the medial basal hypothalamus (MBH) of agonadal male rhesus monkeys using Affymetrix DNA arrays. Cerebral cortex was used as a control. Our results revealed that hypothalamic expression of several genes encoding zinc finger proteins endowed with a Krüppel-associated box (KRAB domain) – known collectively as KRAB Zinc-finger (KZNF) genes – decreased during the juvenile-pubertal transition, coinciding with the pubertal increase in pulsatile GnRH as reflected by LH secretion. Half of the human KZNF genes are clustered within the long arm of chromosome 19 (q13.12-13.43). Quantitative PCR analysis of selected KZNF mRNAs transcribed from the q13 locus (KZNF566, KZNF573, KZNF264, KZNF542, and KZNF587) verified the array results. In addition, hypothalamic expression of two other Zinc-finger genes identified by genome-wide association studies of others as influencing either age of menarche (KZNF462, chromosome 9) or pediatric height (GATAD1, chromosome 7), also decreased at puberty. Although GATAD1 does not contain a KRAB domain, it is remarkable as it represses transcription via an epigenetic mechanism. It first "reads" H3K4me3, a histone mark associated with gene activation, and then forms a transcriptional co-repressor complex. Additional array and qPCR analyses showed that, with exception of KZNF573 and GATAD1, different KZNFs had decreased pubertal expression in intact female monkeys. One of these two (GATAD1) was therefore selected for mechanistic studies. GATAD1 inhibited the promoter activity of genes regulating puberty onset (KISS1, TAC3, EED, PENK and EAP-1), but required the presence of the H3K4me3 demethylase, JARID1A, to exert its repressive action. Overexpressing GATAD1 in the arcuate nucleus of immature rats delayed first ovulation and resulted in ovarian follicular atrophy, supporting the notion that GATAD1 association to transcriptionally active promoters in the MBH via a histone modifying complex may be a component of a repressive mechanism controlling the timing of primate puberty.

Disclosures: J.M. Castellano: None. **S.R. Ojeda:** None. **A. Lomniczi:** None. **C. Toro:** None. **V. Matagne:** None. **T.M. Plant:** None. **M. Tena-Sempere:** None.

Poster

543. Luteinizing Hormone Secretion, Puberty, and Seasonality

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 543.08/OO1

Topic: E.01. Neuroendocrine Processes

Support: BBSRC

Title: The role of the posterodorsal medial amygdala in pubertal timing in female rats

Authors: ***M. HU**[1], X. LI[1], B. P. HANLEY[1], Y. LIN[1], L. POSTON[1], S. L. LIGHTMAN[2], K. T. O'BYRNE[1]
[1]Women's Health,School of Med., King's Col. London, London, United Kingdom; [2]Henry Wellcome Lab. for Integrative Neurosci. & Endocrinol., Univ. of Bristol, Bristol, United Kingdom

Abstract: Puberty is a developmental process initiated by maturation of the hypothalamic-pituitary-gonadal (HPG) axis. The central mechanisms underlying changes in pubertal timing as a consequence of metabolic status or stressful perturbations are not well understood. Obesity is the major risk factor for early onset of puberty, but there is emerging evidence that other factors are involved, amongst these are psychosocial stress. One key brain region is the amygdala, notable for its roles in controlling caloric intake and regulating stress and behavioural reactivity. Early research showed that amygdaloid lesions that included the medial nucleus advanced puberty in rats. More recently, it was shown that a critical site for lesion-induced hyperphagia and obesity in rats is the posterodorsal subnucleus of the medial amygdale (MePD), which may explain the altered pubertal timing by way of an earlier attainment of the critical body mass. It has also been reported that glutamatergic activity increases in the MePD during puberty without a corresponding GABAergic change, suggesting an overall activation of this site at the onset of puberty. The role of endogenous glutamatergic and GABAergic signalling in MePD in pubertal timing remains to be investigated. In the present study, we report that lesioning the MePD (postnatal day 20), using ibotenic acid, advances the onset of female puberty (vaginal opening and first estrus) and increases the rate of weight gain prior to puberty onset in rats receiving a normal diet. MePD lesioned rats fed with a 25% non-nutritional diet also showed a dramatic advancement of puberty, but without the increased rate of weight gain. In both dietary groups the MePD lesion resulted in an increase in socialization (social interaction) and a decrease in play fight (pinning behaviour). We have also shown that chronic infusion (via osmotic mini-pump starting on postnatal day 20) of the GABA receptor antagonist, bicuculline, into the MePD had the same effect as the MePD lesion on both pubertal timing and behaviour without the alteration of weight gain, while infusion of the glutamate receptor antagonist, amino-5-phosphonovaleric acid, had the opposite effect. In conclusion, our results suggest that the MePD alter the timing of puberty onset via a mechanism independent of change in body weight and food intake. Glutamate in this limbic brain area seems to have an advancing effect on puberty, whilst GABA has the opposite.

Disclosures: **M. Hu:** None. **X. Li:** None. **B.P. Hanley:** None. **Y. Lin:** None. **L. Poston:** None. **S.L. Lightman:** None. **K.T. O'byrne:** None.

Poster

543. Luteinizing Hormone Secretion, Puberty, and Seasonality

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Topic: E.01. Neuroendocrine Processes

Support: U54 HD028138

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Charles H. Hood Foundation for Child Health Research Program

Microgrant Program from The Biomedical Research Institute and the Center for Faculty Development and Diversity's Office for Research Careers at the Brigham and Women's Hospital

Title: The central role of substance p/nka in puberty onset and fertility

Authors: *V. M. NAVARRO, S. SIMAVLI, R. CARROLL, U. B. KAISER
Med., Brigham and Women's Hosp. / Harvard Med. Sch., Boston, MA

Abstract: Puberty is a very tightly regulated process that ultimately leads to the reactivation of GnRH pulsatility and attainment of reproductive function. Kiss1 neurons play a crucial role by stimulating GnRH neurons to induce puberty onset, however, the mechanisms determining the activation of Kiss1 neurons in this developmental process remain unknown. Kiss1 neurons are regulated by tachykinins neurokinin A (NKA), neurokinin B (NKB) and substance P (SP). NKB has been involved in the activation of Kiss1 neurons prepubertally and we have recently documented in mice that a) NKA and SP receptor agonists induce gonadotropin release in a kisspeptin dependent manner and b) advance puberty onset, and c) Tac1 mRNA (encoding NKA and SP) expression increases prior to puberty. Here, we extend these findings by characterizing

the reproductive phenotype of Tac1 null mice. Female Tac1-/- mice present delayed puberty onset compared to wild-type (WT) controls, as determined by the age of vaginal opening (VO). The day of first estrus and estrous cycle length was not significantly different among groups; however, Tac1-/- mice displayed a significantly longer time from the first estrus to the initiation of the estrous cyclicity. Moreover, in a fertility test, Tac1 null animals showed a significantly lower number of pups per litter compared to WT controls. Interestingly, male Tac1-/- mice did not show any significant difference in puberty onset (determined by the age of preputial separation). In summary, these findings support the role for substance P and NKA in the stimulation of kisspeptin and GnRH release prior to puberty onset and documents the required presence of these neuropeptides to attain full reproductive capabilities in female mice. Altogether, these data suggest that all three tachykinins may participate in the coordinated regulation of Kiss1 neurons.

Disclosures: V.M. Navarro: None. **U.B. Kaiser:** None. **S. Simavli:** None. **R. Carroll:** None.

Poster

543. Luteinizing Hormone Secretion, Puberty, and Seasonality

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 543.10/OO3

Topic: E.01. Neuroendocrine Processes

Support: Health Research Council NZ 11/404

Title: Increased luteinising hormone pulse frequency in a mouse model of polycystic ovarian syndrome

Authors: *M. PRESCOTT, C. J. MARSHALL, R. E. CAMPBELL
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Abstract: Polycystic ovarian syndrome (PCOS) is characterized by hyperandrogenism, anovulation and cystic ovaries, and is currently the most common endocrine disorder among women of reproductive age worldwide. PCOS is associated with an increase in luteinising hormone (LH) pulse frequency, suggestive of increased gonadotropin-releasing hormone (GnRH) pulse frequency, hypothesized to result from impaired steroid hormone feedback. Prenatal androgen exposure (PNA) of mice on embryonic days 16-18 results in female offspring that present with a PCOS-like phenotype, including hyperandrogenism, disrupted estrous cyclicity and ovarian morphology, and infertility. However, it remains to be determined whether

pulsatile LH secretion is increased in this model. Until very recently, LH measurement has been limited to single samples in mice due to the large sample volume required. The development of a sensitive sandwich ELISA to measure LH in as little as 2µl of whole blood has enabled us to address for the first time whether PNA exposure in mice results in an increase in LH pulse frequency using repeated blood sampling. In order to assess LH pulsatility, vehicle and PNA treated mice were habituated for 4 weeks with daily handling and training with a cardboard tube prior to blood sampling. A 5µl tail blood sample was taken in 6 or 10 minute intervals for 2 hours, diluted in PBS-Tween and immediately frozen. LH levels were then determined by ELISA. LH pulses, identified by peak values greater than 3 standard deviations above baseline and shape, were detected in the majority of the mice sampled. Compared with prenatally vehicle-treated controls in diestrus (n=5), PNA-treated mice (n=6) had a significant increase in LH pulse frequency (1.6± 0.37 vs 3± 0.24/2hr, $p<0.05$), and a significantly reduced pulse interval (74± 11.2 vs 41± 5.4 min, $p<0.05$). Mean baseline LH, peak LH, LH pulse amplitude, and area under the curve was not different between groups. This increase in pulsatile LH in PNA-treated mice provides additional support for the utility of this murine model of PCOS and supports the hypothesis of impaired steroid hormone feedback of the GnRH/LH system in this common form of female infertility.

Disclosures: **M. Prescott:** None. **C.J. Marshall:** None. **R.E. Campbell:** None.

Poster

543. Luteinizing Hormone Secretion, Puberty, and Seasonality

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 543.11/OO4

Topic: E.01. Neuroendocrine Processes

Support: NIH43200

Title: Involvement of kndy neurons in luteinizing hormone surges induced by steroids

Authors: ***C. V. HELENA**[1], N. TOPORIKOVA[3], B. KALIL[4], A. M. STATHOPOULOS[2], J. A. ANSELMO-FRANCI[4], R. BERTRAM[2]
[1]Biol. Sci., [2]Florida State Univ., Tallahassee, FL; [3]Washington and Lee Univ., Lexington, VA; [4]Univ. of Sao Paulo, Ribeirao Preto, Brazil

Abstract: A subset of hypothalamic arcuate neurons that coexpress kisspeptin, neurokinin B and dynorphin (KNDy neurons) has been postulated to be critical for puberty onset and regulation of

luteinizing hormone (LH) secretion. A method for targeted ablation of KNDy neurons was recently developed using the molecular neurotoxin saporin conjugated to the selective NK3R agonist [MePhe7]Neurokinin B (Nk3-SAP). Ovariectomized rats were microinjected bilaterally into the arcuate nucleus with Blank-SAP or Nk3-SAP. One set of rats was transcardiacally perfused 1, 2 or 3 weeks after the injections and immunocytochemistry for kisspeptin was performed in the arcuate nucleus region. The number of KNDy neurons was significantly decreased after 1 week of the toxin injection, however maximal fiber ablation was only achieved 3 weeks after the microinjections. Another group of rats was treated with oil (OVO), estradiol (OVE) or estradiol plus progesterone (OVEP). One week later, rats had their jugular vein cannulated and blood samples were taken at 10am and hourly from 3 until 6pm. Selective ablation of KNDy neurons of OVO rats significantly reduced basal LH levels at all time points studied. Basal LH levels in OVE and OVEP animals did not differ between groups, yet KNDy ablation increased peak LH levels in the afternoon of OVE and OVEP rats. A third group of OVE animals was microinjected with norbinaltorphimine (nor-BNI), a kappa opioid receptor antagonist, directly into the anteroventral periventricular nucleus (AVPV) one hour before the expected LH surge. The blockage of dynorphin receptors intra-AVPV significantly increased the LH surge, similar to the effect of KNDy ablation in OVE rats. Our results suggest that KNDy neurons provide inhibition to AVPV kisspeptin neurons through dynorphin and thus regulate the size of the LH surge induced by estradiol or estradiol plus progesterone.

Disclosures: C.V. Helena: None. **N. Toporikova:** None. **B. Kalil:** None. **A.M. Stathopoulos:** None. **J.A. Anselmo-Franci:** None. **R. Bertram:** None.

Poster

543. Luteinizing Hormone Secretion, Puberty, and Seasonality

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 543.12/OO5

Topic: E.01. Neuroendocrine Processes

Support: FAPESP / Brazil

CAPES / Brazil

Title: Estrogen modulates the interaction of leptin and nitrergic system in the brain areas involved in the control of the reproductive function in females rats

Authors: *L. OLIVEIRA, B. DEL-BIANCO BORGES, C. R. FRANCI
Physiol., Ribeirão Preto Med. School, Univ. of São P, Ribeirão Preto, Brazil

Abstract: Leptin (a known mediator of the energy balance) and nitric oxide (NO) have been identified as mediators in the mechanisms to control the reproductive function. We aimed assess whether estrogen and food condition modulate a possible interaction between leptin and NO. Estrogen-primed and unprimed ovariectomized rats were subjected to the fasting for 48 hours before the experiment or normally fed. Two hours after saline (control) or leptin (3μg/1μl) intracerebroventricular (icv) microinjection, the animals were decapitated or transcardially perfused to remove the encephalon. We assessed neuronal NO synthase (nNOS) content (Western blotting) and nNOS gene expression (RT-PCR) in the medial preoptic area (MPOA) and medial basal hypothalamus (MBH); and nNOS/ phosphorylated-signal transducer and activator of transcription-3 (pSTAT3) co-expression (immunohistochemistry) in the MPOA and hypothalamic nuclei (arcuate - ARC; ventromedial - VMH; dorsal / ventral dorsomedial- dDMH / vDMH; premammilar ventral - PMV). The results were evaluated by multifactorial analysis of variance (ANOVA) followed by post- test of Duncan. Leptin and estrogen did not change the nNOS gene and protein expression in the MPOA and MBH in fed rats, but increased in fasted rats. In all of the areas studied, leptin increased pSTAT3 and the interaction estrogen / fasting increased the action of leptin. Moreover, the leptin increased the percentage of nNOS / pSTAT3 co-expression in the MPOA only in estrogen-primed fasted rats. This co-expression increased in other hypothalamic regions in estrogen-primed and unprimed rats. Leptin increased nNOS-immunereactive cells expression in the VMH, DMH and PMV of estrogen-primed rats. The results indicate that in the fasting state, the interaction leptin and estrogen modifies the nNOS gene and protein expression in the MPOA and MBH. The leptin, in the presence of estrogen, increases the expression of nNOS in areas related to the control of reproductive function, facilitating the activity of the hypothalamic-pituitary-gonadal (HPG) axis. Thus, when occurs the fall of leptin, how in the fasting state, the leptin replacement is effective to increase the expression of nitric oxide that activates the HPG axis, when in presence of estrogen. Estrogen modulates the nitrergic system, the leptin sensitivity and consequently the leptin action on the nitregic system in some hypothalamic areas. vDMH and PMV seem to be the more involved areas in this interaction.

Disclosures: L. Oliveira: None. **B. Del-Bianco Borges:** None. **C. R. Franci:** None.

Poster

543. Luteinizing Hormone Secretion, Puberty, and Seasonality

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 543.13/OO6

Topic: E.01. Neuroendocrine Processes

Support: Biotechnology and Biological Sciences Research Council, UK.

Title: Biphasic influence of substance P on LH secretion in female rats

Authors: *S. Y. LI[1], M. H. HU[1], X. F. LI[1], B. KALIL[2,3], T. M. PLANT[2], K. T. O'BYRNE[1]
[1]Div. of Women's Health, Sch. of Med., King's Col. London, London, United Kingdom; [2]Dept. of Obstetrics, Gynecoloy and Reproductive Sciences, Sch. of Med., Univ. of Pittsburgh, and Magee-Womens Res. Inst., Pittsburgh, PA; [3]Dept. of Physiol., Univ. of Sao Paulo, Ribeirao Preto, Brazil

Abstract: The pulsatile release of gonadotrophin-releasing hormone (GnRH) which results in the intermittent release of luteinising hormone (LH) is a prerequisite for normal reproduction. However, the exact neuronal components of the GnRH pulse generator are still unknown. **KNDy** neurones which co-express the neuropeptides **K**isspeptin, **N**eurokinin B (NKB) and **Dy**norphin A (Dyn) in the hypothalamic arcuate nucleus (ARC) have been postulated to represent part of this neuronal construct. Kisspeptin exerts stimulatory effect and Dyn exerts inhibitory effect on the GnRH pulse generator. NKB acts on neurokinin-3 receptor (NK3R) to stimulate or inhibit LH secretion depending on the sex steroid milieu. Recently, substance P, a neuropeptide belonging to the same family as NKB but acting on NK1R, has been shown to be expressed within the KNDy neurones in human hypothalamus. Furthermore, central administration of substance P alters LH release, but with conflicting results; both stimulatory and inhibitory actions evident. We hypothesize that substance P may be a fourth player in regulating the GnRH pulse generator. To address this, ovariectomized (OVX) rats with or without 17β-estradiol replacement were used. Rats were chronically implanted with bilateral intra-ARC cannulae and cardiac catherters. Serial blood samples were automatically taken every 5 min for 6 h for measuring LH concentrations. We showed that intra-ARC administration of substance P dose-dependently suppressed pulsatile LH release in OVX rats. In contract, intra-ARC administration of substance P in OVX, 17β-estradiol-replacement rats (estradiol level: 38.1 ± 4.2 pg/ml) stimulated LH secretion followed by an inhibition. These data suggest that substance P is involved in the regulation of the GnRH pulse generator in female rats and its effects may be biphasic depending on circulating levels of estradiol.

Disclosures: S.Y. Li: None. **M.H. Hu:** None. **X.F. Li:** None. **B. Kalil:** None. **T.M. Plant:** None. **K.T. O'Byrne:** None.

Poster

543. Luteinizing Hormone Secretion, Puberty, and Seasonality

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 543.14/OO7

Topic: E.01. Neuroendocrine Processes

Support: NIH Grant DK043200

Title: Effect of ovarian steroids in the response to oxytocin of anterior pituitary gonadotrophs from intact and ovariectomized female rats

Authors: *A. E. GONZALEZ-IGLESIAS[1], J. A. ARIAS-CRISTANCHO[2], P. A. FLETCHER[2], R. CRISTANCHO-GORDO[2], R. BERTRAM[2], J. TABAK[2]
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Abstract: The peptide oxytocin (OT) is secreted by hypothalamic neurons and exerts numerous actions related to reproduction. We and others have shown previously that administration of OT to proestrus rats advances the preovulatory prolactin (PRL) and luteinizing hormone (LH) surges. We have also found that perifused anterior pituitary lactotrophs from proestrus rats are more sensitive than those from diestrus to the stimulatory actions of OT on PRL release and intracellular Ca2+ (Ca2+i). Here we sought to evaluate whether similar differences to the action of OT on hormone release and Ca2+i response exist between diestrus and proestrus gonadotrophs. In addition, to determine the influence of ovarian steroids we compared OT-induced responses *in vitro* between cells from ovariectomized (OVX) rats, OVX rats receiving an implant of 17 β-estradiol (OVE) and OVE rats receiving progesterone implants (OVEP). OT stimulated the secretion of LH in a dose-dependent manner in gonadotrophs from diestrus and proestrus rats, but the latter exhibited greater responses and sensitivity. The half maximal effective concentration (EC50) of OT was 17.6 nM and 2.6 nM for diestrus and proestrus, respectively. Both OT-stimulated LH release and Ca2+i transients were stronger in gonadotrophs from steroid-treated OVX rats, which exhibited an increase in the fraction of cells responsive to OT. Taken together, these results show that ovarian steroids influence OT actions on gonadotrophs and suggest that the heightened responsiveness of LH releasing activity to OT on proestrus may be partially accounted for by direct effects at the pituitary.

Disclosures: A.E. Gonzalez-Iglesias: None. **J.A. Arias-Cristancho:** None. **P.A. Fletcher:** None. **R. Cristancho-Gordo:** None. **R. Bertram:** None. **J. Tabak:** None.

Poster

543. Luteinizing Hormone Secretion, Puberty, and Seasonality

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 543.15/OO8

Topic: E.01. Neuroendocrine Processes

Support: NZ Marsden Fund

Health Sciences Career Development Postdoctoral Fellowship

Title: Deletion of estrogen receptor-α (ERα) from RFamide-related peptide-3 (RFRP-3) neurons disrupts the negative and positive feedback effects of estradiol on gonadotrophin secretion

Authors: *M. Z. RIZWAN, M. A. INGLIS, G. M. ANDERSON
Ctr. for Neuroendocrinology, Dept. of Anat., Univ. of Otago, Dunedin, New Zealand

Abstract: Fertility in humans and animals is driven by the pulsatile secretion of GnRH neurons in the hypothalamus. The negative and positive feedback effects of estradiol modulate it, but indirectly since GnRH neurons do not express ERα. RFRP-3, a neuropeptide produced by neurons with cell bodies distributed around the dorsomedial hypothalamus, has been postulated to act upstream of GnRH to modulate the feedback effects of estradiol, and to inhibit the surge of GnRH and gonadotrophins that triggers ovulation. Many RFRP-3 neurons express ERα, project to GnRH neurons and suppress their activity. In support of this, our group has demonstrated that RFRP-3 infusion suppresses the GnRH and gonadotrophin surge in rats (Anderson et al 2009, Endocrinology 150: 1834-40), and that hypothalamic RFRP-3 levels markedly decline just prior to the surge in this species (unpublished observation), presumably to permit its occurrence. To definitively determine the requirement of RFRP-3 for ovulation and fertility, we utilised a new Cre-loxP transgenic mouse model created by our group. RFRP-3-Cre mice were crossed with ERα floxed mice to produce mice with ERα deleted specifically from RFRP-3 neurons. These mice exhibit an advanced first estrus, and disordered subsequent estrous cycles. The aim of the current study was to investigate whether RFRP-3 neurons mediate estradiol negative and positive feedback in female mice. LH levels in diestrus RFRP-3-Cre-ERα knockout (RERKO) animals were approximately three-fold higher than the littermate controls ($p<0.05$, $n=5$). All mice were ovariectomised (OVX) for 7d before measurement of pulsatile LH secretion in frequently collected tail-tip whole blood samples. Chronic low-dose estradiol (E2)-filled capsules were then implanted sub-cutaneously to generate negative feedback. Mean and basal LH concentrations were suppressed to about 30% of OVX values by E2 treatment in control mice ($p<0.05$), although pulse frequency (2-3 pulses/2h) was unaffected. In RERKO mice, however, all pulse parameters remained at OVX levels. When treated with estradiol benzoate to induce a positive feedback response, the RERKO mice showed no evidence of an LH surge ($p<0.05$ vs control peak surge values). It is possible that the latter effect represents a secondary response to impaired

negative feedback prior to the surge. These results highlight the importance of RFRP-3 neurons as key players in the hypothalamic control of fertility via estradiol.

Disclosures: M.Z. Rizwan: None. **G.M. Anderson:** None. **M.A. Inglis:** None.

Poster

544. Sexual Differentiation

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 544.01/OO9

Topic: E.01. Neuroendocrine Processes

Support: NC State University Start Up Funds

Grass Foundation

Title: Sex differences in medium spiny neuron electrophysiological properties in pre-pubertal rat dorsal striatum

Authors: D. M. DORRIS, J. CAO, *J. MEITZEN
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Abstract: Neural sex differences are traditionally associated with areas of the brain directly involved with reproduction in adult, post-pubertal animals. There is growing acknowledgement that sex differences can exist in other brain regions and developmental periods as well. However, the pervasiveness of neural sex differences is still largely unexplored, especially in the pre-pubertal period widely used for electrophysiological recordings. Here we test the hypothesis that passive and active medium spiny neuron (MSN) electrophysiological properties in pre-pubertal rat dorsal striatum (caudate/putamen) differ by sex. This neuron type and brain region was chosen given its prominence and the known sex differences in adult striatum and relevant behaviors/pathologies. We recorded 30 MSNs from males and 31 MSNs from females using whole-cell current clamp technique in Sprague Dawley (CD) rat brain slices. We analyzed electrophysiological properties according to Meitzen et al., 2009. We found that: 1) the slope of the evoked firing rate to current injection curve was increased in MSNs recorded from females compared to males. Likewise, the initial action potential firing rate was increased in MSNs recorded from females compared to males. 2) Action potential afterhyperpolarization peak was decreased and threshold hyperpolarized in MSNs recorded from females compared to males. 3) No sex differences in passive electrophysiological properties were detected. These findings

indicate that MSN excitability is increased in females compared to males, providing a mechanism that may contribute to generating sex differences in striatal-mediated behavior and pathologies. Broadly, these findings demonstrate that sex differences in neuron electrophysiological properties can exist pre-puberty in brain regions not directly related to reproduction.

Disclosures: D.M. Dorris: None. **J. Meitzen:** None. **J. Cao:** None.

Poster

544. Sexual Differentiation

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 544.02/OO10

Topic: E.01. Neuroendocrine Processes

Support: CIHR Grant

Title: Sex hormones and sex chromosomes separately influence brain structure of intact mice as seen by MRI

Authors: *C. CORRE[1], M. FRIEDEL[2], D. A. VOUSDEN[2], A. METCALF[2], S. SPRING[2], L. R. QIU[1], M. R. PALMERT[1], J. P. LERCH[2]
[1]Div. of Endocrinol., [2]Mouse Imaging Ctr. and Program in Neurosci. and Mental Hlth., The Hosp. For Sick Children, Toronto, ON, Canada

Abstract: Males and females exhibit dimorphisms in brain structure and function. To determine the basis for these sex dimorphisms, we investigated the influences of sex hormones and sex chromosomes on brain structure and function in mice. We used the Four Core Genotype (4CG) mice, which can generate both male and female mice with XX or XY sex chromosome complement, allowing decoupling of effects of sex chromosomes from those of the hormonal milieu. In order to determine the influences of sex hormones and sex chromosomes in a normal context, we used intact mice, characterized their endocrine function, and found that during the course of our study males and females displayed physiological levels of sex hormones regardless of chromosome complement. To assess differences in cognitive function, mice were trained on a radial arm maze, and to examine whole brain structure, high resolution ex-vivo MRI was performed. As reported for other cognitive tests, maze training demonstrated strict hormone-dependency with no apparent influence of sex chromosomes on spatial learning and memory. The influence of sex chromosomes on brain structure has been thought of as small in comparison

to that of sex hormones. However, voxel-wise and volumetric analyses of MRI data uncovered influence of chromosomes in several areas. Of 30 regions that displayed variation among the 4CG mice, 15 were exclusively hormone-dependent and 13 were exclusively chromosome-dependent. Only two brain regions displayed structural variation that associated with both chromosomes and hormones, and the combined effects were additive rather than interacting. The structural variation we observed overlapped with previously identified dimorphisms, and also highlighted differences in areas that are less well documented, such as the pons, medulla, cerebral peduncle, fornix, and internal capsule.Understanding the influences of chromosomes and hormones on brain structure and function is important for understanding sex differences, an endeavor that has implications for understanding gender biases observed in the prevalence of psychiatric disorders.

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Poster

544. Sexual Differentiation

Location: Halls A-C

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Topic: E.01. Neuroendocrine Processes

Support: NIH Grant NS047264

NIH Grant T32HD049336

Title: BDNF immunolabeling in SNB motoneurons and levels in target musculature are not dependent on estrogens during postnatal development

Authors: *L. M. RUDOLPH, D. R. SENGELAUB
Indiana Univ., Bloomington, IN

Abstract: The lumbar spinal cord of rats contains the sexually dimorphic, steroid-sensitive motoneurons of the spinal nucleus of the bulbocavernosus (SNB). In males, these motoneurons innervate the perineal muscles bulbocavernosus (BC) and levator ani (LA) that control penile reflexes. We have previously demonstrated that testosterone interacts with brain-derived neurotrophic factor (BDNF) to regulate SNB dendritic morphology in adulthood. For example, after castration of adult males, SNB dendrites undergo significant atrophy, BDNF

immunolabeling decreases in SNB motoneuron somata, and BDNF levels increase in the BC muscle. Blockade of BDNF prevents castration-induced dendritic atrophy, indicating a regulatory role for BDNF. Furthermore, the castration-induced dendritic atrophy and changes in BDNF in SNB motoneurons and target muscles can be reversed by androgen treatment, suggesting that androgens mediate BDNF action to maintain SNB dendritic morphology in adulthood. Developing SNB dendrites are also dependent on gonadal steroids, and require estradiol to support dendritic growth. Early castration prevents SNB dendrite growth, and treatment of castrates with estradiol fully supports SNB dendrite growth during the early postnatal period. This estrogenic support is mediated by the expression of estrogen receptor α in the target musculature through postnatal day (P) 21. In the present study, we assessed if estrogens could mediate BDNF in SNB motoneurons and their target muscle, potentially providing a mechanism for regulating SNB dendrite growth. Male rats were castrated on P7 and left untreated or were given an estradiol implant (0.1 mg) either at the BC muscle or in the interscapular region. Another group of males was castrated on P7 and received a blank implant at the BC muscle; an additional group of gonadally intact males was left untreated. At P21, BDNF was assessed in SNB motoneurons by immunohistochemistry (AB1779, Millipore, Temecula, CA) and muscle BDNF levels were assessed by ELISA (CYT306, Millipore). BDNF immunolabeling in SNB motoneurons did not change in response to castration or estradiol treatment. As observed in adulthood, castration resulted in a significant increase (45%) in BDNF in the BC muscle, and thus may be involved in the failure of SNB dendrites to grow after early castration. However, estradiol treatment or the presence of a blank implant had no effect on the castration-induced increase in muscle BDNF. These data suggest that while BDNF may be involved in SNB dendritic development, the failure of estrogen treatment to alter muscle BDNF levels indicates that estrogen-dependent SNB dendrite growth is not mediated by BDNF.

Disclosures: L.M. Rudolph: None. **D.R. Sengelaub:** None.

Poster

544. Sexual Differentiation

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 544.04/OO12

Topic: E.01. Neuroendocrine Processes

Title: Sex differences of blood in DNA methylation

Authors: *M. INOSHITA[1], S. NUMATA[1], M. KINOSHITA[1], A. TAJIMA[2], I. IMOTO[2], T. OHMORI[1]
[1]The Univ. of Tokushima, Tokushima, Japan; [2]Human Genet., Inst. of Hlth. Biosciences, The Univ. of Tokushima Grad. Sch., Tokushima, Japan

Abstract: Object- : DNA methylation, which is the addition of a methyl group to the cytosine in a CpG dinucleotide, plays an important role in disease as well as in normal development. Accumulating evidence indicates that sex is one of the major influences over DNA methylation. However, only a few genome-wide methylome studies investigating sex differences have been reported, and little is known about sex-biased DNA methylation on autosomes. The aim of the present study was to investigate sex-biased DNA methylation on the autosomes in human blood. Methods- : We performed a genome-wide DNA methylation profiling of the peripheral leukocytes from non-psychiatric controls (N=94; 50 males and 44 females) using Infinium HumanMethylation450 BeadChips, and we examined sex differences in DNA methylation using multiple linear regression analysis with an adjustment for the covariates that we had identified in a surrogate variables analysis. The final data set included 398,753 CpG sites. All subjects were of unrelated Japanese origin, and they signed written, informed consent forms that were approved by the institutional ethics committee of the University of Tokushima Graduate School for participating in this study. Results- : We identified significant sex differences in DNA methylation at 471 autosomal CpG loci at 5-% Bonferroni correction. Some of these loci also showed the same sex-biased DNA methylation patterns in a recent human post-mortem brain study by Xu and colleagues (2013). Conclusion- : We identified differential DNA methylation between males and females in blood at numerous CpG loci on autosomes. This finding suggests that DNA methylation may be responsible for the sexual dimorphism of blood in particular genes, and will shed light on the molecular mechanism of sex differences.

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Poster

544. Sexual Differentiation

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Topic: E.01. Neuroendocrine Processes

Support: NIEHS Grant R01 ES022759

NIEHS Grant F32 ES019404

Title: Gestational and trans-generational effects of Bisphenol-A on vasopressin immunoreactive AVP

Authors: *J. GOLDSBY, J. T. WOLSTENHOLME, E. F. RISSMAN
Univ. of Virginia Sch. of Med., Charlottesville, VA

Abstract: Bisphenol-A is an endocrine disrupting compound used to make resins and plastics. It is found in many commonly used items such as food can linings, plastic bottles, and thermal receipts. Exposure to BPA during development alters steroid hormone function, which in turn affects brain organization and sexual differentiation. We fed female C57BL/6J mice phytoestrogen-free diets that did not (control) or did contain BPA (5mg/kg of diet). Females were mated and when pups were born they were all fostered to dams on control diets to limit BPA exposure to gestation in the first generation. Pairs from the same diet lineages were bred to the third generation with no further BPA exposure. Adult brains from the first (F1) and third (F3) generations were collected, acrolein fixed, then stained for AVP. Three sexually dimorphic regions, the lateral septum (LS), the bed nucleus of the striate terminalis (BNST), and the medial posterodorsal amygdala (MePD) were analyzed and quantified for size of stained area and intensity of staining. In the first generation (n=3 per group) the expected sex difference was noted in the LS, males had more intense AVP immunoreactivity than females. An interaction between diet and sex was found in the BNST whereas the males exposed in gestation to BPA had more intense immunoreactivity than all other groups. In the F3 mice (n=5-6 per group) the expected sex difference in area stained was noted in the LS, and the intensity of the immunoreactivity was affected in this regions by diet (controls > BPA lineages). The same diet effect on intensity was found in the BNST. In the MePD sex differences (males>females) were found for both immunoreactive area and the staining intensity. These pilot data show the some of the expected sex differences in AVP immunoreactivity in both F1 and F3 braind. Moreover, BPA lineage animals (F3) have less AVP present in normally AVP-expressing cells when compared with control animals. Thus, we can conclude that exposure to a low dose of BPA during gestation has a transgenerational effect on production and/or release of AVP in the brain, suggesting a transgenerational effect on brain neuropeptide dynamics. This work was supported by NIEHS grants R01 ES022759 and F32 ES019404.

Disclosures: J. Goldsby: None. **J.T. Wolstenholme:** None. **E.F. Rissman:** None.

Poster

544. Sexual Differentiation

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 544.06/OO14

Topic: E.01. Neuroendocrine Processes

Support: RO1 MH52716-018 to MMM

Title: Deciphering roles for CB1 versus CB2 endocannabinoid receptors in development of the amygdala

Authors: *K. J. ARGUE, M. M. MCCARTHY
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Abstract: The amygdala is a collection of nuclei that are critical for social and emotional behaviors, parameters that differ in males and females and are altered across a broad spectrum of psychiatric disorders. In rats, adolescent males display higher levels of social play behavior compared to females, and previous studies from our group showed that treatment of female rats with the non-specific endocannabinoid agonist WIN-55,212 (WIN) between postnatal days 0-3 (PN0-3) increases the frequency of adolescent play behavior to the level observed in males (PNAS 107; 2010). The differences in play behavior correlate to a sex difference in BrdU+ cells in the developing medial amygdala in which females have more newly born cells compared to males at PN4, and treatment with WIN reduces the number of new cells in females to those observed in males. The endocannabinoid system includes two major receptor types, CB1, which modulates synaptic function through inhibition of neurotransmitter release, and CB2, which is typically associated with the immune system and inflammatory cells. Given their distinct expression patterns, CB1 and CB2 are not thought to work together. In order to more completely understand the role of CB receptors in sexual differentiation of the amygdala and social play, we administered highly selective agonists for each receptor to male and female rat pups from PN0-3. To our surprise, specific agonism of either CB1 or CB2 alone was not sufficient to increase adolescent female social play behavior (ANOVA $p < 0.05$), however combined treatment with specific CB1 and CB2 agonists recapitulates the effect observed with WIN such that dual-agonist treated females exhibited male-like levels of social play (ANOVA $p > 0.05$). Due to the association of CB2 with the immune system, it has been traditionally considered a peripheral endocannabinoid receptor that is not abundant in the healthy brain. Here we show *in situ* hybridization and immunohistochemical evidence that CB2 is abundantly expressed in the developing amygdala and that expression appears to decrease with increasing age. Additionally, we are pursuing an extensive analysis of the role of the endocannabinoid system in mediating sex specific proliferation and differentiation via stereological analysis of astrocyte, inhibitory neuronal, and excitatory neuronal proliferation in the developing amgydala. These studies provide insight into the role of the endocannabinoid system in sexual differentiation of the brain and aid in a more complete understating of the relative contributions of the endocannabinoid receptors in brain development

Disclosures: **K.J. Argue:** None. **M.M. McCarthy:** None.

Poster

544. Sexual Differentiation

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 544.07/OO15

Topic: E.01. Neuroendocrine Processes

Support: NIH Grant R01 MH52716-018

Title: Endocannabinoid-induced phagocytosis in the developing amygdala mediates a sex difference in cell genesis

Authors: *J. W. VANRYZIN[1,2], M. M. MCCARTHY[3,4]
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Abstract: The mechanisms underlying the establishment of sex differences in the developing brain are still largely unknown, but recent evidence suggests an increasing role for non-neuronal cells in this process. Microglia, the resident immune cells of the brain, act in a variety of roles outside of their capacity to survey and respond to insult. In development, microglia are involved in synaptic pruning, tissue homeostasis, and the support and regulation of neuronal precursor populations both through signaling and targeted phagocytosis of viable cells- termed 'phagoptosis'. To further support this, we implicated microglia as an integral component of brain sexual differentiation in the preoptic area, suggesting an important signaling capacity of these cells in organizing sex-specific brain structure and behavior (Lenz et al. J Neurosci 33(7). 2013). Additionally, we reported a sex difference in cell proliferation in the developing rat medial amygdala (MeA) that was mediated by endocannabinoids, with females having higher rates of cell proliferation than males. These differences in cell proliferation correlated to behavioral changes, as female treatment with the CB1/2 agonist WIN55,212-2 exhibited masculinized juvenile social play behavior (Krebs-Kraft et al. PNAS 107(47), 2010). Further investigation revealed a surprising new role for microglia phagoptosis in mediating the number of newborn cells with effects specific to the postnatal MeA. Males displayed twice as many Iba1+ microglia exhibiting a phagocytic morphology as compared to females. These microglia contained one or more processes that ended in a phagocytic cup, but otherwise exhibited thick or ramified processes. Furthermore, exogenous CB1 receptor agonist treatment increased the number of phagocytic microglia in females to levels observed in males. Treatment with the tetracycline

derivative minocycline, an inhibitor of microglial activation, increased male BrdU+ cell counts to female levels, but did not alter the number of female BrdU+ cells. We found that every microglia exhibiting a phagocytic morphology was also DAPI+, a marker for DNA, within the phagocytic cup; thus, we sought to characterize the specific cell types targeted by the microglia. BrdU colocalized to the phagocytic cup, in addition to inhibitory interneuron markers calbindin and nNos. This work was supported by RO1 MH52716-018 to MMM.

Disclosures: J.W. Vanryzin: None. **M.M. McCarthy:** None.

Poster

544. Sexual Differentiation

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 544.08/OO16

Topic: E.01. Neuroendocrine Processes

Title: Sexual dimorphism in the expression of pituitary specific genes during rat development

Authors: *I. BJELOBABA, K. MAREK, S. S. STOJILKOVIC
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Abstract: The developmental increase in GnRH release in hypophyseal portal blood accounts for gradual establishment of hypothalamic-pituitary-gonadal axes in mammalian females and males. Released GnRH reaches pituitary gonadotrophs wherein it facilitates the expression of a gene for its own receptor, Gnrhr, and genes coding for gonadotropin subunits Cga, Lhb, and Fshb. Our experiments with developing rats revealed a progressive and comparable increase in the pituitary Gnrhr expression in females and males, reaching the peak in expression at 4 and 5 weeks of age, respectively, followed by a significant decline during peripubertal period. In males, there was a strong correlation between pituitary Gnrhr vs. Lhb, Fshb, and Cga expression, reflecting similar developmental pattern of gene expression. However, in female pituitaries Lhb, Fshb, and Cga expression did not correlate well with Gnrhr expression, the lack of correlation was not related to variations in gene expression during estrous cycle in postpubertal females, and the level of expression was lower than in males. The expression of Spp1, another gonadotroph specific gene (encoding osteopontin), was also significantly higher in male pituitaries, but correlated well with Gnrhr expression in both males and females. The sexual dimorphism in the expression of gonadotroph-specific genes was established during juvenile period, in parallel with the sexual dimorphism in the expression of a lactotroph specific gene Prl, which was ten fold higher in females than males. These data indicate that the synchronized activity of gonadotroph-

specific genes during development in males reflects a dominant role of GnRHR, whereas in female gonadotrophs GnRHR-controlled gene expression is substantially modified by other factors.

Disclosures: I. Bjelobaba: None. **K. Marek:** None. **S.S. Stojilkovic:** None.

Poster

544. Sexual Differentiation

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Program#/Poster#: 544.09/OO17

Topic: E.01. Neuroendocrine Processes

Support: NIH NS045195

NSF 12-599

NIH RC100158

Title: Sex difference and laterality in the number of astrocytes in the adult posterodorsal aspect of the medial amygdala of mice

Authors: *D. R. PFAU[1], N. J. HOBBS[2], S. M. BREEDLOVE[2], C. L. JORDAN[2]
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Abstract: In rodents, pheromonal signals play a key role in the expression of stereotypical and sex-specific adult sexual behaviors. Vital to the detection and integration of such chemical signals is the posterodorsal aspect of the medial amygdala (MePD) which receives input from sensory pathways transducing pheromonal signals. In adult rats, the MePD is sexually dimorphic, as males have a larger MePD volume than females. The MePD in male rats also contains more and larger neurons than females. Interacting with these sex differences are laterality differences, with X MePD being larger than X MePD but only in males. Astrocytes in the rat MePD are also sexually dimorphic and lateralized, with sex differences in astrocyte number and complexity, but unique to only one side or the other. For example, males have more MePD astrocytes than females on the right side, and more complex astrocytes than females in the left MePD. Furthermore, masculinization of MePD astrocytes depends on functional androgen receptors, since the morphology of MePD astrocytes in XY rats deficient in functional ARs is completely feminized. Here we investigated whether the MePD in adult C57Bl/6J mice is also

sexually dimorphic in its volume and astrocyte morphology. Astrocytes were visualized with an antibody directed against glial fibrillary acidic protein (GFAP). MePD volume in adult male C57 mice is larger and contains more astrocytes than in females. Moreover, the total number of astrocytes is lateralized with their being more in the left hemisphere in females only, so there is a sex difference favoring males only on the right side. Given the sex difference in MePD volume, we also asked whether the density of astrocytes depended on either sex or hemisphere and found that no such differences. Neither did we find a sex difference or laterality in the complexity of astrocytes in mice, contrary to findings in rats. Overall these data show that the MePD is sexually dimorphic in mice, underscoring its possible role in detecting pheromonal signals important for the development and expression of sex-specific social-sexual behaviors.

Disclosures: D.R. Pfau: None. **N.J. Hobbs:** None. **S.M. Breedlove:** None. **C.L. Jordan:** None.

Poster

544. Sexual Differentiation

Location: Halls A-C

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Program#/Poster#: 544.10/OO18

Topic: E.01. Neuroendocrine Processes

Support: NSF Grant IOS1050367

Title: Aggressive behavior in progesterone receptor knockout (PRKO) mice

Authors: *D. LALITSASIVIMOL, C. K. WAGNER
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Abstract: The display of sexually dimorphic behaviors, such as aggression, in adulthood is directed by the differential exposure of males and females to steroid hormones during critical developmental windows. The medial preoptic nucleus (MPN) is sexually differentiated by hormones during development and is important for the display of intermale aggression in adult male rodents. During perinatal development, differential exposure to the testosterone metabolite, estradiol, induces a significant sex difference in progesterone receptor (PR) expression in the MPN, suggesting that PR plays an important role in the sexual differentiation of the MPN and MPN-mediated behaviors. The present study examined intermale aggressive behavior in adult male PR knockout (PRKO) and wildtype (WT) mice using the resident intruder paradigm. PRKO and WT adult males were castrated and implanted with a silastic capsule containing testosterone

propionate (TP) to clamp circulating testosterone levels. PRKO males demonstrated significantly lower levels of aggression compared to WT males. PRKO males exhibited significantly longer latencies to attack ($p<0.05$) and a significantly reduced attack frequency compared to WT males. Ongoing experiments test the hypothesis that whether PR expression during neonatal life is required for the masculinization of aggressive behavior by testosterone. Neonatal female PRKO and WT mice were exposed to TP or oil vehicle from postnatal days 1-7 and in adulthood were ovariectomized, implanted with TP capsules, and tested for the display of male-typical aggressive behavior in response to an intruder. Results to date suggest that the expression of PR is essential for the normal display of intermale aggression in mice.

Disclosures: D. Lalitsasivimol: None. **C.K. Wagner:** None.

Poster

544. Sexual Differentiation

Location: Halls A-C

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Program#/Poster#: 544.11/OO19

Topic: E.01. Neuroendocrine Processes

Title: Serotonin promotes feminization of sexually dimorphic nuclei in a cell phenotype-specific manner

Authors: *A. M. MADDEN, N. E. SHTEYNBERG, A. A. BARTLETT, A. T. PAUL, S. L. ZUP
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Abstract: Testosterone and its metabolites are known to masculinize the brain during a critical perinatal window. Evidence of this can be seen in the relative size of sexually dimorphic brain areas, including the sexually dimorphic nucleus of the hypothalamus (SDN; larger in males), and the anteroventral periventricular nucleus (AVPV; larger in females). Interestingly, serotonin (5HT) also seems to play a role in the development of these nuclei, as both chronic administration of a general serotonin agonist (5-methoxytryptamine; 5MT) given across perinatal development and more acute administration of a 5HT2A/2C-specific agonist (-DOI) given across the second week of life have been shown to feminize these structures in males. Therefore, 5HT2A/2C receptor activation over the second week of life may drive the feminizing effect of serotonin, although that has yet to be shown conclusively. Thus, we injected male and female Sprague-Dawley rat pups with saline, 5MT, or ketanserin (a 5HT2A/2C antagonist; KET) over the second week of life from postnatal day (PND) 8 to PND16. This allowed us to both examine

the importance of (1) the proposed critical window, and (2) the necessity of 2A/2C activation for this effect. On PND18, the brains were collected and processed for calbindin or tyrosine hydroxylase immunostaining and thionin counterstain. The volume of the calbindin+ SDN was found to be larger in males than in females, regardless of treatment (ANOVA; $p<.001$). A similar pattern was found in the calbindin+ cell count and soma size, with males having more and larger calbindin+ cells than females (ANOVA; $p<.001$, and $p<.005$, respectively). Preliminary results from the AVPV suggest that the effect of 5HT on nucleus size may depend on cellular phenotype, as an interaction was observed between sex and drug in this nucleus (ANOVA; $p<.05$). This study highlights the importance of considering cellular phenotype within sexually dimorphic structures, as activation of 5HT2A/2C receptors has been shown to be sufficient for feminization of overall nucleus size in the SDN and AVPV, while the present data suggests that they may not be necessary for development of the specific sex differences in calbindin+ SDN or TH+ AVPV cell number.

Disclosures: A.M. Madden: None. **N.E. Shteynberg:** None. **A.A. Bartlett:** None. **A.T. Paul:** None. **S.L. Zup:** None.

Poster

544. Sexual Differentiation

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Topic: E.01. Neuroendocrine Processes

Support: NSF Grant IOS1050367

Title: Sex differences in the distribution of Calbindin-D28K immunoreactive cells in wildtype and progesterone receptor knock-out (PRKO) mice in adulthood

Authors: B. GROTH[1], D. LALITSASIVIMOL[2], *C. K. WAGNER[3]
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Abstract: Steroid hormones activate nuclear receptors, which as transcription factors can exert powerful influences on fundamental processes of neural development. There is a dramatic sex difference in the expression of progesterone receptor (PR) within the rodent medial preoptic nucleus (MPN) during perinatal life, suggesting that PR is important for the sexual differentiation of this region. The distribution of calbindin-D28 is sexually dimorphic within the MPN of mice (Gilmore et al., 2012) and can be used as a marker of sexual differentiation. The

present study examined the number and distribution of calbindin immunoreactive (CALB-ir) cells in the MPN of adult male and female PR knockout (PRKO) or wildtype (WT) mice. Results demonstrate a significant effect of sex and genotype with females having a significantly fewer CALB-ir cells compared to males and PRKO mice having significantly fewer CALB-ir cells than WT mice. In addition, there are significant differences in the distribution of CALB-ir cells within the MPN. In WT males there is a distinct clustering of CALB-ir cells that is essentially absent in PRKO males with a more dispersed and medial distribution of CALB-ir cells in PRKO males. These results suggest that the expression of PR is critical for the sexual differentiation of the MPN and that the absence of PR activity may alter the normal neural circuitry of this behaviorally important region.

Disclosures: B. Groth: None. **C.K. Wagner:** None. **D. Lalitsasivimol:** None.

Poster

544. Sexual Differentiation

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Topic: E.01. Neuroendocrine Processes

Support: NSF Grant IOS1050367

Title: Effects of exposure to bisphenol A (BPA) during development on progesterone receptor expression in the medial preoptic nucleus of female rats

Authors: A. PHILLIPS[1], K. STRYKER[1], *P. Q. MENNELLA[2], C. K. WAGNER[1]
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Abstract: The expression of progesterone receptor (PR) within the medial preoptic nucleus (MPN) of rodents during perinatal life is highly dependent on the aromatization of testosterone to estradiol in males (Quadros et al 2002). Perinatal females, in the relative absence of gonadal steroid hormones, express extremely low levels of PR in the MPN. PR expression is virtually abolished in neonatal male rats castrated on the day of birth, male rats administered an aromatase inhibitor or in estrogen receptor alpha knockout mice. Conversely, PR expression is induced to levels similar to that of males in neonatal female rats given testosterone or estradiol. In this regard, PR expression within the MPN of neonatal male and female rats serves as a sensitive bioassay for exposure to compounds that exert estrogenic activity and/or disrupt endogenous estrogen action. The present study examined the estrogenic effects of the plastics contaminant,

bisphenol A (BPA) on the fetal and neonatal brain. In Experiment 1, neonatal male and female rats were injected with BPA (5mg/kg) or vehicle On P2-4 and the estrogen receptor antagonist ICI 182,780 (1.5mg/kg) or vehicle on P1-4 and tissue was collected on P5. In Experiment 2, pregnant mothers were administered BPA (10 or 50mg/kg) or vehicle orally by ingestion of a BPA-laced vanilla wafers from gestation day E12-E22 and tissue was collected on the day of birth. Preliminary evidence from both studies suggests that neonatal or maternal exposure to BPA increases the expression of PR in the MPN of females, consistent with the idea that BPA exposure can exert estrogenic-like effects in developing MPN and may alter the sexual differentiation of brain and behavior.

Disclosures: A. Phillips: None. **P.Q. Mennella:** None. **K. Stryker:** None. **C.K. Wagner:** None.

Poster

545. Neuroimmunology: Behavioral Effects

Location: Halls A-C

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Program#/Poster#: 545.01/OO22

Topic: E.02. Neuroimmunology

Support: NIH Grant R01-AG-033028

Title: Age-related impairments in the dynamic regulation of active microglia by astrocytes

Authors: *D. M. NORDEN[1], P. J. TROJANOWSKI[1], F. R. WALKER[2], J. P. GODBOUT[1]
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Abstract: Systemic infection is associated with an increased frequency of behavioral complications in the elderly. In aged rodents, acute activation of the innate immune system causes an exaggerated neuroinflammatory response that is associated with the development of neurobehavioral complications. Our studies have shown that while the anti-inflammatory cytokine IL-10 is highly expressed in the aged brain, microglial activation is prolonged. Recently we showed that astrocytes of adult mice express the IL-10 receptor (IL-10R) and that IL-10 re-directs active astrocytes to produce TGFb, which in turn, attenuates the activation of microglia. Therefore, the purpose of this study was to investigate the degree to which these key cytokine interactions between glia are impaired in the aged brain following systemic immune challenge. First, the morphology and gene expression of astrocytes was determined in the brain of adult (2-3mo) and aged (18-20 mo) BALB/c mice. In the hippocampus of aged mice, there was increased

expression of the astrocyte inflammatory markers vimentin and GFAP. Moreover, there was evidence of significant remodeling and cytoskeletal re-organization of aged astrocytes in the hippocampus. For instance, aged astrocytes had a global enlargement of the GFAP cytoskeleton without a concomitant increase in branch length, indicating that aging provokes significant cytoskeletal hypertrophy. This was also reflected in increased GFAP labeling intensity. There was no evidence of age-related changes in the number of astrocytes. These data suggest that astrocytes have an early stage pathological profile with age. Moreover, astrocytes from aged mice had reduced surface IL-10R expression compared to adults. Next, to investigate the degree to which these changes influence immune regulation, adult and aged mice were injected with LPS and astrocyte and microglial populations were sorted and collected during the resolution phase following LPS (24h). NanoString gene array of these specific populations indicated that astrocytes from adult mice had an mRNA expression profile associated with increased IL-10 signaling including increased IL-10R and TGFb expression. Astrocytes from aged mice, however, failed to upregulate either IL-10R or TGFb mRNA. This lack of regulation by TGFb was associated with decreased TGFb signaling and exaggerated expression of pro-inflammatory mediators in aged microglia. In summary we interpret these data to indicate that astrocytes have an important role in regulating microglia via TGFb signaling and that an impaired IL-10 response in aged astrocytes contributes to age-related deficits in the regulation of active microglia.

Disclosures: D.M. Norden: None. **P.J. Trojanowski:** None. **F.R. Walker:** None. **J.P. Godbout:** None.

Poster

545. Neuroimmunology: Behavioral Effects

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Program#/Poster#: 545.02/OO23

Topic: E.02. Neuroimmunology

Support: NIH Grant AG028271

Title: The effects of surgery and morphine treatment on neuroinflammation and cognitive decline in aged rats

Authors: *R. M. BARRIENTOS, V. M. THOMPSON, M. M. KITT, L. R. WATKINS, S. F. MAIER
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Abstract: Opioids, such as morphine, induce potent analgesia and are the gold standard for the treatment of acute and chronic pain. However, opioids also activate glia, inducing proinflammatory cytokine and chemokine production within the brain. Aging potentiates neuroinflammatory responses to a peripheral challenge, due in large part to sensitized microglia, and this potentiation is especially prominent in the hippocampus. Furthermore, this exaggerated proinflammatory response is causally associated with robust memory impairments. We have previously shown that aged rats that have undergone an abdominal surgery exhibit persistent neuroinflammation in the hippocampus, with striking deficits in the formation of new memories occurring during this neuroinflammatory period. Aged patients who undergo surgery are often treated with morphine for several days to manage post-operative pain. In this study we explore the possibility that the duration of the proinflammatory response following surgery, and the accompanying memory deficit will be further extended in aged rats by morphine treatment. Preliminary data show that rats that have undergone surgery and have received daily morphine injections exhibit a potentiated neuroinflammatory response in the hippocampus compared to rats exposed to either surgery or morphine alone.

Disclosures: R.M. Barrientos: None. **V.M. Thompson:** None. **M.M. Kitt:** None. **L.R. Watkins:** None. **S.F. Maier:** None.

Poster

545. Neuroimmunology: Behavioral Effects

Location: Halls A-C

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Program#/Poster#: 545.03/OO24

Topic: E.02. Neuroimmunology

Support: CONACYT

Title: Absence of mast cells disrupts grooming behavior in mice: Involvement of dopaminergic and histaminergic systems

Authors: *M. I. SOLLOZO DUPONT, F. GUZMAN-MEJÍA, C. GONZÁLEZ-ESPINOSA, C. LÓPEZ-RUBALCAVA
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Abstract: Evidence show that brain mast cells degranulation induces the release of many neuro-active mediators, some of which are involved in behavioural and physiological components of anxiety. Considering that the analysis of grooming microstructure is a useful measure of anxiety

in rodents, this study was designed to determine the potential influence of mast cells on grooming phenotypes, focusing on the dopaminergic system. Mast cells deficient mice (KitWsh/Wsh), congenitally normal mice C57BL/6J (wild-type, WT) and KitWsh/Wsh intraperitoneally reconstituted with WT mast cells were used. In addition, because brain mast cells contribute to the neural pool of histamine and there are several studies revealing histaminergic modulation of emotional behaviors in rodents, we analyzed the effects of histamine microinjections on grooming in KitWsh/Wsh and WT mice. Results showed that KitWsh/Wsh mice had a greater number of grooming patterns and an increased percentage of incorrect transitions in comparison to WT and reconstituted mice. Increased levels of dopamine in striatum were also observed in KitWsh/Wsh. By contrast, DOPAC/DA and HVA/DA were decreased in KitWsh/Wsh in comparison with the other two strains. While KitWsh/Wsh exhibited a strong positive correlation between dopamine levels and incorrect transitions, the correlation was notably negative in WT and KitWsh/Wsh reconstituted mice. When histamine was injected to KitWsh/Wsh, a reduction in the percentage of incorrect transitions in KitWsh/Wsh compared with controls (KitWsh/Wsh administered with saline solution) occurred after the administration of 2.5 and 5.0 μg of histamine. The percentage of incorrect transitions observed in animals administered with both doses of histamine was similar to the value registered by the WT group. In conclusion, our data implicate brain mast cells in the modulation of grooming and provide evidence that these cells are contributing in the regulation of the dopaminergic system. Our results also confirm that mast cell deficiency could alter brain histamine content, with the subsequent impact on behaviour.

Disclosures: **M.I. Sollozo Dupont:** None. **F. Guzman-Mejía:** None. **C. González-Espinosa:** None. **C. López-Rubalcava:** None.

Poster

545. Neuroimmunology: Behavioral Effects

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Topic: E.02. Neuroimmunology

Support: NIMH IRP

Title: Acute but not chronic social stress activates microglia in the prefrontal cortex of CX3CR1-GFP reporter mice

Authors: *M. L. LEHMANN, R. B. SCHEINERT, M. E. DEAN, H. A. COOPER, M. HERKENHAM
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Abstract: Microglia are the innate immune cells in the brain parenchyma and are functionally and morphologically dynamic. Activation (by infection, trauma, or cell impairment) rapidly alters microglia_they retract their processes, produce inflammatory molecules, and can cause pathology. Recent studies have argued that psychosocial stress activates microglia in select brain areas. Data on the effects of psychosocial stress on microglial activation status have been inconsistent. Here we examined the effects of acute and chronic stress on microglial density and phenotype in specific stress-responsive regions of brain using CX3CR1-GFP reporter mice that display strong GFP reporting confined to microglia. We first mapped changes in microglial cell density following acute and chronic stress exposure. Mice were exposed to either two (acute) or fourteen (chronic) days of social defeat stress. Mice were then phenotyped for social behaviors, perfused, and brains examined for changes in microglia number and proliferation. We also isolated microglia from brains and used fluorescence-activated cell sorting of the cell-surface markers CD11b, CD45, and CD68 to determine activation status. Lastly, an *ex vivo* approach was used to address whether acute or chronic stress alters the responses of microglia to pro-inflammatory stimuli. C57BL6/J mice were exposed to acute or chronic stress, and microglia in the prefrontal cortex were isolated and challenged with LPS to probe for stress-modified pro-inflammatory cytokine production. Both acute and chronic stressed mice showed reduced social and hedonic behaviors indicative of depressive-like phenotype. The amount of anhedonic behavioral change was stress-duration dependent. Acute but not chronic stress increased microglia density and proliferation in stress-responsive brain regions, including the medial prefrontal cortex. Acute stress also enhanced expression of CD11b on microglia taken from the prefrontal cortex. Lastly, when stimulated with LPS, peritoneal macrophages from mice exposed to acute stress expressed higher levels of pro-inflammatory cytokines than those from mice exposed to chronic stress or unstressed home cage mice. The data showed that stress had a biphasic effect on microglial activation. Acute stress increased the density, proliferation, and activation of microglia within the prefrontal cortex. After 14 days of stress exposure, microglia appear to have adapted_both the density and levels of activation were comparable to non-stressed mice. The mechanisms and consequences of such microglial adaptation are currently under investigation.

Disclosures: M.L. Lehmann: None. **R.B. Scheinert:** None. **M.E. Dean:** None. **H.A. Cooper:** None. **M. Herkenham:** None.

Poster

545. Neuroimmunology: Behavioral Effects

Location: Halls A-C

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Topic: E.02. Neuroimmunology

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Title: Minocycline treatment blocks LPS-induced impairment in context discrimination memory retrieval

Authors: *J. CZERNIAWSKI, J. F. GUZOWSKI
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Abstract: Neuroinflammation is implicated in cognitive deficits associated with disease, aging, and trauma. During an immune challenge, there is an increase in cytokine levels in the CNS, which can impair certain forms of learning. Recently, we demonstrated that systemic administration of the bacterial endotoxin lipopolysaccharide (LPS) results in elevated mRNA expression of the proinflammatory cytokines IL-1β, TNF-α, and IL-6 in the rat brain and produces a robust impairment in context discrimination memory retrieval. Furthermore, using the Arc/Homer catFISH immediately-early gene imaging method, we observed greater overlap in neuronal ensembles activated in dorsal hippocampus during each context presentation in LPS-treated rats compared to controls, consistent with the notion of impaired pattern separation. These data provide support for a direct link between cytokine mediated changes at the neural circuit activity level with cognitive impairment at the behavioral level. Because these cytokines are produced in the brain by microglia, the present study aimed to determine if blocking microglia with minocycline, a semi-synthetic tetracycline antibiotic, could block the LPS-induced neuroinflammation and cognitive deficits. Young adult male Sprague Dawley rats were trained in context discrimination conditioning (CDC), in which they were placed into 2 similar behavioral chambers (A and A') daily for 3 min each. One of the environments (A) was paired with a brief, mild footshock (0.5 mA, 1s). Upon reaching discrimination criterion, each subject received an intraperitoneal (i.p.) injection of minocycline (50 mg/kg) or saline after the last day of training and a second dose the next day. Thirty minutes after the second dose of minocycline or saline, LPS (150 μg/kg, i.p.) or saline was administered and subjects were tested 6 h later in each of the contexts. Preliminary findings indicate that minocycline treatment blocks the impaired context discrimination memory retrieval and elevated expression of IL-1β in dorsal hippocampus observed in LPS-treated rats. Findings from this study will help provide a direct causal link between cytokine expression in the brain and the observed cognitive and neural circuit activity deficits. Furthermore, because minocycline can readily cross the blood-brain

barrier, it is valuable to determine the mechanistic effects of it in the brain as a putative treatment for human patients with impairments in cognitive function resulting from neuroinflammation.

Disclosures: J. Czerniawski: None. **J.F. Guzowski:** None.

Poster

545. Neuroimmunology: Behavioral Effects

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 545.06/OO27

Topic: E.02. Neuroimmunology

Title: Churg-Strauss syndrome following vaccination against 2010 influenza A (H1N1): A case report

Authors: *H. M. FU
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Abstract: Churg-Strauss syndrome (CSS) is a systemic disorder characterized by asthma, transient pulmonary infiltrates, hypereosinophilia, and systemic vasculitis. This is a rare syndrome of unknown etiology, and several inducing factors such as inhaled allergens, infections, vaccinations, and drugs have been reported. Here we present a 55-year-old female who developed signs and symptoms of this syndrome after receiving H1N1 vaccination with good recovery after steroid treatment.

Disclosures: H.M. Fu: None.

Poster

545. Neuroimmunology: Behavioral Effects

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 545.07/OO28

Topic: E.02. Neuroimmunology

Support: NIH Grant R21 MH101663-01

Title: Sex differences in neonatal immune function and potential implications for developmental delays in learning

Authors: J. I. CAULFIELD, L. S. TERASAKI, *J. M. SCHWARZ
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Abstract: Microglia are the resident immune cells of the brain. Under baseline conditions in the adult brain, microglia express low levels of immune molecules (cytokines and chemokines), they survey the microenvironment, and they interact with surrounding neurons and astrocytes via thin, long processes. Microglia are thus intricately involved in neural and cognitive function. Microglia respond to infection, insult, or injury, with a rapid change in morphology and a robust increase in the production of cytokines and chemokines in order to re-establish homeostasis in the brain. Neurons exhibit a marked sensitivity to the inflammatory signals produced by microglia and if left unchecked, these stimuli can cause neuronal dysfunction, cognitive dysfunction, or even neuronal death. Microglial activation is associated with multiple neurodevelopmental disorders that also have known or suspected immune etiologies, including autism, ADHD, schizophrenia, and cerebral palsy. Notably, all of these disorders exhibit a strong sex bias in males. We recently discovered a striking sex difference, that males have significantly more microglia in the developing hippocampus, cortex and amygdala than females on postnatal day 4 (Schwarz et al. J. Neurochem., 2011). Given these rodent data and the well-known human epidemiological data, we hypothesize that neonatal males may be more vulnerable to an immune challenge than neonatal females, which may have significant consequences neural and cognitive development. To test this hypothesis, we first treated male and female rat pups with a mild Escherichia coli (E.coli) infection on P4 and examined both the peripheral and neuroimmune responses to the infection. We found a significant interaction of sex and infection in the expression of multiple cytokines and neurotropic factors that were dependent upon brain region (hippocampus vs. cerebellum). In contrast, we saw a significant effect of infection on the peripheral immune response that was not dependent upon sex. This suggests that sex-specific effects of neonatal immune activation are particular to the brain and the brain region examined. In a second experiment, we allowed a similar cohort of rat pups to grow till postnatal day 21 at which point we measured anxiety behaviors. We found that neonatally-infected males were significantly more anxious than control males and neonatally-infected or control females (Sex X Infection Interaction: $F_{1,29} = 4.04$; $p < 0.05$), similar to previous reports. On-going experiments will determine the impact of sex and neonatal immune activation on juvenile immune function and the onset of hippocampal-dependent learning that occurs around this young age.

Disclosures: J.I. Caulfield: None. **L.S. Terasaki:** None. **J.M. Schwarz:** None.

Poster

545. Neuroimmunology: Behavioral Effects

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 545.08/OO29

Topic: E.02. Neuroimmunology

Support: NIH Grant NS080585

Title: Interleukin 1 receptor signaling and fadrozole regulate anxiety behaviors in mice

Authors: *K. A. DUNCAN[1], A. FEIGHERY[2], J. JOSIMOVICH[2], J. ORR[2], K. BLACKSHEAR[2], C. J. SALDANHA[4], K. S. HOLLOWAY[3]
[1]Biol., [3]Psychology, [2]Vassar Col., Poughkeepsie, NY; [4]Biol., The American Univ., Washington, DC

Abstract: Activation of the immune response through proinflammatory cytokines influences a range of physiological and behavioral responses. These responses include fever, inflammation, reduced locomotor activity, sleep disorders, and interestingly diminished social interactions. Specifically neuroinflammation, mediated by Interleukin 1 (IL-1β), elicits changes within the CNS that are implicated in emotional disorders such as depression and anxiety. In the present study, we investigated the role of the IL-1 signaling pathway in anxiety-like behaviors. We then tested the effect of decreased estrogen on anxiety-like behaviors. Previous data has shown that estrogen-mediated neuroprotection is through a decrease in inflammation, thus we hypothesized that decreased circulating estrogens by the use of aromatase inhibition would alter anxiety-like behaviors. Adult male mice (IL-1 receptor knockouts (IL-1R KO) and wild type C57BL/6) were tested for measures of both sickness and anxiety-like behaviors two hours following an injection of LPS or vehicle. IL-1R KO mice differ in their anxiety response prior to treatment. This effect is further exacerbated following LPS treatment and immune system activation. For studies examining the role of aromatase inhibition and anxiety-like behaviors, Fadrozole was given at the same time as the LPS injection. Interestingly, Fadrozole decreased anxiety behaviors in all mice, but the effect was greater in wild-type versus IL-1R KOs. These data suggest that IL-1 signaling is associated with anxiety-like behaviors and steroid hormone levels can ameliorate this effect.

Disclosures: K.A. Duncan: None. **A. Feighery:** None. **J. Josimovich:** None. **J. Orr:** None. **K. Blackshear:** None. **C.J. Saldanha:** None. **K.S. Holloway:** None.

Poster

545. Neuroimmunology: Behavioral Effects

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Program#/Poster#: 545.09/OO30

Topic: E.02. Neuroimmunology

Support: NIH Grant MH096224

Title: Microglia possess a circadian clock that controls inflammatory responses independent of glucocorticoids

Authors: *L. K. FONKEN, M. G. FRANK, M. M. KITT, L. R. WATKINS, S. F. MAIER
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Abstract: The circadian system regulates many physiological functions including inflammatory responses. For example, mortality following administration of lipopolysaccharide (LPS) varies depending on the time of immunostimulation in mammals. The effects of more subtle challenges on the immune system and cellular changes underlying circadian differences in inflammatory responses are not well understood. Thus, we investigated whether a sub-septic dose of LPS injected in the light or dark phase would differentially affect rats' sickness responses, and whether these differences were related to circadian oscillations in microglia. Adult male Sprague-Dawley rats were injected with 100ug/kg of LPS during either the middle of the light or dark phase. Rats injected during the light phase displayed elevated sickness behaviors and hippocampal cytokine production compared to rats injected during the dark phase. Because microglia are the primary immune cell of the CNS we next sought to determine whether microglia demonstrate circadian regulation of inflammatory factors. Hippocampal microglia isolated from adult rats rhythmically expressed inflammatory factors and circadian clock genes. There were robust rhythms of TNFα and IL1β mRNA expression in hippocampal microglia, with peak cytokine gene expression occurring during the middle of the light phase. Moreover, microglia isolated during the light phase were more reactive to immune stimulation; *ex vivo* treatment with LPS resulted in an exaggerated cytokine response in microglia isolated during the light phase. Finally, we explored whether glucocorticoids mediate circadian responses in microglial. Time of day differences in clock and inflammatory gene expression persisted in microglia isolated from adrenalectomized rats. However, treating microglia with corticosterone *ex vivo* induced Per1 expression. Therefore, circadian clock gene expression in microglia appears to be entrained by, but oscillate independent of, glucocorticoids. Taken together, these findings demonstrate that microglia possess a circadian clock that influence inflammatory responses.

These results indicate time of day is an important factor to consider when planning surgeries or immunotherapies.

Disclosures: L.K. Fonken: None. **M.G. Frank:** None. **M.M. Kitt:** None. **L.R. Watkins:** None. **S.F. Maier:** None.

Poster

545. Neuroimmunology: Behavioral Effects

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 545.10/OO31

Topic: E.02. Neuroimmunology

Title: Social Influences on Neuroinflammation after Ischemia

Authors: *M. M. GAUDIER-DIAZ, N. ZHANG, M. ZHOU, A. C. DEVRIES
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Abstract: Social support is associated with improved cardiovascular and cerebrovascular health in humans. Likewise, affiliative social interactions reduce neuronal damage after both experimental stroke and cardiac arrest in rodents. The goal of this project is to determine whether the brains of socially isolated mice are "primed" to respond to cerebral ischemia in a way that increases neuronal damage. The overarching hypothesis is that socially isolated mice mount a greater pro-inflammatory response to cerebral ischemia than socially housed mice, which in turn leads to increased neuronal death. To test this hypothesis, male mice were housed in pairs with an ovariectomized female (n=20) or individually (n=20) for 7 days prior to the induction of 8 minutes of cardiac arrest followed by CPR (CA/CPR). Following resuscitation, the mice were returned to their original housing conditions. Twenty-four hours later, IL-1β was significantly elevated in the hippocampus of socially isolated CA/CPR mice relative to pair housed mice. In a second experiment, *in vitro* oxygen glucose deprivation (OGD) was used to determine whether a trajectory for increased neuroinflammation among socially isolated mice is already established at the time of the ischemic injury. The brains of male pair housed (n=8) or socially isolated mice (n=8) were collected and sectioned into eight slices, 4 of which were exposed to an OGD solution containing (in Mm) 125 NaCl, 25 NaHCO3, 1.25 NaH2PO4, 3.5 KCl, 2 CaCl2 and 1 MgCl2, bubbled with 95%N2/5%CO2, and 4 of which were exposed to control condition for 15 minutes. To determine whether there is a housing-induced difference in the pro-inflammatory response to OGD, tissue was collected for qPCR of pro-inflammatory cytokines after 120 min (n=8/group) of *in vitro* reperfusion. There was a significant increase in TNF-α and IL-6 mRNA

expression in the OGD sections relative to the control sections, but no significant effect of housing. Additional later time points are currently under investigation. In summary, *in vivo* CA/CPR data demonstrate that the brains of socially isolated mice are more susceptible to ischemia-induced damage than brains from pair housed mice; nevertheless, *in vitro* data does not support priming of the pro-inflammatory cytokine response during OGD as a likely mechanism.

Disclosures: M.M. Gaudier-Diaz: None. **N. Zhang:** None. **M. Zhou:** None. **A.C. DeVries:** None.

Poster

545. Neuroimmunology: Behavioral Effects

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Topic: E.02. Neuroimmunology

Support: RO1 DA 12104

RO1 DA 022935

RO1 DA031202

K05DA033881

Title: Dynamics of TLRs and chemokine expression in the regulation of inflammatory immune cells migration into the CNS: A potential mechanism for neuropathogenesis in drug abuse/HIV model with secondary infection

Authors: *R. DUTTA, S. ROY
Univ. of Minnesota, Minneapolis, MN

Abstract: Accelerated neuropathological complications were reported in human immunodeficiency virus (HIV) infected patients with opioid (morphine) abuse and/or addiction. Even though in HIV infected opioid dependent individuals, the neurological damages are more pronounced in the presence of secondary opportunistic infection, the mechanism associated with this clinical condition is poorly understood. In this study we demonstrate that systemic co-infection with S. pneumoniae may be the contributing factor in the increased prevalence of HIV associated neurocognitive disorder (HAND) among opioid dependent population. Flow cytometry and adoptive transfer experiments demonstrates differential leukocyte migration

(CD3+, F4/80+ and Ly6C+ cells) with specific chemokine expression into the CNS of mice treated with morphine in combination with HIV-1 Tat and/or S. pneumoniae. Dissemination of S. pneumoniae to CNS takes place through the 'Torjan Horse' model using F4/80+ and Ly6C+ monocyte subpopulation as transporter across blood brain barrier. Trafficking of immune cells is mediated through CNS chemokine production which facilitates chemokine receptor-dependent accumulation of leukocytes at the sites of infection. We observed significant induction of CCL5 following HIV-1 Tat treatment, however infection with S. pneumoniae led to preferential induction of CXCL12. Morphine potentiated both Tat HIV-1 and S. pneumoniae mediated chemokine induction. Our pervious published data demonstrate that morphine treatment potentiate Toll like receptors (TLR) expression in microglia. Therefore, the role of TLR in chemokine ligands induction was further investigated. Our present data showed a significant role of TLR2 in CD3+CCR5+ migration and however activation of both, TLR2 and 4 was necessary for the migration of monocyte subtypes with chemokine receptors (CCR5 and CXCR4). Our present study, in addition to the previous one, emphasizes the contribution of systemic infection as a potential neuroinflammatory mediator responsible for exacerbated HAND in opioid drug abusers.

Disclosures: R. Dutta: None. **S. Roy:** None.

Poster

545. Neuroimmunology: Behavioral Effects

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UTHSCSA Dept of Psychiatry Friends Research Grant

Title: Kynurenine metabolism mediates inflammation-induced cognitive dysfunction and impulsivity

Authors: *J. C. O'CONNOR[1], J. M. HEISLER[1], J. MORALES[1], D. A. CRUZ[2], P. M. THOMPSON[2]
[1]Pharmacol., [2]Psychiatry, UTHSCSA, San Antonio, TX

Abstract: The dorsolateral prefrontal cortex (dlPFC) is the critical brain region that regulates working memory, cognitive flexibility, planning and impulse control (i.e. executive function). Imaging and postmortem studies suggest that dlPFC dysfunction is a core neurobiological feature of many neurodevelopmental and neuropsychiatric diseases. While the molecular mechanisms underlying dlPFC dysfunction remain poorly understood, proinflammatory activation of the kynurenine pathway(KP) has been identified as a putative pathogenic factor. Here, we demonstrate that proinflammatory cytokine transcripts were increased in the dlPFC of subjects with mood disorders compared to matched controls, and both tumor necrosis factor(TNF)-α and microglia upregulation are strongly associated with motor impulsivity and cognitive inflexibility. Metabolism of tryptophan along the kynurenine pathway was also increased in subjects with mood disorders and associated with dlPFC dysfunction. In mice, we subsequently determined that endotoxin-induced inflammation increases microglial activation and kynurenine metabolism within the medial prefrontal cortex (mPFC), which is the functionally analogous region to human dlPFC, and impairs PFC-dependent cognitive flexibility and motor impulsivity. Mice lacking indoleamine 2,3-dioxygenase, the rate limiting enzyme of the KP, are protected cognitive impairment. Together, these data identify the KP as a key factor in the neuropathology of mood disorders and as a novel therapeutic target.

Disclosures: J.C. O'Connor: None. **J.M. Heisler:** None. **D.A. Cruz:** None. **J. Morales:** None. **P.M. Thompson:** None.

Poster

545. Neuroimmunology: Behavioral Effects

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Functional Neurorecovery Pilot Grant, Department of Physical Medicine and Rehabilitation, UAB

Title: Hippocampal neuroinflammation and cognitive decline as a consequence of acute myocardial ischemia-reperfusion

Authors: *B. JOHNSTON[1], K. E. EVONUK[1], M. E. YOUNG[2], T. M. DESILVA[1]
[1]Dept. of Physical Med. and Rehabil., Univ. of Alabama At Birmingham, Birmingham, AL; [2]Div. of Cardiovasc. Diseases, Dept. of Med., Univ. of Alabama at Birmingham, Birmingham, AL

Abstract: An emerging literature has demonstrated the need to further understand the impact of cardiovascular disease (CVD) on cognitive decline. Human studies have documented lesions in brain regions critical to learning and memory, and a correlation between impaired cognitive function and adverse cardiac events. To better understand the mechanisms of CVD-related cognitive decline, and propose therapeutic strategies to ameliorate cognitive decline in this rapidly expanding patient population, it is essential to validate animal models that recapitulate the neuropathology in these phenotypes. We utilized two murine models of ischemia-reperfusion (I/R), an open- and closed-chest model. For the open-chest model, the left anterior descending (LAD) coronary artery of 20 week old C57BL/6 male mice were ligated for 30 minutes followed by reperfusion. An occluding device was implanted in the closed-chest model without immediate ligation. One week later, the LAD was occluded for 30 minutes followed by reperfusion. Seventy-two hours post-surgery the hippocampus was assessed for reactive glia. Using stereological methods, every 10th serial section of the CA1, CA3, and dentate gyrus subfields of the hippocampus from bregma -1.06 mm to bregma -3.64 mm were counted to determine GFAP and Iba-1 positive cell estimates. Activated astrocytes and microglia in all subfields of the hippocampus in both I/R models were increased relative to sham controls. To investigate how vascular permeability may change in response to I/R, mice received tail-vein injections of FITC-dextran and TRIC-albumin 72 hours post-I/R. An increase in fluorescent intensity for both markers was observed in the hippocampus of mice subjected to I/R. Consistent with an increase in activated glia and vascular permeability within the hippocampus a deficit in hippocampal-dependent learning was observed. Contextual fear-conditioning demonstrated a decrease in freezing behavior in mice 2 months post I/R relative to sham. These data suggest that following a cardiac-ischemic event, rapid inflammatory responses in the hippocampus contribute to subsequent impairment in hippocampal-dependent learning and memory.

Disclosures: B. Johnston: None. **K.E. Evonuk:** None. **M.E. Young:** None. **T.M. DeSilva:** None.

Poster

545. Neuroimmunology: Behavioral Effects

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Topic: E.02. Neuroimmunology

Support: University of Missouri Research Board (UMRB) grant

UMKC School of Pharmacy Bridge Funding

Title: Cerebral expression of IFN-α reduces weight gain with low plasma leptin in mice

Authors: *J. WANG, Y. DAI
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Abstract: Interferon-alpha (IFN-α) is an innate immune mediator. It is permeable to the blood-brain barrier, and peripheral IFN-α can act at the central nervous system. Chronic treatment of IFN-α has been shown to result in neuropsychiatric complications. To determine the impact of long-term IFN-α stimulation in the brain, astrocyte-targeted IFN-α transgenic (GFAP-IFNα) mice with C57BL/6J background was generated by backcross-breeding. Selective expression of IFN-α transgene in the brain is confirmed as stimulation of prototypic IFN-stimulated genes is found exclusively in the brain, but not in peripheral tissues. Body weight monitoring reveals a profound difference in the body weight trajectory between adult wildtype and transgenic mice. Compared with controls, the body weight gain is significantly reduced in the transgenic mice after 6 months of age. Such reduction is progressive, and the weight gap between the two groups of mice becomes greater, from 9.7% at 6 months of age to 31.7% at 12 months of age. Measurement of blood glucose indicates a low glucose level in transgenic mice (119.7 ± 6.1) compared with the controls (150.6 ± 10.8) ($p < 0.02$). However, feeding behavior tests show unchanged food or water intake between the two groups. ELISA reveals a remarkable decrease in plasma leptin concentration in mice with IFN-α expression ($p < 0.001$). Western blots show a significant increase in STAT1 expression and activation in the brain regions confirming IFN-α-triggered JAK/STAT signaling. Nevertheless, cerebral expression and activation of the molecules implicated in leptin signaling such as STAT3, Akt, Erk1/2 remains unchanged between GFAP-IFNα and control mice. In conclusion, our findings demonstrate that chronic exposure of the brain to antiviral cytokine IFN-α would decrease body weight gain, and such effect may be mediated by dysregulated leptin signaling.

Disclosures: J. Wang: None. **Y. Dai:** None.

Poster

545. Neuroimmunology: Behavioral Effects

Location: Halls A-C

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Program#/Poster#: 545.15/PP4

Topic: E.02. Neuroimmunology

Title: Effect of cold shock on neural function and behavior

Authors: *H. M. CHARLES, K. A. KILLIAN
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Abstract: During its lifetime, an animal can be exposed to varying environmental temperatures, and large fluctuations in temperature can influence survival. Even if survival is not directly comprised, exposure to temperature extremes could have lasting impacts on an animal's physiology and behavior. At low temperatures, many insects enter a state of immobility called chill coma from which an insect can recover. Our goal was to characterize the long-term effects of a single cold shock on the behavior, neural function and immune function of male *Acheta domesticus* crickets. Crickets, 9-12 days in age past the adult molt, were placed into individual conical tubes submerged in an alcohol bath at 0C for 6 hr. After removal to 20°C, cold-shocked males took approximately 2.5 hr to recover and right themselves. To examine the effects of the shock on general neural function, a set of age-matched cold-shocked (CS, n=24) and non-cold-shocked control (NCS, n=24) males were given a series of mechanical touches 24 hr after shock. CS males produced significantly fewer jumps in response to such stimulation (p=0.005). We also assessed long term effects on agonistic behavior. Seven days after shock, a pair of age- and weight-matched CS and NCS males was placed in an arena separated by a barrier. After 15 min, the barrier was lifted and each fight scored. All pairs fought and fights reached a level of aggression similar to that previously observed for fights between control males. However, CS males were more likely to become subordinate (67%, n=21 pairs) in such trials. We examined the effects of a cold-shock on immune function since a previous study in our lab suggested a direct link between immune function and aggression. CS males had significantly lower hemolymph activity of the enzyme phenoloxidase (PO) 7 days after shock. PO converts DOPA into melanin during the insect encapsulation response; PO activity in each blood sample was thus measured as change in absorbance per min. CS males exhibited a significantly lower total blood PO activity relative to NCS males (n=8 both groups; CS: 2.15 Δ absorbance/min; NCS: 7.52 Δ absorbance/min; p<0.0001). We also examined direct effects of a cold shock on the brain by injecting CS and NCS males with BrdU 48 hr after shock. We observed a 10-fold greater number of BrdU+ cells directly below the neural lamella on the brain surface of CS males (n=5) *vs*. NCS

males (*n*=5). We hypothesize these are perineurial glia proliferating in response to the shock; we are currently performing experiments testing this idea. Our results thus support that exposure to a single cold shock can have lasting impacts on brain function, immune function and behavior.

Disclosures: H.M. Charles: None. **K.A. Killian:** None.

Poster

545. Neuroimmunology: Behavioral Effects

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Topic: E.02. Neuroimmunology

Support: 1SC2 MH085472 NIMH/NIGMS

69172-0038 PSC CUNY

61214-0039 PSC CUNY

65379-0043 PSC CUNY

Title: Mold exposure alters adult new neuron migration in the mouse hippocampus

Authors: *A. LOPEZ[1], K. PAGE[1,2], E. NORMAND[3], B. SHAUKAT[1], N. ADAMS[3], K. LIN[3], L. BICKERTON[3], C. F. HARDING[2,3], C. L. PYTTE[1,2]
[1]Psychology, Queens College, CUNY, Flushing, NY; [2]Grad. Center, CUNY, New York, NY; [3]Hunter College, CUNY, New York, NY

Abstract: Exposure to environmental mold is a growing concern for individuals living or working in water-damaged buildings. In addition to a suite of somatic effects, mold exposure is also associated with cognitive impairments. Alarmingly, neurologists cannot differentiate between cognitive deficits in patients exposed to mold and those with mild traumatic brain injury. Prior research in our lab has found that mold exposure leads to a decrease in hippocampal-dependent memory in a contextual fear task. We have also demonstrated that mold exposure leads to a significant decrease in hippocampal new neuron survival assessed in mature (~35-day old) neurons. Moreover, numbers of mature new neurons were negatively correlated with numbers of cells expressing the inflammatory interleukin-1 beta (IL-1β). Here we further characterize the effects of mold on hippocampal neurogenesis. To determine whether mold inhalation specifically targets the survival of mature neurons, we quantified numbers of younger

hippocampal neurons (<30 days old) expressing doublecortin (DCX) in mice treated with mold. New neurons are primarily incorporated into the granular layer of the dentate gyrus; however, in damaged brains, ectopic neuron incorporation is seen, particularly in the hilar region. Therefore, we also assessed the relative numbers of mature new neurons seen in the granular layer and hilus. C57BL/6 mice were given intranasal instillations of 1) intact Stachybotrys spores (IN), 2) extracted Stachybotrys spores that had toxins removed and proteins denatured leaving skeletal elements (EX), or 3) saline vehicle (VEH). Mice were treated 3x per week for 6 weeks. We injected mice with bromodeoxyuridine (BrdU) 31-37 days and 3 hours before sacrifice. Immunohistochemistry was used to visualize cells expressing BrdU and the neuronal marker Hu. We also labeled cells expressing doublecortin and the inflammatory marker, IL-1β. We found that numbers of mature new neurons were negatively correlated with IL-1β, whereas numbers of young neurons were positively correlated with IL-1β. This suggests that inflammatory effects of mold exposure decrease new neuron survival specifically in older neurons (by age 31-37). Increased numbers of young DCX-expressing neurons may reflect compensation for cell death of older cohorts. In addition, mice exposed to mold spores had fewer new neurons in the granular layer and greater numbers of new neurons in the hilus than vehicle controls. These findings suggest that impaired hippocampal-dependent learning may also be associated with aberrant new neuron migration in addition to decreased new neuron numbers.

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Poster

545. Neuroimmunology: Behavioral Effects

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CUNY CIRG 1937 to CFH & CP

Research Centers in Minority Institutions Award RR-03037 from the NCRR to Hunter College

Title: Environmental mold exposure leads to spatial memory deficits

Authors: *D. LIAO[1], C. HARDING[1,2], R. PERSAUD[1], K. LIN[1], K. PAGE[2,3], C. L. PYTTE[2,3]
[1]Psychology, Hunter College, CUNY, New York, NY; [2]Behavioral & Cognitive Neurosci. Doctoral Program, Grad. Center, CUNY, New York, NY; [3]Psychology, Queens College, CUNY, New York, NY

Abstract: An estimated 40% of American buildings contain mold. Exposure to environmental mold can cause motor impairments, chronic fatigue, and cognitive deficits. To our knowledge, no animal research has been published examining how mold causes these problems. We developed a mouse model to determine the physiological mechanisms behind these neurobehavioral issues. Adult male C57BL/6 mice were given intranasal instillations (3X/week) of 1) intact, toxic *Stachybotrys* spores (IN), 2) extracted *Stachybotrys* spores with their toxins removed and proteins denatured (EX), or 3) the saline vehicle. Our basic hypothesis is that mold inhalation activates an innate immune response, leading to brain inflammation and consequent behavioral impairment. Because innate immune activation specifically impairs hippocampal function, we examined the effects of mold exposure on performance in the Morris water maze using a two-day protocol that compared the mouse's ability to find the nonvisible platform after four visible training trials 24hr previously. As predicted, mold exposure caused significant deficits in spatial memory. After 4.5-5.5 weeks treatment, EX mice performed significantly worse in finding the hidden platform compared to VEH or IN mice. EX mice showed the greatest deviation from their performances during visible training trials. They took significantly longer to reach the hidden platform and used longer paths. Performance in locating the visible platform predicted performance in locating the hidden platform for VEH mice. The same was not true for the spore-treated groups. Greater durations/path lengths and inconsistent performance suggest memory impairment in EX and IN mice. Performance on the water maze was inversely correlated with numbers of interleukin-1β (IL-1β)-immunoreactive cells in hippocampal CA1 (r^2 = -0.56, p = 0.01). This relationship was stronger for the spore-treated groups, consistent with spatial memory impairment resulting from brain inflammation due to mold treatment. Spatial memory deficits on both the last training trial (r^2 = 0.52, p = 0.004) and the first testing trial (r^2 = 0.59, $p < 0.001$) also correlated positively with weight gain during the first three weeks of treatment. Once again, this relationship is stronger for the spore-treated mice. This was not surprising since energy reserves modulate immune responses. It is unclear why treatment with spore skeletons (EX) caused greater impairment than treatment with intact, toxic spores (IN). Clearly, the spore skeleton is sufficient to elicit adverse cognitive effects. These findings are consistent with brain inflammation as a cause of neurobehavioral dysfunction.

Disclosures: D. Liao: None. **C. Harding:** None. **R. Persaud:** None. **K. Lin:** None. **K. Page:** None. **C.L. Pytte:** None.

Poster

545. Neuroimmunology: Behavioral Effects

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1SC2 MH085472 from NIMH

69172-0038 PSC CUNY

61214-0039 PSC CUNY

65379-0043 PSC CUNY

Title: Mold-induced changes in microglial morphology: A method for quantifying dimensions

Authors: *K. PAGE[1,2], M. NAGAI[3], T. DESAI[4], C. F. HARDING[1,3], C. L. PYTTE[1,2]
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Abstract: Histologically, microglia are frequently divided into two phenotypes: ramified and amoeboid. However, this classification is clearly limiting and much debate surrounds where the dividing point for the two categories should be. I propose to assess microglial phenotypes along a continuum. This method was used to analyze microglial morphology in the dorsomedial granular dentate gyrus of mice exposed to intact toxic mold spores, spore skeletons, or control vehicle. IBA-1-expressing cells were traced with closed area contours using NeuroLucida software (MBF). These contours provide valuable information about the cell such as area, roundness and distance to the nearest cell. Across all animals, increased numbers of microglia were correlated with larger microglial cell bodies ($p=0.03$). Numbers of IL-1β-expressing cells were also correlated with larger microglia ($p=0.04$). Together, these findings indicate that brains with higher numbers of microglia also had larger, more inflamed, microglia. We also assessed microglia distribution by comparing the number of cells per area with average distance between cells. In the control condition, cell density was positively correlated with cell distance, suggesting microglia were evenly distributed throughout the granular layer of the dentate gyrus. However, following mold exposure there was no correlation between cell density and cell distance. We believe this is represents a change in microglial territoriality following mold-exposure. To determine if different measures of microglial phenotypes predicted outcomes in a

hippocampal-dependent memory task, values of morphological measures were correlated with behavioral measures from the same animals. Increased numbers of microglia and larger microglia both were correlated with decreased memory in a contextual fear task 24 hours post training ($p=0.049$, $p=0.046$). Taken together, these data suggest this method of quantifying soma size of microglial cells can provide useful information pertaining to the activation state of the cells. Furthermore, morphological measurements can be useful to help identify specific characteristics of microglia corresponding to neurological and behavioral measures in an effort to better understand what phenotypic changes relate to experimental outcomes.

Disclosures: K. Page: None. **M. Nagai:** None. **T. Desai:** None. **C.F. Harding:** None. **C.L. Pytte:** None.

Poster

545. Neuroimmunology: Behavioral Effects

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 545.19/PP8

Topic: E.02. Neuroimmunology

Support: Duke University Bass Connections Grant

National Science Foundation Graduate Research Fellowship

Title: Developmental programming of body weight, neuroinflammation, and behavior by western diets

Authors: *M. G. WILEY[1], J. L. BOLTON[2], L. A. SIMMONS[3], B. RYAN[2], S. TRUONG[2], S. D. BILBO[2]
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Abstract: Obesity is now a multigenerational epidemic. Alarmingly, maternal obesity during gestation/lactation can "program" offspring long-term for increased obesity themselves, along with increased vulnerability to mood disorders. Emerging evidence suggests that this programming by perinatal diet is propagated via inflammatory mechanisms, specifically linked to two components that are enriched in a "Western diet": saturated fatty acids and branched-chain amino acids (BCAAs). We have previously shown that maternal high-fat diet (HFD) can "prime" microglia, the primary immune cells of the brain, and result in elevated levels of

proinflammatory cytokines within the hippocampus of adult offspring, in conjunction with increased anxiety. BCAAs are known to compete with tryptophan transport across the blood-brain barrier, thus resulting in decreased serotonin production and increased anxiety. In the current study, we tested the hypothesis that the HFD and BCAA dietary components would synergize to result in exacerbated brain and behavioral consequences in offspring. We placed female mice on one of 4 diets: 1) high-fat diet (HFD), 2) low-fat diet (LFD), 3) HFD supplemented with BCAA (HFD+BCAA), or 4) LFD supplemented with BCAA (LFD+BCAA) for 6 weeks prior to breeding, resulting in the following weight pattern: HFD+BCAA > HFD = LFD+BCAA > LFD. HFD+BCAA dams had pups with significantly lower birth weights, and qPCR analysis of offspring brains at postnatal day (P)1 revealed that HFD and HFD+BCAA pups had decreased expression of microglial markers (e.g., Iba-1, CD11b), suggesting that microglial colonization and maturation may be delayed or altered in these groups. At weaning (P28), HFD pups weighed more than LFD, whereas both BCAA groups weighed significantly less. Despite placement on a LFD at weaning, HFD and HFD+BCAA offspring showed signs of increased anxiety- and depressive-like behavior as adults. Analyses are currently ongoing to determine whether microglia are primed long-term by these perinatal diets, as well as whether peripheral infiltrating macrophages play a role in the neuroinflammation of the adult offspring (as has been observed in other models), and what, if any, are the consequences for the serotonergic system. In sum, perinatal diet, particularly HFD, can program body weight, microglial development, and behavior of offspring into adulthood.

Disclosures: **M.G. Wiley:** None. **J.L. Bolton:** None. **L.A. Simmons:** None. **B. Ryan:** None. **S. Truong:** None. **S.D. Bilbo:** None.

Poster

545. Neuroimmunology: Behavioral Effects

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Topic: E.02. Neuroimmunology

Support: NIH Grant R21NR012845-01A1

Title: Inflammation-induced fatigue: Exploring neurobiological mechanisms and potential treatments

Authors: *D. BONSALL[1], H. KIM[1], A. M. PETRONZIO[1], P. C. MOLYNEUX[1], T. E. SCAMMELL[2], M. E. HARRINGTON[1]
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Abstract: The induction of fatigue is a common response of an animal to systemic challenge by pathogens. This response is in part mediated through action of the pro-inflammatory cytokine interleukin-1 beta (IL-1). We have characterized a dose of peripherally-administered IL-1 that can reduce wheel-running and general locomotor activity in middle-aged (6-12 mo) C57Bl/6 mice, without induction of other signs of sickness, such as fever, muscle ache or anhedonia (as measured with abdominal temperature probes, pre-treatment with the analgesic buprenorphine and through sucrose preference respectively). Here we report our studies of the effects of IL-1 on two possible candidate pathways implicated in fatigue, the orexin-containing neurons of the lateral hypothalamus and the circadian rhythmic neurons in the suprachiasmatic nucleus (SCN), as well as the impact of several possible pharmacological treatments. Prior research has shown that LPS- and chemotherapy-induced reductions in general locomotor activity were associated with fewer Fos-positive orexin neurons and could be reversed by i.c.v. administered orexin (1,2). Here we first replicated prior work showing reduced wheel use in orexin-/- mice. We further demonstrate reduced wheel-running activity (normalized to baseline) following i.p. administration of 400ng IL-1 in middle-aged male and female orexin-/- mice, equivalent to the response seen in wild-type controls. This suggests that orexin is not necessary for IL-1-induced reductions in wheel-running. Given that patients with fatigue show dampened daily cortisol rhythms and disruptions in sleep-wake cycles, we hypothesized fatigue may be associated with deficits in circadian output from the SCN. We used mPer2luc/+ mice (3) to show altered SCN responses to shifted light-dark cycles following chronic administration of IL-1. Our results suggest that following IL-1 administration, the SCN's ability to drive coordinated output rhythms may be impaired. Given that the availability and success of therapeutic treatments for fatigue is currently limited, we examine the effectiveness of three potential clinical treatments in our animal model of fatigue. These include the stimulants modafinil and methylphenidate as well as the histamine H3 inverse agonist/antagonist pitolisant. We demonstrate the varying success of different potential treatments in restoring locomotor activity following IL-1 administration. These studies will allow us to determine possible neural pathways through which IL-1 induces fatigue. 1.Grossberg et al., J Nsci (2011) 31:11376-86. 2. Weymann et al., Brain Behav Immun (2014) 37:84-94. 3. Yoo et al. PNAS (2004) 101:5339-5346

Disclosures: D. Bonsall: None. **H. Kim:** None. **A.M. Petronzio:** None. **P.C. Molyneux:** None. **T.E. Scammell:** None. **M.E. Harrington:** None.

Poster

545. Neuroimmunology: Behavioral Effects

Location: Halls A-C

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Program#/Poster#: 545.21/PP10

Topic: E.02. Neuroimmunology

Title: Chronic T. gondii infection in the Nurr1-null heterozygous mice exacerbates elevated open field activity and disrupts sensorimotor gaiting

Authors: *J. B. EELLS[1], A. VARELA-STOKES[2], S. X. GUO-ROSS[2], E. KUMMARI[2], H. M. SMITH[2], E. COX[2], D. S. LINDSAY[3]
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Abstract: Infection with *Toxoplasma gondii* is common in humans (approximately 30% of the global population) and is a significant risk factor for schizophrenia. Since prevalence of *T. gondii* infection is far greater than prevalence of schizophrenia (around 0.5-1%), genetic risk factors are likely also necessary to contribute to schizophrenia. To test this concept in an animal model, Nurr1-null heterozygous (+/-) mice and wild-type (+/+) mice were evaluated using an emergence test, activity in an open field and in a novel object, response to bobcat urine and prepulse inhibition of the acoustic startle response (PPI) prior to and 6 weeks after infection with *T. gondii*. In the emergence test, *T. gondii* infection significantly decreased the amount of time spent in the cylinder. *Toxoplasma gondii* infection elevated open field activity in the female +/- mice, but not the female +/+ mice, and in both the male +/+ and +/- mice, but with a significantly greater effect in the male +/- mice. Additionally, *T. gondii* infection disrupted PPI only in the male +/- mice. The +/+ female mice showed a significant aversion to bobcat urine which was lost after infection with *T. gondii*. Antibody titers to *T. gondii* were a critical variable associated with changes in open field activity, with a significant increase in activity at low and medium antibody levels but no effect at high antibody levels. These data are the first to show disrupted sensorimotor gating with *T. gondii* infection and demonstrate that the Nurr1 +/- genotype predisposes mice to *T. gondii* induced alterations in dopamine-related behaviors that are correlated with symptoms of schizophrenia. We propose that these alterations in murine behavior are due to further exacerbation of the altered dopamine neurotransmission and identification of mechanisms driving those alterations will help to elucidate how *T. gondii* infection contributes to the development of schizophrenia.

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Poster

545. Neuroimmunology: Behavioral Effects

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SfN-IBRO

Title: The effect of interleukin 1-beta (IL-1β) on memory reconsolidation is mediated by a reduction in glutamate release, calcium influx and AMPA phosphorylation. Modulation by alpha-melanocyte-stimulating hormone (α-MSH)

Authors: *I. MACHADO[1], P. GONZALEZ[1], A. VILCAES[2], M. LASAGA[3], T. SCIMONELLI[1]
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Abstract: The immune system is an important modulator of learning, memory and neural plasticity. Interleukin 1β (IL-1β), a pro-inflammatory cytokine, significantly affects several cognitive processes. Previous studies of our group have demonstrated that the intrahippocampal administration of IL-1β impairs reconsolidation of contextual fear memory. This effect was reversed by the melanocortin alpha-melanocyte-stimulating hormone (α-MSH). The mechanisms underlying the effect of IL-1β on memory reconsolidation have not been established yet. Our results demonstrate that IL-1β produced a significant decrease in the glutamate release from dorsal hippocampus synaptosomes after reactivation of the fear memory. Examination of the cytosolic Ca2+ using Fluo-3AM revealed that the inhibition of glutamate release could be attributed to a reduction in voltage-dependent Ca2+ influx. Also, western blot analysis demonstrated that IL-1β reduced the phosphorylation of GluR1 AMPA subunit. The intrahippocampal administration of α-MSH can modulate these effects. Our results establish a possible mechanism involved in the detrimental effect of IL-1β on memory reconsolidation and also that α-MSH may exert a beneficial modulatory role in preventing IL-1β effects.

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Poster

545. Neuroimmunology: Behavioral Effects

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Title: IL-1 receptor antagonism in the basolateral amygdala reduces binge-like ethanol intake of C57BL/6J mice

Authors: *S. A. MARSHALL[1,2,3], J. A. RINKER[4,3], J. D. CASACHAHUA[4], D. T. LYSLE[4], T. E. THIELE[4,3]
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Abstract: The transition from experimental binge alcohol consumption to ethanol abuse and dependence is thought to be a result of neuroplastic changes that fundamentally alter both biological and behavioral functions. The ability of chronic alcohol exposure to alter the neuroimmune system has recently come to the forefront. Maladaptations within the neuroimmune system have been shown to underlie addictive-like behaviors in other drugs of abuse including opioids. Moreover, problems within the neuroimmune system are associated with several alcoholism comorbidities such as depression and pain dysregulation. It has previously been established that excessive ethanol consumption alters the neuroimmune system of human alcoholics, but the implications of these changes, particularly a shift in the cytokine profile, have yet to be determined. Our lab has recently found that following the “drinking in the dark” (DID) paradigm, there are increases in IL-1β mRNA and immunoreactivity in the

basolateral amygdala (BlA). The current study furthers this research by measuring ethanol consumption following antagonism of the IL-1 receptor in the BlA. Male C57BL/6J, underwent stereotaxic bilateral cannulae placement into the BlA and were given a week of recovery before DID procedures. Each DID trial, mice were allowed two hours of access to 20% ethanol beginning for three days, however, on the fourth day, or test day, mice were given four hours of ethanol access. Animals underwent DID procedures twice, receiving either IL-1 receptor antagonist (IL-1Ra) or saline on the test day in a 2 x 2, Latin-square design. Tail blood samples were collected at the end of each test session for blood ethanol concentration (BEC) determination. Following all testing, animals were perfused to ensure accurate cannulae implantation. T-tests were used to determine if IL-1R antagonism had a significant effect on consumption and BECs. IL-1Ra reduced ethanol consumption by 57%, reducing average consumption from 4.3g/kg to 2.5g/kg. The reduced consumption attributed to IL-1Ra manipulation also resulted in reduced BECs (Saline = 87.5±9.9; IL-1Ra = 46.0± 7.6). These data align with other findings that anti-inflammatory agents such as ibudilast and minocycline can reduce ethanol self-administration; however, this study indicates a specific role of IL-1β and IL-1 receptor signaling in the BlA in alcohol consumption. The actions of IL-1Rathat lead to reduced consumption still warrant investigation; however, this data suggest that manipulating the neuroimmune system remains a viable target for alcohol use disorder therapeutics. (Support by NIH grants AA022048, AA013573, AA015148, AA011605 & NIGMS GM000678).

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Poster

545. Neuroimmunology: Behavioral Effects

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Topic: E.02. Neuroimmunology

Support: NIH/NIA AG034113

Title: Meningeal sinuses: Key site for T cells in regulating brain function?

Authors: *A. LOUVEAU[1,2], S. GADANI[1], N. DERECKI[1], T. HARRIS[1], J. KIPNIS[1]
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Abstract: Meningeal sinuses: key site for T cells in regulating brain function ? Antoine Louveau, Sachin Gadani, Noel Derecki, Tajie Harris, and Jonathan Kipnis. Recent studies have enlightened the role of T lymphocytes in learning and memory and more precisely the pro-cognitive effect of accumulation of IL-4 secreting CD4 T cells in the meninges. However, the specific localization of T cells within the meningeal space and the signal that drives them into the meninges under physiological condition remains poorly understood. Using confocal microscopy of brain slices and dura/arachnoid whole mount, we were able to show that the vast majority of T cells reside in the close vicinity to or even within the brain sinuses located in the dura matter. T cells were also found in other meningeal compartments, such as dura, arachnoid, pia and choroid plexus, but at a much lower density. Interestingly, after a cognitive task, we measured a specific increase of T cells density within the sinuses but not in the other areas of the meninges suggesting that the sinuses are the major site for T cell function in the meninges. Immunostaining and *in vivo* injection of fluorescent CD45 antibody indicated an abluminal localization of the T cells suggesting that the sinus might be a gate for T cell trafficking into the meninges. Moreover, qPCR analysis of whole meninges and choroid plexus from naive and trained mice showed specific increase of CCL5, CCL8 and CXCL9 in the meninges after cognitive task performance, suggesting a chemokine-induced recruitment of T cells during cognitive task. Overall, this study will allow the development of new tools to study the structurally and functionally poorly understood brain sinuses and decipher the path of T cell entry into the meninges under physiological conditions. Understanding the physiological paths, may shed a new light on etiology of neuroinflammatory conditions, such as Multiple Sclerosis.

Disclosures: A. Louveau: None. **S. Gadani:** None. **N. Derecki:** None. **T. Harris:** None. **J. Kipnis:** None.

Poster

545. Neuroimmunology: Behavioral Effects

Location: Halls A-C

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Program#/Poster#: 545.25/PP14

Topic: E.02. Neuroimmunology

Title: Chronic fatigue syndrome from vagus nerve infection: A psychoneuroimmunological hypothesis

Authors: *M. B. VANELZAKKER[1,2]
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Abstract: Chronic fatigue syndrome (CFS) is an often-debilitating condition of unknown origin. There is a general consensus among CFS researchers that the symptoms seem to reflect an ongoing immune response, perhaps due to viral infection. Thus, most CFS research has focused upon trying to uncover that putative immune system dysfunction or specific pathogenic agent. However, no single causative agent has been found. Here, I describe a new hypothesis for the etiology of CFS: infection of the vagus nerve. When immune cells of otherwise healthy individuals detect any peripheral infection, they release proinflammatory cytokines. Chemoreceptors of the sensory vagus nerve detect these localized proinflammatory cytokines, and send a signal to the brain to initiate sickness behavior. Sickness behavior is an involuntary response that includes fatigue, fever, myalgia, depression, and other symptoms that overlap with CFS. The vagus nerve infection hypothesis of CFS contends that CFS symptoms are a pathologically exaggerated version of normal sickness behavior that can occur when sensory vagal ganglia or paraganglia are themselves infected with any virus or bacteria. Drawing upon relevant findings from the neuropathic pain literature, I explain how pathogen-activated glial cells can bombard the sensory vagus nerve with proinflammatory cytokines and other neuroexcitatory substances, initiating an exaggerated and intractable sickness behavior signal. According to this hypothesis, any pathogenic infection of the vagus nerve can cause CFS, which resolves the ongoing controversy about finding a single pathogen. The vagus nerve infection hypothesis offers testable hypotheses for researchers, animal models, and specific treatment strategies.

Disclosures: M.B. VanElzakker: None.

Poster

545. Neuroimmunology: Behavioral Effects

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Topic: E.02. Neuroimmunology

Support: NIH Grant 5U42OD010918-15

Title: The gut microbiota as an important intercurrent variable in behavioral research

Authors: A. C. ERICSSON, D. J. DAVIS, C. L. FRANKLIN, *C. E. HAGAN
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Abstract: The NIH has highlighted concern about the reproducibility of research, particularly preclinical research using animal models. Our group has established that there is significant variability in enteric microbial ecology among different vendors and genetic backgrounds of mice. Given that host-microbe interactions are gaining recognition as important factors shaping neurodevelopment, brain function, and behavior, we hypothesized that the gut microbiota could be a source of variability that could compromise reproducibility in studies of brain function and behavior. To test this hypothesis, we used a multi-dimensional experimental approach involving microbiota transfers, next-generation sequencing, cell culture, behavioral testing, and measures of peripheral immune function. Specifically, we sought to determine how two different vendor-specific microbial communities affect brain function and behavior when transferred into genetically identical mice. Our results suggest that a more complex microbiota confers enhanced stress resilience and reduced anxiety. This conclusion is based on measurements of behavior, serum cytokines, serum corticosterone, and cytokine production by splenocytes, microglia, and astrocytes. These results underscore the need to consider the gut microbiota as an important intercurrent variable in behavioral research.

Disclosures: A.C. Ericsson: None. **D.J. Davis:** None. **C.L. Franklin:** None. **C.E. Hagan:** None.

Poster

545. Neuroimmunology: Behavioral Effects

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Topic: E.02. Neuroimmunology

Support: NIMH Grant R01 MH090127

Title: Neurotoxic kynurenine metabolism mediates inflammation induced behavioral despair in mice

Authors: *J. M. PARROTT[1,2], L. REDUS[1], J. C. O'CONNOR[1,2]
[1]Pharmacol., Univ. of Texas Hlth. Sci. Ctr. At San Antonio, San Antonio, TX; [2]Ctr. for Biomed. Neurosci., San Antonio, TX

Abstract: Mounting evidence indicates that the kynurenine pathway of tryptophan metabolism plays an important role in mediating the behavioral effects of inflammation, which has implications for a number of neuropsychiatric disease populations. Both human and rodent studies have demonstrated that increased kynurenine metabolism is associated with depressive symptoms or preclinical depressive-like correlates, and inhibition of the rate limiting kynurenine pathway enzyme, indoleamine 2,3 dioxygenase, prevents the development of most inflammation-induced depressive-like behaviors. However, the kynurenine pathway consists of two functionally distinct metabolic branches with either excitatory or inhibitory effects on glutamatergic neurotransmission. To determine if inflammation results in an excitatory imbalance in kynurenine metabolism, wild type (WT), heterozygous or homozygous kynurenine 3-monooxygenase (KMO) deficient mice were treated with either lipopolysaccharide (LPS, 0.5mg/kg) or saline. KMO is the rate limiting enzyme for the subsequent generation of excitatory and potentially neurotoxic metabolites, and following peripheral immune challenge with LPS, pro-inflammatory cytokine and KP enzyme expression, including KMO, was up-regulated in the brains of WT mice in a time-dependent manner. Consistent with previous reports, a robust depressive-like phenotype was observed in WT mice 24h post-LPS. However, KMO deficient mice were specifically protected from LPS-induced behavioral despair measured in the tail suspension test, not anhedonia (sucrose preference) or anxiety-like behaviors (open field test). Metabolite analysis confirmed that KMO-dependent kynurenine metabolism was increased in the brains of LPS-challenged WT mice, and KMO deficient mice failed to exhibit any metabolic activity along this branch. Finally, mice lacking the hydroxyanthranilic acid dioxygenase (HAAO) gene, which is responsible for producing the final excitatory kynurenine metabolite (quinolinic acid), were challenged with LPS. Similar to KMO deficient mice, LPS did not induce depressive-like behavior in these mice as assessed in the tail suspension test. Together these results suggest that excitatory kynurenine metabolites are important mediators of inflammation induced behavioral despair.

Disclosures: **J.M. Parrott:** None. **L. Redus:** None. **J.C. O'Connor:** None.

Poster

545. Neuroimmunology: Behavioral Effects

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Topic: E.02. Neuroimmunology

Support: The Ohio State University Startup Funds

Title: Mast cells in the neonatal brain: Sex differences, biochemical mediators and early life programming of social and anxiety behavior

Authors: *K. M. LENZ, A. GALAN
Psychology, The Ohio State Univ., Columbus, OH

Abstract: Innate immune cells in the brain regulate many processes that are necessary to normal neurodevelopment, including cell genesis, cell death, synaptogenesis and synaptic pruning. Another such process is brain sexual differentiation. We have previously characterized major sex differences in the number and activation profile of innate immune cells in the developing rodent preoptic area, including microglia and mast cells. Both ameboid microglia and mast cells are twice as numerous in neonatal male rats than in females (Lenz et al., 2013, J Neurosci). In the preoptic area, activation of both microglia and mast cells contributes to sex differences in the number of dendritic spine synapses and resultant masculinization of adult reproductive behavior (Lenz et al., 2013). A majority of mast cells are located within the hippocampal parenchyma as well as in the pia mater between the hippocampus, thalamus, and amygdala during development. We have recently found a similar sex difference in both mast cells and microglia in the developing hippocampus, with males having approximately twice as many ameboid microglia and mast cells as females on postnatal day (PN) 4 (quantified using Stereoinvestigator). We have begun to assess the crosstalk between microglia and mast cells during brain development and the consequences of this crosstalk for neuronal and behavioral development. Pharmacological activation of mast cells using Compound 48/80 (administered icv) during the first four postnatal days produced a male-typical activated microglial morphology in the female hippocampus, which was assessed on Iba1-stained tissue using computer-based morphometry software (Neurolucida). Neonatal stimulation of mast cell activation in females also led to an increased number of newly-born BRDU-positive cells in the hippocampus on PN4, which is a masculinized phenotype. Mast cell activation early in life also programs behaviors that depend upon the brain regions in which mast cells are found, including juvenile social play and anxiety behaviors, and males are more affected than females. Currently, we are assessing whether mast cell activation produces resultant changes in brain levels of mast cell derived mediators, including serotonin, histamine, proteases and inflammatory cytokines. Since mast cells are the main cellular mediator of the allergic response, we are testing whether allergic challenge of maternal dams during pregnancy leads to activation of mast cells in the fetal brain and resultant changes in the development of socioaffective behaviors. Together, these data show that mast cells play a crucial role in normal development of brain and behavior.

Disclosures: K.M. Lenz: None. **A. Galan:** None.

Poster

545. Neuroimmunology: Behavioral Effects

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Program#/Poster#: 545.29/PP18

Topic: E.02. Neuroimmunology

Support: NIH Grant R00AG040194

Title: Diet-induced obesity prevents spatial learning deficits following bacterial endotoxin exposure

Authors: *S. SETTI[1], A. LITTLEFIELD[2], C. DIAZ[2], A. JONES[2], S. JOHNSON[2], R. A. KOHMAN[2]
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Abstract: Diet-induced obesity (DIO) is associated with altered immune function. Activation of the immune system induces a series of behavioral changes, collectively known as sickness behavior, as well as impairs cognitive function. Prior research has shown that DIO can exaggerate and prolong the behavioral response to an immune challenge (Pohl et al, 2013), but whether DIO alters the learning deficits associated with inflammation is unknown. The current study examined whether DIO would influence the development of cognitive deficits following an immune challenge with the endotoxin, lipopolysaccharide (LPS). Adult female C57BL/6J mice were free-fed either a high-fat diet (HFD; 60% fat) or a control diet (CD; 10% fat) for a total of 5 months. After 4 months, mice were systemically injected with either LPS or saline, and 4 hours later tested for spatial-learning in the Morris water maze (MWM). One month later, mice received the same treatment (i.e., LPS or saline) they received prior to the MWM task. Four hours after treatment, brain, adipose, and spleen samples were collected. Behavioral data show that LPS administration impaired spatial learning in the CD mice, but diet-induced obese mice failed to develop cognitive deficits. Data collection is still in progress to determine whether the lack of a behavioral response in the DIO mice reflects a modified inflammatory response. Preliminary results show that hippocampal expression of the proinflammatory cytokines interleukin-1β and interleukin-6 did not significantly differ between the DIO and CD mice basally or following LPS administration. However, DIO mice failed to show an LPS-induced increase in expression of CD74, a molecule involved in trafficking major histocompatibility complex II (MHCII) proteins, in the hippocampus. Currently, our data indicate that DIO prevents the development of cognitive deficits following an immune challenge and may modify aspects of the neuroinflammatory response.

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Poster

546. Stress: Cellular Consequences

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

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Topic: E.05. Stress and the Brain

Title: A comparative analysis of GSK3β serine 9 and serine 389 inhibitory phosphorylation following acute challenge, variate stress, or voluntary exercise

Authors: *B. D. HARE[1], T. M. THORNTON[2], M. RINCON[2], D. M. JAWORSKI[3], W. A. FALLS[1]
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Abstract: Glycogen synthase kinase 3β (GSK3β) is a constitutively active protein kinase expressed throughout the central nervous system. Inhibition of GSK3β has been a therapeutic target for stress related disorders since its inhibition by lithium was discovered. GSK3β inhibition via increased phosphorylation at its N-terminus, serine 9 (S9), is associated with reduced depression-like behaviors. Similarly, GSK3β-S9 phosphorylation is increased by exercise, a behavior associated with reduced anxiety. GSK3β inhibition at its C-terminus, serine 389 (S389), by p38 MAPK was recently demonstrated by the Rincon laboratory. Strikingly, GSK3β S389-mediated inhibition is independent of S9 phosphorylation. To date, the role of GSK3β S389 phosphorylation in depression- and anxiety-like behaviors has not been examined. Acute forced swim and fluoxetine administration replicated previously reported GSK3β S9 phosphorylation effects but failed to modulate GSK3β S389 phosphorylation, demonstrating the independence of S389- and S9-mediated GSK3β inhibition. GSK3β phosphorylation was next examined following 14 days of variate stress or 14 days of exercise, both of which are metabolic challenges that induce hypothalamic pituitary adrenal axis activation, but are associated with different behavioral outcomes, increased and decreased anxiety respectively. Variate stress was associated with elevated GSK3β S389 phosphorylation in the hippocampus, amygdala and bed nucleus of the stria terminalis (BSNT). GSK3β S9 phosphorylation was increased in the BNST, and decreased in the amygdala following both exercise and variate stress exposure. Our findings suggest that S9 and S389 phosphorylation of GSK3β are differentially modulated by variate stress and voluntary exercise. Notably, changes in GSK3β S389 phosphorylation were most evident in the variate stress group suggesting that metabolic challenge alone is not sufficient to produce changes in S389 phosphorylation. Analysis of upstream regulators of GSK3β

phosphorylation is ongoing, as is analysis of the association of GSK3β with DNA damage following variate stress or voluntary exercise.

Disclosures: B.D. Hare: None. **T.M. Thornton:** None. **M. Rincon:** None. **W.A. Falls:** None. **D.M. Jaworski:** None.

Poster

546. Stress: Cellular Consequences

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 546.02/PP20

Topic: E.05. Stress and the Brain

Title: How the stress-induced protein DRR1 modulates actin dynamics

Authors: *A. KRETZSCHMAR[1], J.-P. SCHÜLKE[1], M. MASANA[3,2], M. B. MÜLLER[3,2], A. R. BAUSCH[4], T. REIN[1]
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Abstract: Being previously known as a potential tumor suppressor, DRR1 was recently characterized as a direct link between stress, actin dynamics and cognition at our institute. It was shown to localize to actin-rich cellular structures and primarily to synapses in neurons. While it decreases neurite outgrowth and reduces LTP magnitude and spine density, mice with viral DRR1-overexpression show enhanced cognitive performance (Schmidt et al., 2011). These findings suggest a protective function of DRR1 counteracting adverse stress effects. Its relevance becomes evident as failing of stress coping imposes an increased risk for depression, anxiety or post-traumatic stress disorder. Currently, we are dissecting the molecular mechanism, cellular and synaptic function of this intriguing protein with *in vitro* and *ex vivo* studies. It exerts a three-fold effect on actin dynamics by bundling filaments, inhibiting their elongation but also enhancing nucleation of new filaments. As the most prominent cytoskeletal component at the synapse, actin is a major player in many processes impacting on synaptic transmission like synaptic shape, neurotransmitter vesicle release and postsynaptic receptor distribution. However, up to now, a profound mechanistic understanding of the pathway from stress to neuronal reorganization and cognitive performance remains elusive. Assuming that the mechanism of DRR1 is not only significant for coping with chronic stress but also during tumor development

and progression, elucidating DRR1's mechanism of action could contribute to several physiologically relevant processes.

Disclosures: A. Kretzschmar: None. **J. Schülke:** None. **M. Masana:** None. **A.R. Bausch:** None. **T. Rein:** None. **M.B. Müller:** None.

Poster

546. Stress: Cellular Consequences

Location: Halls A-C

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Program#/Poster#: 546.03/PP21

Topic: E.05. Stress and the Brain

Support: NIMH IRP Research Program Project 1ZIAMH002386

NARSAD Young Investigator Grant 21356

Title: Stress-mediated signaling by the Gs-coupled GPCR PAC1 at the adrenomedullary synapse: Role of a novel camp sensor, NCS/Rapgef2

Authors: *A. C. EMERY[1], N. STROTH[1,2], T. MUSTAFA[1], L. E. EIDEN[1]
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Abstract: The neuropeptide PACAP is released at the adrenomedullary synapse, during stress, to mediate epinephrine secretion from, and promote catecholamine biosynthesis in, chromaffin cells (Stroth et al. *Endocrinology* **154**: 330, 2013). PACAP signaling also mediates stimulus-transcription coupling for additional bioactive peptides and proteins whose expression is up-regulated during the stress response (Ait-Ali et al., *Cell Mol. Neurobiol.* **30**: 1441, 2010). We now identify the neuroprotective peptide stanniocalcin 1 (Stc1) as up-regulated in wild-type, but not PACAP-deficient mouse adrenal gland after restraint stress, and characterize its regulation by PACAP via a novel cAMP-dependent pathway in primary bovine chromaffin cells (BCCs) in culture. Stc1 mRNA is increased ~3-fold within one hr following acute restraint stress in C57Bl/6N mice, and this increase is maintained across 6 hr of restraint and reversed upon cessation of restraint. PACAP-deficient mice exhibit wild-type basal expression levels, but no significant increase in Stc1 mRNA expression, following 6 hr of restraint. In primary BCC cultures, treatment with PACAP causes up-regulation of Stc1 gene expression mimicked by treatment with 8-CPT-cAMP, a cell-permeable cAMP analog. A novel cAMP sensor, the

neuritogenic cAMP sensor (NCS) has been identified as the protein product of the Rapgef2 gene (Emery and Eiden, *FASEB J.* **26**: 3199, 2012; Emery et al. *Sci. Sig*., **6**: ra51, 2013), and cAMP elevation causes activation of both NCS/Rapgef2, linked exclusively to ERK phosphorylation, and PKA, linked exclusively to CREB phosphorylation in the NS-1 pheochromocytoma cell line (Emery et al., *J. Biol. Chem*., **289**: 10126, 2014). Likewise, in primary BCCs (which like mouse chromaffin cells express the PAC1 receptor), either 8-CPT-cAMP or PACAP cause both CREB and ERK phosphorylation. Stimulation of ERK phosphorylation and up-regulation of Stc1 mRNA levels initiated by PACAP, forskolin, or 8-Br-cAMP are concomitantly blocked by inhibition of MEK with U0126 in BCCs. Additional cAMP-responsive genes in BCCs, including those encoding galanin (GAL) and vasoactive intestinal polypeptide (VIP) conform to this pharmacological response pattern. On the other hand, multiple transcripts, such as that encoding substance P (Tac1), show PACAP-dependent induction that is blocked by H89, indicating PKA-CREB-dependent regulation. We conclude that the stress transmitter PACAP activates both NCS/Rapgef2 and PKA via cAMP elevation, leading to ERK and CREB phosphorylation and activation of two sets of cAMP response genes encoding proteins with distinct functions, in chromaffin cells.

Disclosures: A.C. Emery: None. **N. Stroth:** None. **T. Mustafa:** None. **L.E. Eiden:** None.

Poster

546. Stress: Cellular Consequences

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

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Topic: E.05. Stress and the Brain

Support: FIRM Grant 4600 R14358

APC Grant 07/CE/B1368

APC Grant 12/RC/2273

SFI Grant 12/IA/1537

Title: The omega-3 polyunsaturated fatty acid docosahexaenoic acid (DHA) as a novel strategy for stress-related psychiatric disorders: reversal of corticosterone-induced changes in cortical neurons

Authors: *M. PUSCEDDU[1,2], Y. M. NOLAN[3], H. F. GREEN[4], P. KELLY[5], T. G. DINAN[2,1], J. F. CRYAN*[3,1]
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Abstract: Chronic exposure to stress can exert long-lasting changes on the brain increasing vulnerability to mental illness. Corticosterone (CORT) is the main rodent stress hormone that has been shown to exert negative effects on neuronal morphology and viability across a number of brain regions. Understanding the molecular and cellular basis of susceptibility and resilience to stress may open up novel therapeutic strategies for disorders such as depression and anxiety. Growing evidence suggests that omega-3 polyunsaturated fatty acids (PUFAs) may have a beneficial effect on health including mental health. Indeed, being constituents of the cellular membrane, they might play a critical role in the development and function of the central nervous system. However, the ability of PUFAs to abrogate the stress-induced toxic effects on neurons has not been well investigated. To this end, we studied the protective effect of the omega-3 PUFA docosahexaenoic acid (DHA), against CORT-induced cellular changes in a mixed cortical primary culture. We first characterized the effect of CORT (75, 100, 150, 200uM) at different time points (24, 48, 72 hours) in a mixed cortical primary culture over 10 days *in vitro* (DIV), prepared from rats at postnatal day 1-2. Cells were then pretreated with DHA (3, 6uM) at 2DIV. CORT (72 hours) induced a dose-dependent reduction in cellular viability as assessed by MTT. Moreover, we demonstrated that CORT (200uM - 72 hours) decreased the percentage composition of neurons whilst increasing the percentage of astrocytes as assessed by B-III tubulin and GFAP immunostaining, respectively. In contrast, DHA (6uM but not 3uM) attenuated CORT (200uM)-induced cell death (72 hours). This translated into a capacity for DHA to prevent neuronal death as well as astrocytes overgrowth following chronic exposure to CORT. Furthermore, DHA (6uM) reversed CORT-induced neuronal apoptosis as assessed by TUNEL, and attenuated CORT-induced reductions in BDNF and CREB mRNA expression. Finally, DHA inhibited CORT-induced down-regulation of GR expression on b-III tubulin-positive neurons. In conclusion, this work supports the view that DHA may be beneficial to ameliorate stress-related cellular changes in the brain and may be a beneficial strategy for stress-related psychiatric disorders.

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Poster

546. Stress: Cellular Consequences

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Topic: E.05. Stress and the Brain

Support: The Academy of Finland

Sigrid Juselius Foundation

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Magnus Ehrnrooth's Foundation

Title: The role of tyrosine hydroxylase-2 producing neurons of zebrafish in stress response

Authors: S. SEMENOVA, Y.-C. CHEN, *P. A. PANULA
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Abstract: The tyrosine hydroxylase (TH) gene is duplicated in zebrafish, and tyrosine hydroxylase 1 (TH1) is more closely related to mammalian THs than TH2. The largest groups of th2-expressing cells are found in the hypothalamus of zebrafish. Expression of the th2 gene is affected by social stress and adverse environmental conditions in adult and larval fish. Otherwise, little is known on the function of TH2-producing neurons and the regulation of their activity. We generated antibodies targeting zebrafish TH2 to study the morphology, projections and contacts of TH2 neurons. The antibodies were produced in hens (IgY) and rabbits injected with a GST-tagged N-terminal fragment of TH2. These antibodies also labeled TH1-producing cells, revealing the whole population of dopaminergic neurons in the zebrafish brain. Western blotting of adult zebrafish brain samples with rabbit or hen antibodies revealed both TH1 and TH2 protein bands, and affinity purification of the serum/IgY did not eliminate immunoglobulins reacting with TH1. TH2-producing cells, identified by labeling with the new TH2 antiserum only and not with a specific TH1 antiserum, comprised all neuron groups which express th2 mRNA. They received contacts from histaminergic and orexinergic neurons. None of the TH2-producing cells were immunopositive for serotonin. The first cells containing TH2 but not TH1 were detected in the larval zebrafish hypothalamus around 84 hours postfertilization. Immunoreactivity of TH2 cells with the new antiserum was abolished by morpholino oligonucleotide knockdown of th2 expression. Expression mapping of the immediate early gene c-fos combined with immunostaining for TH2 was used to characterize the response of zebrafish neurons to various types of stimuli. Handling stress induced c-fos expression in dopaminergic cells of the posterior tuberculum of larval fish. Changes in the ionic strength or pH of the environment, as well as addition of ammonium salts to the incubation medium, induced strong c-fos expression in the hypothalamus, including its caudal part where the TH2 cells are found. Neurons in the nuclei of lateral and posterior recess of the hypothalamus were activated by handling and antagonistic social interaction in adult fish. However, few of the c-fos expressing

cells were immunopositive for TH2. Therefore, TH2 cells of zebrafish are probably involved in long-term adaptive response to stress rather than in immediate brain reactions to stimuli.

Disclosures: S. Semenova: None. **P.A. Panula:** None. **Y. Chen:** None.

Poster

546. Stress: Cellular Consequences

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 546.06/PP24

Topic: E.05. Stress and the Brain

Support: FAPESP Grant 13/14199-3

CNPq

Title: Activation of noradrenergic neurons in the locus coeruleus by different stress paradigms

Authors: *I. R. DOS SANTOS[1], N. PESTANA-OLIVEIRA[2], J. A. ANSELMO-FRANCI[3], C. M. LEITE[3]
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Abstract: Acute stress can elicit endocrine, sympathetic and behavioral changes which allow the body to avoid sudden changes in the mechanisms that maintain the homeostasis. The stress response involves changes in the activation of noradrenergic (NE) system, primarily in the locus coeruleus (LC) neurons, increasing noradrenaline release in many stress-responsive brain regions and thereby mediating the feed forward drive to hypothalamic-pituitary-adrenal system responses to stress. Ovarian steroids may modulate stress response by acting on the LC. Since it is known that the stress response depends on the stress paradigm, stress magnitude, and the level of circulating plasma estradiol and that LC is activated by some of acutely stressful stimuli, but not by others, this study aimed to evaluate whether different acute stress paradigms applied on the morning of proestrus, when the estradiol levels are already high, could differentially activate the NE neurons of LC. At 10:00 h, proestrus female rats were submitted to one of the four stress paradigms: 1) restraint, 2) exposure to a cat, 3) uterine cervix stimulation or 4) all types of stress applied together. The rats were perfused 90 minutes after the beginning of stress section. The brains were removed and sections containing the LC were submitted to immunohistochemistry to

access tyrosine hydroxylase (TH) and c-Fos immunoreactive (-ir) neurons. Restraint stress, cervical stimulation and the association of all stress paradigms induced a similar increase in the number of Fos/TH-ir neurons in the LC; however this increase was not observed in the group exposed to the predator. These results suggest that the LC NE system plays an important role in mediating stress responses induced by restraint, uterine cervix stimulation and the association of all stress paradigms, but it seems not to participate in response to predator exposure. Interestingly, the association of all stress paradigms was not able to amplify the activation of LC neurons, suggesting that LC neurons are maximally activated after the exposure to one kind of stress and the association of all stress cannot overcome this response.

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Poster

546. Stress: Cellular Consequences

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Topic: E.05. Stress and the Brain

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ECRIP

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CWPW grant program

Childrens Health and Research Foundation

Title: Post transcriptional regulation of adrenal TH gene expression contributes to the maladaptive responses triggered by insulin-induced recurrent hypoglycemia

Authors: *B. B. NANKOVA[1], N. KIRTOK[2], O. CHAN[3], C. STERLING[4], A. TANK[4], E. F. LAGAMMA[5]
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Rochester, NY; [5]Pediatrics, Biochem. and Mol. Biol., New York Med. Col. and Regional Neonatal Center, Maria Fareri Childrens Hosp., Valhalla, NY

Abstract: Acute metabolic stress (insulin-induced hypoglycemia) triggers a counter-regulatory response during which release of catecholamines (epinephrine), activation of tyrosine hydroxylase (TH) enzyme and compensatory biosynthesis of catecholamines occur in the adrenal medulla. However, recurrent hypoglycemia (RH) in humans (consequence of the tight glycemic control in individuals with type 1 diabetes) and in animal models compromise the physiological and behavioral defenses against subsequent glucoprivation episode, including progressive loss of circulating epinephrine response. The molecular mechanisms underlying this maladaptive response to repeated stress are largely unknown. We hypothesized that the impaired epinephrine release following RH reflects altered regulation of adrenal catecholamine biosynthesis. To test this hypothesis we compared the effect of single daily and twice-daily episodes of insulin - induced RH on adrenal epinephrine release and production (as evidenced by altered TH gene transcription, steady state TH mRNA levels, Ser40-phosphorylated- and total TH enzyme molecules) in normal SD rats. Antecedent treatments were: once daily saline (1xSal); once daily insulin (1xRH); twice daily saline (2xSal) and twice daily insulin (2xRH) for 3 days. Counter-regulatory hormonal responses were monitored during hypoglycemic-hyperinsulinemic clamp on day 4 for all experimental groups. To analyze changes in TH gene expression in dissected adrenal medulla, sets of animals from each experimental group were sacrificed on day 4 before (at 0 time point), during the clamp (at 30 and 60 min) or after 3 hrs recovery period (time point for maximal TH mRNA induction). The counter regulatory responses, relative TH transcription and TH mRNA levels and Ser40-TH phosphorylation (marker for enzyme activation) were induced to a similar extent in 1xSal, 2xSal and 1xRH groups. The observed defective epinephrine release in 2xRH (reported before by others for this animal model of hypoglycemia-associated autonomic failure, HAAF) was associated with reduced more than 40% abundance of TH mRNA and transient Ser40-phosphorylation of TH. Accumulation of total TH protein was observed only following the recovery period in 1xRH animals. Thus, adrenal TH gene expression is subjected to differential regulation depending on the metabolic stress paradigm. Our results suggest that novel posttranscriptional mechanisms controlling TH mRNA and activated TH enzyme turnover contribute to the impaired epinephrine responses and may provide new therapeutic targets to prevent HAAF.

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Poster

546. Stress: Cellular Consequences

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 546.08/QQ2

Topic: E.05. Stress and the Brain

Support: Other Support - Graphic Era University

Title: Role of dexras1 and stress in triggering of type 2 diabetes mellitus

Authors: *A. THAPLIYAL[1], R. VERMA[1], M. THAPLIYAL[2], P. ANTHWAL[1], T. KAPOOR[1], P. SEMWAL[1]
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Abstract: Data from various sources project India to become, slowly, the "diabetes capital of world" as the number of diabetics is increasing steadily. In a sharp contrast, the number of patients of younger age groups, between the ages of 30 to 45, has increased tremendously. It has been generally observed that the lower age limit for diagnosis of diabetes in India is well into 30's. In case of type 2 diabetes mellitus, a healthy individual, after a certain time gap suddenly shows symptoms. This transition of a healthy to a diabetic individual should occur due to some molecular event. We carried out investigations to check our hypothesis that does dexras1, a monomeric G-protein, along with stress have any role in the triggering of type 2 diabetes. The basis of our hypothesis was that it is well known that Dexras1 is localized within pancreatic β-cells. It has been demonstrated that Dexras1 modulates insulin secretion in pancreatic β-cells and involves protein kinase A, protein kinase C, and ERK1/2 pathways. Today's lifestyle creates many stress conditions and stress up-regulates corticosteroid levels and this in turn influences. Our in-vitro data suggests that exposure of insulin secreting cell line to corticosteroid hormone (and dexamethasone) increases dexras1 expression. If the dexras1 expression increases is for a longer time period and continuous, it creates an imbalance of calcium ions inside the cells. This imbalance is sensed by the cells and somehow triggers slow cell death as dexras1 can interfere in calcium homeostasis. Further investigations into the molecular details are ongoing but our data suggests that under in-vitro conditions, long exposure to stress might be the trigger point for type 2 diabetes mellitus which is mediated by calcium imbalance and the exposure time is of great relevance. This finding will have profound effect on prevention of onset of diabetes mellitus at earlier age and suggests that lifestyle, especially stressful conditions might have a major role in triggering of type 2 diabetes mellitus and hence the brain connection is the most important factor.
KEY WORDS: Dexras1, type 2 diabetes mellitus, calcium, stress, event initiation signaling cascade

Disclosures: **A. Thapliyal:** None. **R. Verma:** None. **M. Thapliyal:** None. **P. Anthwal:** None. **T. Kapoor:** None. **P. Semwal:** None.

Poster

546. Stress: Cellular Consequences

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 546.09/QQ3

Topic: E.05. Stress and the Brain

Title: Temporal course of the serotonergic system of the medial amygdala in male rats subjected to restraint stress

Authors: *M. R. GONZALEZ LOPEZ[1], N. L. GARCÍA-SALDÍVAR[1], R. DOMÍNGUEZ[2], S. E. CRUZ-MORALES[1]
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Abstract: The amygdala is involved in the coordination of behavioral, autonomic and endocrine responses to stress and receives serotonergic innervations from the dorsal raphe. The medial nucleus of the amygdala has been involved with the stress response, especially with psychological stressors. Restraint stress (R) increase the 5-HT levels in the central amygdale, but not in medial amygdale nucleus. It is known that 5- HT may be an important mediator in the response to inescapable stress. The aim of present study was to evaluate in male rats the effect of exposure 60 minutes of restraint in serotonergic system of the medial amydala nucleus evaluated 0, 1, 24, 48 or 72 hr after restraint. Male Wistar rats (250-270 g) , were assigned to 6 independent groups: an unstressed control group (C) and five groups subjected to R during 60 min , and sacrificed at different times after the R: 0 , 1, 24 , 48 or 72 hr. Samples of the medial amygdala nucleus were taken to measure [5 - HIAA] and 5- HT by HPLC. The serotonergic activity was calculated as [5-HIAA]/ [5-HT]. Significant differences were observed in [5 -HT], 1 and 24 hr after R the concentration were similar to C group, a significant increase was detected after 48 and 72 hr, and no changes were detected at time 0 because the sensitivity was below of the method. For the [5 -HIAA] groups at time 0 and 24 hr showed low concentrations compared to C group, and a tendency to recovery was observed in 48 and 72 hr. Serotonergic activity decreased at time 0, increased at time 1, and a recovery was clear since 24 hr, no significant differences were observed at 48 and 72 hrs compared with C group. The results show that at time 0 the [5-HT] and [5 -HIAA] is low, this effect may be explained to the inhibition of neurotransmitter synthesis induced by R. Previously we reported that in the medial raphe nucleus the exposure to 15 min of restraint, to the elevated plus maze and to elevated T-maze decreased [5HIAA]. Some authors have observed increases in [5HT] in the central nucleus of the amygdala , with no effect in the medial nucleus in studies conducted in the dark phase, however these studies were realized at different times during the day. The forced swim stress increases

serotonergic activity in amygdale at time 0, but not assess of serotonergic activity was done beyond 24 hr. We conclude that the serotonergic system in the medial amygdala is very sensitive to the restraint effects and inhibits the synthesis 5HTand that recovery of serotonergic systems is time dependent.

Disclosures: M.R. Gonzalez Lopez: None. **N.L. García-Saldívar:** None. **R. Domínguez:** None. **S.E. Cruz-Morales:** None.

Poster

546. Stress: Cellular Consequences

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 546.10/QQ4

Topic: E.05. Stress and the Brain

Support: R01- MH074811

Title: Prenatal protein malnutrition in adult rats under stress results in increased activation of inhibitory interneurons in the anterior cingulate and medial prefrontal cortex

Authors: *X. WANG[1], A. C. AMARAL[2], F. MORTAZAVI[2], J. A. MCGAUGHY[3], D. J. MOKLER[4], J. R. GALLER[5], R. J. RUSHMORE[2], D. L. ROSENE[2]
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Abstract: Exposure to prenatal protein malnutrition (PPM) increases the risk of a number of neuropsychiatric disorders that are associated with inhibitory processes, including depression, schizophrenia and attention deficits in humans. Previous studies using a rat model of PPM and c-Fos as a marker of neuronal activation have found that neurons in anterior cingulate cortex (ACC) and medial prefrontal cortices (mPFC) respond excessively to restraint stress. Surprisingly, parallel studies in this same model using 2-deoxyglucose as a marker of metabolic activity show that ACC and mPFC in resting state are hypo-active. While there are several possible explanations for these differential observations, one possibility is that the c-Fos positive neurons might be largely GABAergic and hence damping down overall activity of the ACC and mPFC. In this study, subjects were P90 Long-Evans male rats born from mothers that were fed isocaloric diets containing either 25% protein (control) or 6% protein (malnourished), cross-fostered at birth to control mothers and maintained on normal laboratory chow after weaning and

throughout adulthood. We replicated the restraint stress paradigm but used double-label immunohistochemistry to identify c-Fos positive neurons, combined with parvalbumin to identify inhibitory interneurons. Numbers of single and double-labeled neurons in the ACC and mPFC were quantified with unbiased stereology. Statistical analysis demonstrated that there was no effect of PPM on the total number of neurons or on the number of parvalbumin neurons in either stressed or unstressed subjects. However, PPM rats showed a significant increase in the number of c-Fos positive neurons as well as in the number of inhibitory parvalbumin positive neurons double labeled with c-Fos within the stress condition. The total numbers of c-Fos positive neurons or the total numbers of double-labeled neurons in unstressed subjects were not different in the two nutrition groups. This suggests that PPM altered the excitability of these inhibitory interneurons either directly or by altering their connectivity. [Supported by: R01-MH074811]

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Poster

546. Stress: Cellular Consequences

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Topic: E.05. Stress and the Brain

Support: NIH 2R01

MH066958

the Pierson Endowment, Tulane University

Title: Tonic and phasic endocannabinoid heterosynaptic modulation in hypothalamic magnocellular neuroendocrine cells

Authors: S. DI, I. R. POPESCU, *J. G. TASKER
Tulane Univ., New Orleans, LA

Abstract: Recent evidence suggests that different endocannabinoids (eCBs) may be responsible for the effects of tonic and evoked eCB release at some synapses. Thus, activity-dependent eCB effects are often dependent on 2-arachidonoylglycerol (2-AG) synthesis and tonic CB1 receptor activation is mediated by anandamide (AEA) at some GABA synapses in hippocampal slice

cultures. Here, we tested for the differential dependence on 2-AG of evoked vs. tonic eCB actions at GABA synapses onto magnocellular neurons using whole-cell patch clamp recording recordings in acute brain slices. We tested for the 2-AG dependence of both depolarization- and glucocorticoid-induced suppression of GABA release in vasopressin (VP) and oxytocin (OT) neurons recorded in slices from dehydrated rats, in which reduced astrocytic coverage of neuronal membranes allows eCBs to spill over from glutamate synapses onto GABA synapses. Bath application of tetrahydrolipstatin (THL), a diacylglycerol lipase antagonist that blocks 2-AG synthesis, blocked both the depolarization- and glucocorticoid-induced suppression of GABA release in both VP and OT neurons, suggesting that evoked eCB actions are mediated by on-demand 2-AG synthesis in these cells. We next tested for the dependence of tonic CB1 receptor activation on 2-AG synthesis. Unlike the evoked eCB actions, tonic CB1 receptor activation was insensitive to changes in glial coverage and to blockade of 2-AG synthesis, suggesting that it was mediated by constitutive AEA release. Thus, AEA is released tonically at GABA synapses and is insensitive to glial buffering, while 2-AG is released phasically in an activity-dependent manner and its access to GABA synapses is regulated by astrocytes.
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Disclosures: S. Di: None. **I.R. Popescu:** None. **J.G. Tasker:** None.

Poster

546. Stress: Cellular Consequences

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 546.12/QQ6

Topic: E.05. Stress and the Brain

Title: Fluorescent visualization of central osmosensitive areas activated by acute osmotic stimulation in c-fos-eGFP transgenic rats

Authors: *Y. UETA, T. ARITOMI, K. SHOGUCHI, T. MATSUURA, M. YOSHIMURA, T. ISHIKURA, T. MARUYAMA, H. HASHIMOTO
Univ. Occupat, Environml Hlth, Japan, Kitakyushu, Japan

Abstract: We have generated transgenic rats that express the c-fos and eGFP fusion gene after adequate stimuli. In the present study we examined the effects of acute osmotic stimulation on the induction of the expression of the c-fos and eGFP fusion gene in the forebrain and brainstem of the transgenic rats. The expression of the c-fos and eGFP fusion gene was observed by fluorescent microscopy for eGFP fluorescence and immunohisotochemistry for Fos protein.

Ninety min after intraperitoneal administration of hypertonic saline in c-fos-eGFP transgenic rats, GFP fluorescence was appeared markedly in the circumventricular organs such as organum vasculosum of the lamina terminalis, median preoptic area and subfornical organ that are known to be osmosensitive areas, the paraventricular and supraoptic nuclei, and the area postrema and the nucleus of the solitary tract in the brainstem. The immunostaining for Fos protein was almost merged with GFP fluorescence induced by acute osmotic stimulation. The c-fos-eGFP transgenic rats are powerful tool to study neuronal circuits in the central nervous system after adequate stimuli.

Disclosures: Y. Ueta: None. **T. Aritomi:** None. **K. Shoguchi:** None. **T. Matsuura:** None. **M. Yoshimura:** None. **T. Ishikura:** None. **T. Maruyama:** None. **H. Hashimoto:** None.

Poster

546. Stress: Cellular Consequences

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 546.13/QQ7

Topic: E.05. Stress and the Brain

Support: CIHR 2011087-3

NSERC 98181

Title: Inhibition in the lateral septum increases sucrose overeating in rats with a history of repeated food restriction and stress

Authors: *A. MITRA, C. LENGLOS, J. CALVEZ, G. GUEVREMONT, E. TIMOFEEVA
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Abstract: Introduction: The Lateral septum (LS) is interconnected to the limbic, hypothalamic and reward centers of the brain to regulate coordinated behaviors such as stress response, feeding behavior and motivational states. LS lesion escalates sweet taste preference. Neuronal activity in LS is enhanced by stress and attenuated by access to sucrose. Stress responses elicit essential behavioral and physiological changes to ensure survival but chronic stress may become maladaptive. Role and mechanisms by which the LS regulate chronic stress-induced changes in dietary preference are largely unknown. Methods: We subjected rats to chronic food restriction with intermittent access to sucrose and weekly foot shock stress session to evaluate the ingestive

behavior along with molecular and electrophysiological effects on LS. Microinjections of GABA-B receptor agonist in LS were performed to see its effect on sucrose intake. Results: Treatment leads to increased consumption of sucrose with enhanced lick numbers, lick-cluster number and its duration. It also amplified GAD-67 mRNA expression, decreased firing rate of LS neurons, consequently reducing c-fos mRNA expression in the LS. Additionally, baclofen-induced LS inhibition increased sucrose intake and decreased anorectic stress effects. Conclusion: Food restriction and chronic stress induced LS inhibition leads to compulsivity and increased incentive value of palatable food, eventually switching rats to a stable sucrose-seeking and -consuming phenotype. This shifted preference towards high palatable food to counteract stress effects comes at a cost of maladaptive feeding behavior that could lead to harmful metabolic consequences including diet-induced obesity.

Disclosures: A. Mitra: None. **C. Lenglos:** None. **J. Calvez:** None. **G. Guevremont:** None. **E. Timofeeva:** None.

Poster

546. Stress: Cellular Consequences

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 546.14/QQ8

Topic: E.05. Stress and the Brain

Support: University of Queensland Research Grant

Title: Clonidine alters the response to chronic stress: Effect on neuron number and seizure susceptibility

Authors: *E. W. H. MU, K. BORGES, N. A. LAVIDIS
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Abstract: Central noradrenaline plays a key role in modulating the stress response in the amygdala, however the role of α2-adrenoceptors following chronic stress has not been well-characterized. This study firstly examined the effects of chronic stress on seizure susceptibility and neuron morphology in the basolateral amygdala (BLA) and central amygdala (CeA). Secondly, by using α2-adrenoceptor agonist clonidine and antagonist idazoxan, the role of α2-adrenoceptor in these changes was determined. Four-week old male Wistar rats were divided into 6 groups (n=8 per group), 3 control and 3 stressed groups. Each group received daily subcutaneous injections (1mL/kg) of vehicle (0.9% saline), idazoxan (1mg/kg), or clonidine

(1mg/kg). Stressed groups were restrained for 4 hours daily post-injection. Following 21 days of treatment, rats were subcutaneously injected with pentylenetetrazol (PTZ, 70mg/kg) to induce seizures. Seizure behaviors including number of myoclonic twitches and latency to twitches were recorded. Once forelimb clonus was exhibited, rats were culled with pentobarbital and perfusion fixed with 4% paraformaldehyde. Brains were sectioned coronally and Nissl-stained. Unbiased stereological analysis of pyramidal neurons and glial cells in the BLA and CeA was completed. In saline-treated rats, stress increased pyramidal neuron number in the BLA by 25 ± 2% ($p<0.05$), while pyramidal neuron number decreased in the CeA by 50 ± 5% ($p<0.05$) compared to control. Clonidine further increased BLA pyramidal neuron number during stress by 38 ± 3% ($p<0.05$), while restoring pyramidal neuron number to control in the CeA. No significant change was observed in glia cell morphology. In the PTZ seizure model, stress had no significant effect on seizure behaviors. Chronic clonidine increased latency to first myoclonic twitch in control and stressed rats by 46-49% compared to saline-treated rats ($p <0.01$). Latency to clonic seizure increased in control clonidine rats by 60 ± 16% compared to saline-treated rats ($p<0.01$). There was no significant change in latency for clonidine-treated stressed rats. No significant difference was observed in seizure behavior or neuron counts in idazoxan groups. In summary, chronic stress changed pyramidal neuron number in the BLA and the CeA. Clonidine counteracts this stress response in the CeA, but not in the BLA. Chronic stress affected the physiological response to chronic clonidine treatment, alleviating its seizure protective effect. Interfering with α2-adrenoceptor activity long term can change synaptic transmission by remodeling neural circuitry and can cause morphological and behavioral changes.

Disclosures: **E.W.H. Mu:** None. **K. Borges:** None. **N.A. Lavidis:** None.

Poster

546. Stress: Cellular Consequences

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 546.15/QQ9

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: Fundación Miguel Alemán

CONACYT CB:2010-155255

CONACYT 165271

Title: Effect of acute stress on GSK3 α and β activity in a menopause model in rats

Authors: *M. A. HERNANDEZ[1], C. LOPEZ-RUVALCABA[2], E. ESTRADA-CAMARENA[3]
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Abstract: The reduction of ovaric estrogens levels and the chronic stress exposition, are two factors that could promote depressive like behaviours in rodents. Hormonal changes are associated with reduction of active PI3k and Akt kinases, which are in turn modulators of Glycogen synthase kinase-3 beta activity (GSK3β). Recent studies showed that neurodegeneration and cell death are events associated with the rise of both GSK3β expression and GSK3β activity in the hippocampus of ovariectomized rats (OVX). Because of the administration of 17-β estradiol and antidepressant drugs promote the reduction of GSK3β levels in different brain regions (prefrontal cortex, hippocampus and striatum) of OVX animals, we suggest that acute stress and the reduction of estrogens induced by ovariectomy, could attenuate the PI3K/Akt survival pathway. The increase of the phosphorylated-inactive GSK3β may provide a valuable pharmacological tool to produce neuroprotective and antidepressant actions in animal models of menopause. However, the response of GSK3α to acute stress remains unknown. The aim of this study was analyze the effect of the acute stress on the GSK3α and GSK3β activation in the hippocampus and dorsal raphe nucleus of OVX Wistar rats. Female Wistar rats (250-300 g) were assigned in two groups: OVX and SHAM (non ovariectomized). Both SHAM and OVX groups were also subdivided into control (without stress) and experimental groups, according to the following acute stress treatments induced by forced swimming test (FST): a unique five (5 min) or fifteen min test (15 min), and an initial 15 min pre-test followed 24 hours later by a 5 min test. Animals were sacrified by decapitation 30 min after FST. Hippocampus (HC) and dorsal raphe nucleus (DRN) were collected in order to analyze by Western blot the expression of phosphorylated and active-non phosphorylated isoforms of GSK3 (GSK3α and GSK3β). Results showed that OVX reduced the phosphorylation of GSK3α and GSK3β in HC compared to SHAM group ($p<0.05$). OVX plus FST at 5 min, decreased the phospho- GSK3α levels in HC and DRN regions ($p<0.05$) but not GSK3β. FST is an acute stressor that could attenuate the GSK3α phosphorylation but not in GSK3β.

Disclosures: M.A. Hernandez: None. **C. Lopez-ruvalcaba:** None. **E. Estrada-camarena:** None.

Poster

546. Stress: Cellular Consequences

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 546.16/QQ10

Topic: E.05. Stress and the Brain

Title: The expression of the oxytocin-monomeric red fluorescent protein 1 fusion gene in the hypothalamus and spinal cord after acute nociceptive stimulation in transgenic rats

Authors: *T. MATSUURA, M. KAWASAKI, Y. MOTOJIMA, H. SUZUKI, M. YOSHIMURA, J.-I. OHKUBO, T. MARUYAMA, H. HASHIMOTO, H. OHNISHI, A. SAKAI, Y. UETA
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Abstract: Our scientific understanding of the chief roles of the neurohypophysial hormone oxytocin (OXT) is to stimulate milk ejection and uterine contraction. OXT is also involved in several physiological and pathological social functions such as feeding, social recognition, anxiety and stress responses. On the other hand, several lines of evidence have suggested that OXT plays an important role in pain modulation. Previous studies showed that a population of parvocellular OXTergic neurons of the paraventricular nucleus (PVN) projects their axons to the spinal cord. However, little is known about the neuronal spinal networks responsible for OXT effects. In the rat spinal cord, OXT binding sites as well as OXT receptor expression are located in the dorsal root ganglion, laminaeI-II, intermediolateral and intermediomedial gray mater, lamina X, and in the parasympathetic regions. In the present study, we assessed the effects of acute nociceptive stimulation on OXT- monomeric red fluorescent protein 1 (mRFP1) expression in the hypothalamus, posterior pituitary (PP) and spinal cord, and examined the role which OXT plays in acute nociceptive effects. The transgenic rat that expresses the OXT and mRFP1 fusion gene used here previously showed to be a powerful tool to visualize OXT dynamics after adequate stimuli. To examine the effects of acute nociceptive stimulation on OXT-mRFP1 expression, OXT-mRFP1 transgenic rats were subcutaneously injected with formalin at the bilateral hindpaws. We revealed that there was a significant increase in OXT-mRFP1 fluorescent intensity in the parvocellular and magnocellular divisions of the PVN and supraoptic nucleus of the hypothalamus. We have assessed whether increased hypothalamic OXT may be associated with the spinal pathway of the acute pain by influencing mechanical nociceptive threshold.

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Poster

547. Stress: Neurodevelopmental Aspects

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 547.01/QQ11

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: F.M. Kirby Foundation

Title: Dopamine D3 receptor contributes to the adult behavior deficits induced by repeated stressful experiences during preadolescence

Authors: J. H. SEO, *E. V. KUZHIKANDATHIL
Pharmacol. & Physiol., Rutgers -New Jersey Med. Sch., NEWARK, NJ

Abstract: The transition from adolescence to adulthood is associated with many behavioral changes. In humans and rodents repeated or chronic stress during preadolescence has long-lasting effects and results in development of behavioral disorders, such as depression, anxiety and substance abuse, in adults. While it has been shown that chronic or repetitive stress during postnatal development increases the likelihood of developing depression and anxiety-related disorders as adults, the underlying molecular mechanisms are not well understood. Here we investigated the role of the dopaminergic system, in particular the dopamine D3 receptor, in mediating the long-lasting effect of repeated stress during preadolescence. To assess the role of D3 receptors, we used the drd3-EGFP reporter mice that express the enhanced green fluorescent protein in cells that express endogenous D3 receptors. Male drd3-EGFP reporter mice, subjected to a 5-day, 2-hour per day repeated restraint stress during the preadolescence period (P35 to P40) exhibited depression and anxiety-related behaviors as adults (>P90). The mice stressed during preadolescence exhibited a decrease in number of center zone entry in an Open Field test compared to non-stressed animals. The stressed animals also showed significantly lower latency to immobility and increased total immobility time in Forced Swim test. More importantly, systemic administration of, 10 mg/kg SB-277011-A, a D3 receptor-selective antagonist, ten minutes before each of the repeated restraint stress episodes, prevents the development of depression and anxiety-related behaviors as adults. The behavior data also correlated with changes in D3 receptor expression and signaling function in specific brain regions in adult mice that were subjected to stress during preadolescence. The expression and function of the D3 receptor was downregulated in adult mice that were subjected to stress during preadolescence. Our results suggest that dopamine D3 receptor and its signaling pathways play an important role in mediating the long-lasting effect of repeated stress experienced during preadolescence.

Disclosures: J.H. Seo: None. **E.V. Kuzhikandathil:** None.

Poster

547. Stress: Neurodevelopmental Aspects

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

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Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: the Office of Naval Research (ONR) N00014-12-1-0366

the Pritzker Neuropsychiatric Disorders Research Consortium

the Hope for Depression Research Foundation

the Biology of Drug Abuse training grant T32 DA007268

Title: A “multiple hit” model of affective disorders in rats selectively bred for differences in emotional reactivity

Authors: *C. AYDIN, K. FROHMADER, A. MEDINA, S. J. WATSON, Jr, H. AKIL
molecular and behavioral neuroscience institute, Universty of Michigan, Ann Arbor, MI

Abstract: In humans, certain temperamental characteristics can predict the propensity for mood disorders and substance abuse. One such personality trait, novelty-seeking behavior, is modeled in rats by identifying outbred rats as high (HR) versus low (LR) responders based on their locomotor reactivity to the mild stress of a novel environment. Compared to LRs, HRs display lower anxiety- and depression-like behaviors and higher vulnerability for drug seeking behavior. To ascertain the genetic and developmental underpinnings of these phenotypes, our laboratory has employed a selective breeding strategy to amplify and segregate these naturally occurring differences generating two lines, the bred HR (bHR) and bred LR (bLRs) rats. We have shown that these lines exhibit stable, predictable and profound differences in multiple facets of affective behavior suggesting a pervasive difference in emotionality. Here we ask how environmental interventions might interact with the genetic and epigenetic differences that bHRs and bLRs exhibit to alter affective behavior: Would the combination of genetic vulnerability and environmental “hits” alter the adult phenotypes to produce either a greater propensity for depressive behavior or addictive behavior? This series of studies focused on the impact of chronic stress exposure during adolescence, a time where environmental changes can have a great impact in the human population. This manipulation is followed by additional stressors in adulthood. We ask: Do “multiple hits”_genetic vulnerability, adolescent stress and adult stress, alter either depression-like behavior or vulnerability to substance abuse? We will report on the behavioral consequences of the multiple hit model using a battery of tests including social

interaction, sucrose preference and cocaine sensitization. We will also describe some of the neural correlates of these interventions. In particular, we will focus on the Fibroblast Growth Factor (FGF) system, given that this system is basally different in the bred lines and is critical both during development and in adulthood in modulating affective and addictive behaviors.

Disclosures: C. Aydin: None. **K. Frohmader:** None. **A. Medina:** None. **S.J. Watson:** None. **H. Akil:** None.

Poster

547. Stress: Neurodevelopmental Aspects

Location: Halls A-C

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Program#/Poster#: 547.03/QQ13

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: NIH Grant P30 HD003352

NIH Grant R01 MH043454

Rath Foundation Wisconsin Distinguished Graduate Fellowship

Title: Early-Life effortful control buffers the relationship between rumination and internalizing in adolescents: Behavioral and neural evidence

Authors: *C. WESTBROOK[1], C. BURGHY[2], D. BUSSAN[2], M. J. ESSEX[2], R. J. DAVIDSON[2]
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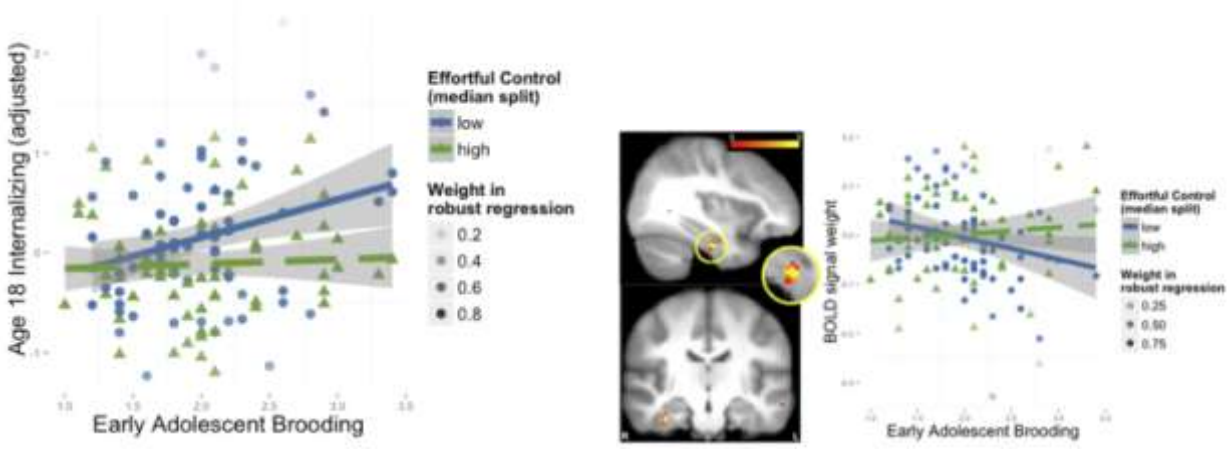
Abstract: Effortful control in childhood and brooding rumination in adolescence have both been demonstrated to have effects on the development of internalizing psychopathology. However, it's unknown whether these two variables interact to predict internalizing. Using data from 136 participants in a longitudinal study, we investigated whether childhood effortful control moderated the effects of early-adolescent brooding on the development of internalizing symptomatology in late adolescence. Additionally, we used fMRI during an implicit emotion regulation task to examine whether the interaction between brooding and effortful control affected neural responding to negative images. We found that childhood effortful control moderated the relationship between early-adolescent brooding and late-adolescent internalizing, and this relationship remained significant when controlling for concurrent brooding and prior internalizing. This interaction corresponded to increased activity in parahippocampal gyrus

during negative images, and activity in this region correlated with decreased internalizing symptomatology. This cluster of activation also demonstrated functional connectivity to other brain areas involved in emotional responding and memory, such as hippocampus and amygdala. Our results provide evidence for a moderating role of childhood effortful control on adolescent brooding rumination, and suggest a possible neural mechanism of this effect in the context of emotion regulation.



Disclosures: C. Westbrook: None. **C. Burghy:** None. **D. Bussan:** None. **M.J. Essex:** None. **R.J. Davidson:** None.

Poster

547. Stress: Neurodevelopmental Aspects

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 547.04/QQ14

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: Medical Research Council Doctoral Training Grant

Medical Research Council *In vivo* Strategic Skills Award

Title: Differential gene expression of components of the hypothalamic-pituitary adrenal axis signalling in juvenile and adult mice

Authors: *A. SADLER, S. J. BAILEY
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Abstract: Stressful life events are known to correlate with an increase in the risk of depression in humans. In depressed adults, there is overactivity of the hypothalamic-pituitary-adrenal (HPA) axis in up to 50% of patients, who show both an increase in cortisol levels as well as reduced negative feedback inhibition. Conversely, this increase in cortisol is not always reported in adolescent depression (Kaufman et al. 2001. Biol. Psychiat. 49(12): 980-1001). Reduced expression of glucocorticoid receptor (GR) and mineralocorticoid receptor (MR) in the hypothalamus of depressed adults has been reported (Pariante and Lightman, 2008, Trends Neurosci. 31(9) 464-468). Similar reductions in GR and MR expression have been shown in mice following chronic stress. In both cases this reduced expression corresponds with impaired negative feedback inhibition of the HPA axis seen by non-suppression of glucocorticoid release in the dexamethasone suppression test. However, less is known about the expression of key components of HPA signalling in juvenile mice and whether this changes in response to stress. Here, we have determined gene expression patterns of components of HPA signalling in adult (9-10 weeks old) and juvenile (4-5 weeks old) BALB/cAnNCrl mice (Charles River, UK) both at baseline and following a repeated stress procedure. Expression of GR, MR, CRH, CRHR1, CHRH2, AVP and V1b were determined in the hypothalamus, hippocampus, prefrontal cortex and pituitary gland of both adult and juvenile mice using quantitative RT-PCR. Blood samples were taken from the tail vein at baseline and following stress, and neuroendocrine function was determined using ELISAs. In control, non-stressed animals, juvenile mice showed increased AVP expression, and decreased CRHR1 expression, in the hypothalamus, compared with adult mice. These data suggest there may be differences in HPA function between adult and juvenile mice that may have repercussions for stress-responsiveness and the development of depression-like behaviours.

Disclosures: **A. Sadler:** None. **S.J. Bailey:** None.

Poster

547. Stress: Neurodevelopmental Aspects

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 547.05/QQ15

Topic: E.05. Stress and the Brain

Support: NeuroNET Seed Grant from the University of Tennessee

Title: Effects of adolescent social play deprivation on responses to social stress and dendritic morphology in the ventral medial prefrontal cortex

Authors: C. A. BURLESON, R. W. PEDERSON, S. SEDDIGHI, *M. A. COOPER
Dept. of Psychology, Univ. of Tennessee, Knoxville, TN

Abstract: Social play is an important feature of animal development as the deprivation of social play leads to altered dendritic morphology in the prefrontal cortex as well as deficits in social and non-social behavior, including inappropriate responses to intraspecific aggression and increases in anxiety-like behavior. Although neural activity in the prefrontal cortex controls coping with stress, it is unknown whether play deprivation affects responses to social stress in adulthood. We used a model of social defeat in Syrian hamsters, called conditioned defeat, to investigate whether social play deprivation leads to impaired dendritic morphology in the ventral medial prefrontal cortex and increased defeat-induced social avoidance. Male hamsters were weaned at post-natal day 21 (P21) and either housed with peers in groups of 3-4 or housed with their mother. At P42, play-deprived animals were reintroduced to peer groups. At P70, animals received 3, 5 minute social defeats in the home cage of a larger resident aggressor or control animals were placed in the empty home cages of resident aggressors. The next day, animals were tested for conditioned defeat in a 5 minute social interaction test with a non-aggressive intruder and then immediately tested for social avoidance of a familiar resident aggressor in a Y-maze. Then brains were collected for Golgi-Cox staining and analysis of dendritic length and dendritic bifurcations in select brain regions. Preliminary data indicate that social defeat produces robust social avoidance compared to non-defeated controls. Analyses of the effects of social play deprivation on conditioned defeat and dendritic morphology in the infralimbic cortex are in progress. This study will address whether social play functions to organize the ventral medial prefrontal cortex to facilitate appropriate responses to social stress in adulthood.

Disclosures: C.A. Burleson: None. **R.W. Pederson:** None. **S. Seddighi:** None. **M.A. Cooper:** None.

Poster

547. Stress: Neurodevelopmental Aspects

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 547.06/QQ16

Topic: E.05. Stress and the Brain

Support: NIMH 093981

Title: Different patterns of neuronal activity in adolescent compared to adult females exposed to repeated social stress

Authors: S. LUZ[1], H. SCHWARZBACH[2], G. KELLY[1], R. VALENTINO[3], *S. BHATNAGAR[4]
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Abstract: Although social stress has adverse consequences for physical and mental health throughout life, its impact may be particularly relevant during adolescence, a time of substantial growth and reorganization of brain circuits. Furthermore, social stress is particularly relevant to adolescent females because their primary source of stress is their social relationships. Our previous work indicates that repeated social stress produces a different behavioral impact in adolescent compared to adult female rats. We sought to determine whether social stress during adolescence activates neural circuits underlying defensive behaviors differently in adolescence compared to adulthood in female rats. Adolescent (ages d28-35) or adult (d70-77) Sprague Dawley female rats were exposed to social stress by placement in the cage of a lactating female. Initial investigation was followed one of the following: supine defeat, five attacks on the intruder rat by the resident rat, or 15 min of resisting defeat, at which point a wire partition was used to separate the two rats. The intruder rat remained in this cage for the remainder of 30 min before returning to her home cage. This was repeated for 7 days and intruder rats were sacrificed 60 min after the 7th defeat. We examined neural activity as assessed by the presence of the Fos protein in the medial amygdala, a region important in mediating defensive behaviors, and the paraventricular thalamus, a region important in adaptation to repeated stress. We used cluster analysis to identify sub-populations of rats that are defeated rapidly (short latency) and rats that resist being defeated that exhibit long latencies to defeat. In adult male rats, short latency rats cope passively and exhibit indices of vulnerability to stress whereas long latency rats cope more actively and exhibit resilience. Preliminary data indicate that defeat resulted in increased numbers of Fos-expressing cells in the medial amygdala and a trend to increased numbers in stressed adult females compared to stressed adolescent females. The increased activation in the adult compared to adolescent stress group was due primarily to adult rats that exhibited rapid defeat (short latency). In the paraventricular thalamus, there were no effects of stress in either adolescence or adulthood when subgroups were combined. However, in adolescent rats exhibiting longer latencies to be defeated, the number of Fos-stained cells was significantly lower compared to short latency adolescent rats. These results indicate that activation of neural structures important for defensive behaviors in females is age dependent and determined by coping strategies

Disclosures: S. Luz: None. **H. Schwarzbach:** None. **R. Valentino:** None. **S. Bhatnagar:** None. **G. Kelly:** None.

Poster

547. Stress: Neurodevelopmental Aspects

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 547.07/QQ17

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Title: Effect of early-life enriched environment treatment on depression-like behavior in mice lacking BDNF expression through promoter IV

Authors: S. JHA[1,2], *K. SAKATA[1]
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Abstract: Promoter IV-driven expression of brain derived neurotrophic factor (BDNF), a major neuronal growth factor, is implicated in the pathophysiology of major depression. We previously reported that mice lacking expression of BDNF through promoter IV (knockin IV: BDNF-KIV mice, Sakata et al., PNAS, 106, 5942-5947, 2009) exhibit depression-like phenotypes (Sakata et al., Genes Brains and Behavior, 9, 712-721, 2010). We further demonstrated that 3 weeks of enriched environment treatment (EET) normalized the defective phenotypes caused by BDNF promoter IV deficiency_depression-like behavior and decreased BDNF levels_ in young-adult (2-5 month old) mice (Jha et al., Translational Psychiatry, e40, 2011). The effects of EET over the life span, particularly during early life, remain unknown. Since early-life development involves dynamic and long-lasting epigenetic processes, we hypothesized that EET provided during early-life development would have maximal antidepressive effects that would endure into later life due to enhanced and long-lasting expression changes in BDNF. Here, as a first step to test this hypothesis, we addressed the effects of early-life EET on depression-like behavior and BDNF protein levels. Mice were raised with or without EET since birth (postnatal 0: P0). Their depression-like behavior was measured by the tail suspension test (TST) and open field test (OFT) at 2 months of age (P60). EET since birth reduced depression-like behavior at P60 in BDNF-KIV mice, as indicated by reduced immobility in the TST and increased explorative activity in the OFT. We further examined if the antidepressive effects of early-life EET endure after 1 month of discontinuance of EET. BDNF-KIV mice raised with EET until P60 and then transferred to standard cages until P90 showed a sustained decrease in immobility in the TST and a trend towards an increase in explorative locomotor activity in the OFT. These results suggest that the antidepressive behavioral effects of early-life EET can endure after discontinuance of the treatment. ELISA measurements confirmed that early-life EET increased BDNF protein levels in the hippocampus and prefrontal cortex (the depression related brain regions) of KIV mice at P60. The induction of BDNF was greater for EET provided in early life than in adulthood. We are

currently investigating whether these BDNF increases endure after discontinuance of EET. In conclusion, early-life EET produces lasting antidepressive behavioral effects and robust induction in BDNF levels, suggesting its usefulness as a potent and lasting prevention/treatment against depression.

Disclosures: S. Jha: None. **K. Sakata:** None.

Poster

547. Stress: Neurodevelopmental Aspects

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 547.08/QQ18

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Title: Effects of chronic fluoxetine and paroxetine treatment on affective behavior in male and female adolescent rats

Authors: *Z. R. HARMONY, S. E. EATON, M. J. STONE, L. VANSA, C. A. CRAWFORD
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Abstract: Selective serotonin reuptake inhibitors (SSRIs) are the most commonly prescribed class of antidepressant drugs, largely because of their effectiveness and favorable side-effect profiles. Unfortunately, the use of SSRIs in pediatric populations is limited due to reduced efficacy and their tendency to induce suicidal ideation in adolescents. While the cause of this reduced efficacy is unknown we previously found that repeatedly treating adolescent rats with fluoxetine (FLX) or paroxetine (PAX) affects affective behaviors when measured 24 h after 10 consecutive days of drug treatment. Specifically, both SSRIs decreased time spent in the open arms of an elevated plus maze (EPM) and time spent in the light compartment of a light/dark box but did not alter sucrose preference. Thus the purpose of the present study was to determine if increasing the time of treatment would alter these SSRI-induced changes in affective behavior. To this end, male and female Sprague-Dawley rats were injected with PAX (2.5, 5, or 10 mg/kg, ip), FLX (5 or 10 mg/kg, ip), or vehicle for 30 consecutive days starting on postnatal day (PD) 30. Rats were assessed for depressive-like behavior using a sucrose preference test and anxiety-like behavior using both EPM and light-dark box procedures. Behavioral testing began 24 h after the last drug treatment (i.e., on PD 60) in all rats. Sucrose preference and behavior in the light/dark box were measured on PD 60 and behavior on the elevated plus maze was assessed 48 hr later. Total sucrose intake and sucrose preference were found to significantly decrease in rats receiving FLX (10 mg/kg). In addition, male rats ingested more sucrose than female rats

regardless of drug treatment. Interestingly, neither SSRI produced anxiety-like behavior after the 30 day treatment. These data suggest that chronic treatment during the adolescent period with PAX and FLX does not enhance anxiety-like behavior. However, the chronic treatment used in the present investigation produced an anhedonic effect not seen with the 10-day drug administration procedure.

Disclosures: Z.R. Harmony: None. **S.E. Eaton:** None. **M.J. Stone:** None. **L. Vansa:** None. **C.A. Crawford:** None.

Poster

547. Stress: Neurodevelopmental Aspects

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 547.09/QQ19

Topic: E.05. Stress and the Brain

Support: NSERC

Title: Age and sex differences in serum cytokine levels following exposure to a bacterial endotoxin

Authors: *J. ROOKE[1], D. KOLMOGOROVA[1], R. WENG[2], L. KANE[1], J. LIANG[2], N. ISMAIL[1]
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Abstract: Exposure to stressors can have long lasting consequences, both on the brain and behaviour, especially during critical periods of development. Puberty is a period of sexual maturation where the brain is undergoing important reorganization and developmental processes. Previous research has shown that an injection of the bacterial endotoxin, lipopolysaccharide (LPS), can cause pubertal mice to be less receptive to hormonal treatments in adulthood compared to adults who were injected with LPS. This study looked at if there were age- or sex-specific differences in serum cytokine levels following exposure to this endotoxin to investigate if differences in immune response could be a contributing factor to later alterations in brain and behaviour. We hypothesized that pubertal mice would show greater serum cytokine concentration levels than adults. Additionally, we anticipated females would show higher levels than males, due to previous research noting females' acute reactions to immune challenges. Six week and ten week old male and female CD-1 mice were injected with either saline or LPS, and then euthanized for trunk blood collection. These samples were analyzed with a multiplex

Luminex immunoassay to determine serum concentrations of TNFα, IL-10, IL-12(p70), IL-1β, and IFNγ. Results showed that there are significant sex differences in cytokine concentrations for TNFα, IL-10, IL-12(p70), and IFNγ, where females had higher concentrations than males for all except TNFα, where males had higher concentrations than females.

Disclosures: **J. Rooke:** None. **N. Ismail:** None. **D. Kolmogorova:** None. **R. Weng:** None. **L. Kane:** None. **J. Liang:** None.

Poster

547. Stress: Neurodevelopmental Aspects

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 547.10/QQ20

Topic: E.02. Neuroimmunology

Support: NIH Grant MH093854

Title: Differential activation of microglia in the mouse brain following an immune challenge may contribute to vulnerability to stressors during puberty

Authors: ***M. K. HOLDER**, J. D. BLAUSTEIN
Univ. of Massachusetts, Amherst, Amherst, MA

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 an increased risk for affective disorders. In female mice, the experience of 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 within the brain, 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 have previously demonstrated that microglia display a more hyper-ramified, or activated, phenotype in the hippocampus, amygdala and hypothalamus during the pubertal period, as compared with adulthood. These data suggest that microglia have an increased sensitivity to stressors during the pubertal period as compared to adulthood. Here, we examined the activation of microglia following administration of LPS to pubertal (6wk) and adult (8 wks) 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. In all brain regions examined, LPS treatment increased Iba1-immunoreactivity (ir). In the hippocampus, the age of animal was without effect on the increase in Iba1-ir following LPS exposure. However, in the amygdala and hypothalamus, we observed more Iba1-ir following LPS treatment in 6 week-old mice, compared to 8 week-old mice. These data suggest that the differential activation of microglia during the pubertal period may underlie the alteration in hormone-responsive affective behaviors in adulthood. Supported by MH 093854 from the National Institutes of Health.

Disclosures: M.K. Holder: None. **J.D. Blaustein:** None.

Poster

547. Stress: Neurodevelopmental Aspects

Location: Halls A-C

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Program#/Poster#: 547.11/QQ21

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: NRF-2013R1A1A1005580

NRF-2005-0049404

NRF-2010-0021190

Title: Prefrontal-limbic change in dopamine turnover by acupuncture in maternally separated rat pups

Authors: *S.-T. KIM, D. KIM, H. JEON
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Abstract: The present study investigated the possible role of acupuncture in alleviating depression-like behavioral changes and examined changes in the levels of serotonin (5-HT), dopamine (DA), and their metabolites in the hippocampus (HP) and prefrontal cortex (PFC) of maternally separated rat pups. On postnatal day 15, rat pups were maternally separated and received acupuncture stimulation at acupoint HT7 or ST36 once a day for 7 days. Then, on postnatal day 21, a tail suspension test was performed, and the HP and PFC were harvested. Levels of 5-HT, 5-hydroxyindole-3-acetic acid (5-HIAA), DA, and 3,4-dihydroxyphenylacetic acid (DOPAC) in the tissue were then measured by high-performance liquid chromatography

analysis. The total duration of immobility in maternally separated rat pups increased after maternal separation, and this increase was alleviated by acupuncture stimulation at HT7. The 5-HIAA/5-HT ratio and the levels of 5-HT and 5-HIAA were not significantly changed, but those of DA and the DOPAC/DA ratio were significantly lower after maternal separation. The maternal separation-induced reduction of the DOPAC/DA ratio significantly improved after acupuncture stimulation at HT7.

Disclosures: S. Kim: None. **D. Kim:** None. **H. Jeon:** None.

Poster

547. Stress: Neurodevelopmental Aspects

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 547.12/QQ22

Topic: A.07. Development of Motor, Sensory, and Limbic Systems

Support: NIH-MH80603

NIH-MH091451

NIH-DA00325

NIH-HD33402

NIH-DC09910

Title: The presence of the mother alters the valence of cues associated with painful stimuli and regulates changes in gene expression in the amygdala of infant rats

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Abstract: In human infants, maternal contact reduces the pain and stress associated with noxious procedures. Simlarly, in animal models the presence of the dam while the infant is exposed to painful or stressful stimuli reduces the stress response and aversiveness of the noxious stimuli (Sullivan et al., 2000; Wiednemayer et al., 2005). Paradoxically, one might expect that cues

associated with infant pain are avoided, but these cues are powerfully attractive to the infant when the mother is present during pain and only aversive when she is not. Activation of amygdala by shock at this age occurs in the absence of the mother and is necessary for the aversion learning. In the presence of the mother, however, the amygdala is quiescent. The mechanisms underlying the dampening of pain by the mother, and which induce the change in valence, are not known. We explored the complex mechanisms that mediate the infant response to a mild noxious stimulus (shock) with or without the dam by assaying changes in gene expression (Affymetrix microarrays) in the amygdala in 12 day old rat pups shocked when the mother present or absent. Methods are as described previously (Barr et al., 2009; Sarro et al., 2013). Raw array data from the "pain" condition (shock vs no shock) and the "Mom present" condition (shock with/without the anesthetized dam) were preprocessed using standard methods and Rank Products analysis was used for assessing differential expression [corrected probabilities (pfp) <.01]. These results were then used for functional analyses. There were several hundred genes whose expression was altered in the pain condition and fewer than 100 in the mother present condition. Of the latter genes, about two-thirds overlapped with the pain treatment group and about one-third were unique to the mother present condition. Multiple functional classes were altered by each condition; changes included genes related to forebrain development, axon guidance, cyclic nucleotides and G-protein coupled receptor signaling. These functional groups differed for genes unique to the pain condition, unique to the mother condition, or that were shared between the two experimental conditions. Thus, the presence of the mother alters the valence of stimuli (odors) associated with noxious stimulation from aversion to preference, reduces the magnitude of induced changes in gene expression, and modifies the functional consequences of those gene expression changes.

Disclosures: G.A. Barr: None. **R.M. Sullivan:** None.

Poster

548. Sleep: Mechanisms and Molecules I

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 548.01/QQ23

Topic: E.08. Biological Rhythms and Sleep

Support: NIH Grant F32HL116077-01A1

NIH Grant G12MD007602

Title: Changes in sleep architecture in response to remote pre-conditioning and ischemic stroke: Impact of Bmal1

Authors: *A. J. BRAGER, T. YANG, J. EHLEN, R. MELLER, K. PAUL
Neurobio., Morehouse Sch. of Med., Atlanta, GA

Abstract: Whole-body knockdown of Bmal1 is with an increase in total daily sleep amounts. Here, we investigate the impact of whole-body knockdown of Bmal1 on ischemic and sleep regulatory pathways. Male wild-type (WT) and Bmal1 knockout (KO) mice received EEG/EMG tether implants for a multi-step experiment. After a 24 h EEG/EMG recording, mild (or sham) ischemia was induced by tourniquet of the left hindlimb across two 10 minute intervals separated by 10 minutes of release (n=6/genotype and treatment). This procedure, also known as pre-conditioning, was followed by a 72 h EEG/EMG recording. Afterwards, harmful ischemia was induced by occlusion of the middle cerebral artery (MCAO) for 1 h and was followed by another 72 h EEG/EMG recording. Infarct volume was determined from a tetrazolium chloride (TTC) stain performed on 0.5 mm-thick coronal brain sections. At baseline, Bmal1 KOs had 9.5+2.2% more daily sleep (NREM and REM) and more sleep-wake fragmentation vs. WTs ($p<0.01$, both). Daily sleep amounts remained unchanged from baseline levels in Bmal1 KOs exposed to mild (0.6+7.2%, from baseline; $p>0.05$) and harmful ischemia (5.2+9.3%, from baseline; $p>0.05$) whereas daily sleep amounts increased by 33.1+11.3% from baseline levels in WTs exposed to mild ischemia ($p=0.02$) and by 32.0+17.7% from baseline levels in WTs exposed to harmful ischemia ($p=0.03$). Total infarct volume in the forebrain (+1.6 to 0.6 from bregma) was larger in pre-conditioned vs. sham animals (32.5+6.9% vs. 12.6+3.6%; $p=0.02$). There was a 50% mortality rate in pre-conditioned Bmal1 KOs vs. 20% in pre-conditioned WTs that was due to microhemorrhaging in the forebrain. This study suggests that Bmal1 is necessary for changes in daily sleep amounts in response to mild and harmful ischemia and modulates the extent of survival induced by harmful ischemia.

Disclosures: A.J. Brager: None. **T. Yang:** None. **J. Ehlen:** None. **R. Meller:** None. **K. Paul:** None.

Poster

548. Sleep: Mechanisms and Molecules I

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Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 548.02/QQ24

Topic: E.08. Biological Rhythms and Sleep

Support: Swedish Research Council (grant no. 2887)

Karolinska Institutet, Sweden

Knut&Alice Wallenberg foundation, Sweden

Ruth&Arwid Wolff foundation, Sweden

Title: Increased mRNA expression and putative release of neuropeptide S after sleep deprivation in rat

Authors: *C. ADORI[1], S. BARDE[1], S. VAS[2], R. K. REINSCHEID[3], G. BAGDY[2], T. HÖKFELT[1]
[1]Karolinska Inst. Dept. of Neurosci., Stockholm, Sweden; [2]Semmelweis University, Dept. of Pharmacodynamics, Budapest, Hungary; [3]Univ. of California, Dept. of Pharmaceut. Sci., Irvine, CA

Abstract: The 20 amino acid 'neuropeptide S' (NPS) is expressed by limited number of neurons in the brainstem. The NPS receptor (NPSR) is, however, widely expressed in the rodent brain. The unique behavioral response of animals after NPS administration, namely the simultaneous anxiolytic and arousal promoting effects make the NPS-NPSR system an interesting potential drug target in mood related- and sleep disorders. Here we applied the single platform-on-water sleep deprivation on Wistar rats and examined the role of NPS in the regulation of sleep and arousal with anatomical methods. Brains were analyzed after 72 hours of sleep deprivation and a subsequent 3-hour rebound sleep. NPS and NPSR1 expression was detected by quantitative *in situ* hybridization. NPS immunoreactivity (IR) was visualized by immunohistochemistry and was quantified by densitometry. Potential targets of NPS-IR fibers were examined by double-labeling and confocal microscopy, with special attention on brain regions/nuclei known to be involved in sleep regulation. NPS-level was determined by radioimmunoassay. The highest expression of NPS was found in the peri-coerulear region and in a cell cluster laterally to the external part of lateral parabrachial nucleus (LPN) and dorsolaterally to the Kölliker-Fuse nucleus (KF cluster). A moderate expression level was detected in the LPN and around the fourth ventricle. Moderate-dense innervations of NPS-IR fibers was found in several brain areas known to be involved in sleep/arousal regulation, including the preoptic area, dorsomedial nucleus and perifornical area in the hypothalamus; PVN in the thalamus; TMN and vlPAG. NPS-IR nerve terminals had frequent close contacts with GABAergic/galaninergic neurons in the VLPO area and TH-IR neurons in several dopaminergic nuclei. The NPS expression was significantly increased in the peri-coerulear cluster but not in the LPN or KF clusters after the deprivation. There was no such a significant increase in case of the large pot (stress control) animals. The expression level in the peri-coerulear cluster returned close to control levels after the 3-hour rebound sleep. The NPS-IR fiber density was significantly decreased after the sleep deprivation in the preoptic area and a parallel increase of NPS-level was found in the same region. The expression of NPSR1, however, did not alter significantly in the preoptic area or in the rhomboid thalamic nucleus. Our

results suggest a differential response of NPS expressing neuron clusters after sleep deprivation and emphasize the role of the peri-coerulear cluster in the modulation of arousal. The results also emphasize the importance of preoptic area in NPS action on sleep.

Disclosures: C. Adori: None. **S. Barde:** None. **S. Vas:** None. **R.K. Reinscheid:** None. **G. Bagdy:** None. **T. Hökfelt:** None.

Poster

548. Sleep: Mechanisms and Molecules I

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 548.03/QQ25

Topic: E.08. Biological Rhythms and Sleep

Support: J. Christian Gillin, M.D. Research Grant, Sleep Research Society Foundation

R01 NS073613

RO1 HL116845

Title: Acute inhibition of catecholaminergic A1/C1 neurons reduces genioglossus muscle activity in behaving mice

Authors: I. RUKHADZE[1,2], P. M. FULLER[1], *V. B. FENIK[3]
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Abstract: Introduction: State-dependent activity of brainstem catecholaminergic (CA) neurons has been suggested to mediate sleep-related hypotonia of upper airway muscles in Obstructive Sleep Apnea patients. We hypothesized that A1/C1 neurons contribute to CA-mediated excitation of the genioglossus (GG) muscle, a major dilator of the upper airway. To test this hypothesis, we employed a pharmacogenetic approach comprising designer receptors exclusively activated by designer drugs (DREADD) to delineate the effects of acute inhibition of A1/C1 neurons on GG muscle activity during natural sleep-wake states. Methods: We used transgenic mice in which Cre recombinase is expressed in CA neurons under the dopamine beta-hydroxylaze (DBH-cre) promoter. The DREADD channel construct was packaged into an AAV vector (EF1a-DIO-hM4Di-mCherry-AAV10; hM4Di-AAV) and bilateral injections (50 nl) of hM4Di-AAV were placed into the A1/C1 cell groups in n=4 mice. One week after injections of

hM4Di-AAV, mice were implanted with electrodes for recording the cortical electroencephalogram (EEG) and neck and GG electromyograms (EMGs). Four weeks after hM4Di-AAV injections the animals were habituated to the recording chamber for two consecutive days. Following chamber habituation all animals received injections of saline or the hM4Di ligand, CNO (0.3 mg/kg; IP) at 12:00 PM. The recordings were analyzed from 10:00 AM to 5:00 PM. Sleep-wake behaviour of the animals was scored in 10 s-long epochs as wake (W), non-rapid-eye-movement (NREM) or rapid-eye-movement (REM) sleep and GG/neck EMGs were quantified separately for each state. Results: State duration and mean amplitudes of neck/GG EMGs were analyzed within 1 hour periods just before and 2.5 hours after the saline/CNO injections and compared using two-way repeated measures ANOVA. GG activity could be fully analyzed in n=3 animals. In these animals, inhibition of A1/C1 neurons decreased GG activity ($F_{2,1,1}=17.7$; $p=0.052$) but was without effect on neck muscle activity ($F_{2,1,1}=3.12$; $p=0.22$) during NREM sleep. There was no significant effect on durations of W, NREM or REM sleep (n=4). Conclusions: Our results suggest that A1/C1 neurons have excitatory effect on GG muscle activity and that they contribute to the maintenance of GG tone during NREM sleep. These studies, for the first time, implicate medullary A1/C1 neurons in the regulation of GG activity in behaving animals.

Disclosures: I. Rukhadze: None. **P.M. Fuller:** None. **V.B. Fenik:** None.

Poster

548. Sleep: Mechanisms and Molecules I

Location: Halls A-C

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Program#/Poster#: 548.04/QQ26

Topic: E.08. Biological Rhythms and Sleep

Support: Dept. of Veterans Affairs (VA merit, RWM)

MH039683 (RWM)

HL095491

MH094803 (REB)

NS079866 (RB)

Title: Suppression of cortical activation from both gamma band auditory stimulation and hypercarbia-induced arousals from sleep by optogenetic inhibition of basal forebrain parvalbumin-containing GABA neurons

Authors: *R. W. MCCARLEY, S. THANKACHAN, J. W. CORDEIRA, T. KIM, J. M. MCNALLY, J. T. MCKENNA, R. BASHEER, R. E. STRECKER, R. E. BROWN
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Abstract: INTRODUCTION. We hypothesized that basal forebrain (BF) parvalbumin GABA (pvGABA) neurons form a key final common pathway for cortical activation from both sensory and visceral stimuli. We used the 40 Hz auditory steady state response (ASSR) as sensory stimuli and measured the resulting activation of cortical gamma band oscillations (GBO, ~40 Hz). Visceral stimuli were hypercarbia (10% CO2), to model obstructive sleep apnea and its cortical activation and arousal from sleep. **METHODS.** For optogenetic inhibition, we bilaterally injected a viral vector with the proton pump ArchT and a green fluorescent protein marker (AAV-FLEX-ArchT-GFP) into the BF of parvalbumin-Cre mice (n=12), and histologically verified transduction. Inhibition was induced by 532 nM bilateral laser illumination preceding and during the 500 ms ASSR and 30 sec hypercarbia stimuli and was compared with no illumination in the same animal. **RESULTS.** Projections of BF pvGABA neurons to frontal cortex was confirmed by GFP-labeled fiber tracing. **Auditory Stimuli**. In each of 8 successfully transduced mice ArchT inhibition during wakefulness of BF PV cells attenuated ASSR-elicited GBO (binomial pbackground). **Hypercarbia.** With bilateral ArchT BF PV inhibition, NREM EEG arousal latencies with hypercarbia in 5 mice were significantly increased (6.5±0.8s without ArchT, 13.1±1.7s with ArchT, paired t-test, p=0.002), an increase of 101.5%. Additionally, under control conditions, arousals occurred at a mean ambient CO2 level of 6.3±0.6%, but when bilateral ArchT BF PV inhibition was applied, the CO2 level for arousal was significantly increased to 8.2±0.8% (paired t-test, p=0.025). **CONCLUSION.** Inhibition of BF pvGABA neurons confirms their key role in cortical activation from both sensory (auditory) and visceral (respiratory) stimuli.

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Poster

548. Sleep: Mechanisms and Molecules I

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Program#/Poster#: 548.05/QQ27

Topic: E.08. Biological Rhythms and Sleep

Support: Washington State University Faculty Seed Grant to Eva Szentirmai

Title: Macrophages are required for normal recovery sleep after sleep loss

Authors: C. AMES[1], *E. SZENTIRMAI[2]
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Abstract: We have previously shown that brown adipose tissue (BAT) heat production is a sleep-inducing signal and sleep loss increases the thermogenic activity of BAT. Recovery sleep after sleep loss is attenuated in BAT deficient, UCP-1 knockout (KO) mice. BAT is activated by norepinephrine (NE) released from postganglionic sympathetic neurons and from a recently recognized macrophage population, the alternatively activated (M2) macrophages. The heat-producing capacity of BAT is severely impaired in the absence of M2 macrophages. We hypothesized that intact macrophage function is also required for recovery sleep after sleep loss as well as for normal sleep responses to cold challenge. Male C57BL/6 mice (n = 15) were instrumented for sleep and body temperature recordings. After 10 days of recovery, baseline sleep and temperature were measured for two days. On the experimental day, the control group (n = 7) received intraperitoneal (ip) injections of isotonic saline and the experimental group (n = 8) was injected with clodronate-containing liposomes in a dose of 0.2 ml/mouse to elicit systemic macrophage depletion. All injections were performed 5-10 minutes before dark onset. Sleep-wake activity and body temperature were measured to determine the acute effects of macrophage depletion as well as its effect on recovery sleep after sleep deprivation. Forty-two h after clodronate administration, mice were sleep deprived by gentle handling during the last 6 h of the light phase. In a separate experiment, the effect of macrophage depletion on cold exposure-induced sleep changes were tested. Starting from dark onset, environmental temperature was reduced to 10oC for 24h. Sleep-wake activity, body temperature and motor activity were recorded for 24 hours after each manipulation. Macrophage depletion elicited an immediate robust increase in non-rapid-eye movement sleep (NREMS) and decrease in rapid-eye movement sleep (REMS). NREMS increased by 110 ± 38.9 min and REMS decreased by 30 ± 6.8 min across 24 h after clodronate treatment. NREMS intensity was suppressed during the initial 12 h after injection. Sleep deprivation elicited rebound increases in NREMS, REMS and SWA in both groups of mice, however, the increases were significantly, ~ 50% smaller in the macrophage-depleted mice. In response to cold exposure, macrophage-depleted mice lost significantly more NREMS and REMS than control mice. Present findings support the hypothesis that intact M2 macrophage function in BAT is required for compensatory sleep after sleep loss and cold-tolerance.

Disclosures: C. Ames: None. **E. Szentirmai:** None.

Poster

548. Sleep: Mechanisms and Molecules I

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Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 548.06/QQ28

Topic: E.08. Biological Rhythms and Sleep

Support: HFSP RGP0004/2013

Title: Early signs of activation in the chick embryo cholinergic system

Authors: *A. CHAN, M. POMPEIANO
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Abstract: The dorsal pontine tegmentum (DPT) contains cholinergic neurons that are mostly active during waking and rapid eye movement (REM) sleep, and in adult mammals these regulate cortical excitability by activating cholinergic neurons of the basal forebrain (BF) (Jones 2008; Brown et al. 2013). In birds (chickens), well-developed cholinergic neurons are present by the second half of embryonic development (von Bartheld and Bothwell 1992), and a REM-sleep-like EEG pattern can be recorded a few days before hatching (Martinez-Gonzalez et al. 2012). Extended waking-like states only appear at or after hatching, but can be transiently induced in late-stage embryos (Balaban et al. 2012). To examine whether cholinergic neurons may regulate either the development of REM sleep in chick embryos or the emergence of waking-like states, we studied the developmental expression of cFos (a marker of neuronal activation) in cholinergic neurons of the BF and DPT. Fertilized eggs were incubated using standard conditions (37.5°C, 55-60% relative humidity). Embryos (in ovo) and hatchlings were anesthetized (isoflurane) and intracardially perfused with fixative. Brains were frozen and cut at a cryostat. Immunofluorescence double-labelling experiments were conducted by incubating brain sections of the BF and the DPT with primary anti-choline acetyltransferase (Millipore) and anti-cFos antibodies (Santa Cruz Biotechnology) overnight and then with secondary fluorescent antibodies. Activation of BF cholinergic neurons (as determined from cFos expression) were first detectable at E16, decreased at E20 and increased again at P1,with a developmental pattern similar to what was previously seen in orexin neurons (Pompeiano et al. 2013). Activation of DPT cholinergic neurons were first detected at E18, reached a peak in pipped (air-breathing) embryos, and declined to basal levels in awake hatchlings. cFos expression was also seen in non-cholinergic cells in both the BF and DPT cholinergic areas. These results suggest that BF cholinergic neurons may be involved in the development of waking-like states in chick embryos, while DPT cholinergic neurons may be involved in the emergence of sleep-like states in the final few days before hatching. Surprisingly, DPT cholinergic neurons seem to play a less substantial role in

supporting waking in chickens than in mammals. Ongoing experiments are increasing the time window and sample sizes of these observations. Further work is also necessary to better characterize the neurotransmitter phenotypes of the active non-cholinergic neurons in both the BF and the DPT, which may also contribute to the development of sleep and waking regulation.

Disclosures: A. Chan: None. **M. Pompeiano:** None.

Poster

548. Sleep: Mechanisms and Molecules I

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 548.07/QQ29

Topic: E.08. Biological Rhythms and Sleep

Support: NIH Grant 5R25GM069621-11

National Institute on Minority Health and Health Disparities Grant 8G12MD007592

Title: Dopamine d2 receptor plays a key role in sleep and circadian activity

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Abstract: Sleep is critical in maintaining body homeostasis and its disruption affects many physiological and behavioral processes. Furthermore, abnormal sleep patterns are typically observed in people with neuropsychiatric disorders such as autism, attention deficit hyperactivity disorder (ADHD), depression, and schizophrenia. Interestingly, those disorders are associated with anomalous dopamine systems. Dopamine is a major neuromodulator in humans and other species including flies and is involved in reward, movement control, cognition and sleep regulation. The goal of our study is to investigate the mechanism by which dopamine regulates sleep and circadian activity. In this study, we focus on D2 receptor in *Drosophila* melanogaster. Sleep and circadian activity of D2 mutant and the flies with overexpressed D2 were measured with the *Drosophila* Activity Monitor (DAM) system. Immunohistochemical studies were also performed on the dissected brain to visualize dopamine receptor expression. We have found that the flies with hypomorphic mutation in dD2R (D2 dopamine receptor) or overexpressed dD2R in the mushroom bodies, a brain structure critical for learning and memory, had morphological and behavioral phenotypes. We are currently analyzing the phenotypes in detail and investigating

how different factors such as sexual dimorphism and social isolation or interaction affect the morphological and circadian phenotypes in wild type and dD2R mutants.

Disclosures: I. Mercado: None. **P. Sabandal:** None. **K. Han:** None.

Poster

548. Sleep: Mechanisms and Molecules I

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Program#/Poster#: 548.08/QQ30

Topic: E.08. Biological Rhythms and Sleep

Support: HFSP RGP0004/2013

Title: Melanin-concentrating hormone neurons in the chick embryo: ontogeny and signs of prenatal activation

Authors: *M. POMPEIANO[1], A. YIP[1], J. BIRD[1], K. E. GODDEN[1], B. S. SEOK[1], D. MARTINEZ-GONZALEZ[2]
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Abstract: Melanin-concentrating hormone (MCH) neurons are found in the dorsal and lateral hypothalamus in rodents and birds. They send diffuse projections throughout the brain that regulate a variety of physiological and behavioral functions, including rapid eye movement (REM) sleep, during which they are most active. The developmental expression of MCH has been studied in rodents, but no information is available for birds. In order to better understand the possible role(s) of MCH neurons in the developing avian brain, we studied MCH expression in chick embryos of different ages and 1 day old chicks. Since a REM-sleep-like EEG pattern can be recorded from chick embryos a few days before hatching (Martinez-Gonzalez et al. 2012), we wondered whether MCH neurons may regulate REM sleep in chick embryos. Therefore, we studied the developmental expression of cFos (a marker of neuronal activation) in MCH neurons. Fertilized eggs were incubated at 37.5°C, 55-60% relative humidity. Embryos (in ovo) and hatchlings were anesthetized (isoflurane) and intracardially perfused with fixative. Cryostat-cut sections were exposed to the primary anti-proMCH antibody (Santa Cruz Biotechnology), a secondary biotinylated antibody and the ABC system with DAB as a chromogen. Double-labelled slides were incubated with primary anti-proMCH and anti-cFos antibodies (Santa Cruz Biotechnology) and then with secondary fluorescent antibodies. A substantial number of lightly-

labeled MCH neurons were first reliably seen at embryonic day (E) 10 in the posterior hypothalamus. The number of labeled neurons and the intensity of staining strongly increased by E12 and older ages. MCH neurons were generally seen in a medial, periventricular location (as in other, non-mammalian vertebrates) at more anterior and dorsal levels. At more posterior levels, they extended from the hypothalamic periventricular organ laterally into the lateral hypothalamic area (as in mammals), medial mammillary nucleus and retromammillary area. cFos expression was virtually absent in MCH neurons at E12, was present at a low level at E16 (~5%), reached a peak at E20 (~20%), and was present at decreased levels in awake 1-day-posthatching chicks. These results suggest that the distribution of MCH neurons in chickens is more complex than previously described, and intermediate between that of other non-mammalian vertebrates and mammals. The presence of a substantial proportion of active MCH neurons towards the end of embryonic development suggests that these neurons may play a functional role in sleep development. We propose that MCH neurons could be involved in the emergence of REM sleep in chick embryos.

Disclosures: **M. Pompeiano:** None. **K.E. Godden:** None. **B.S. Seok:** None. **D. Martinez-Gonzalez:** None. **A. Yip:** None. **J. Bird:** None.

Poster

548. Sleep: Mechanisms and Molecules I

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Program#/Poster#: 548.09/QQ31

Topic: E.08. Biological Rhythms and Sleep

Title: Brown adipose tissue plays a central role in sleep responses to systemic inflammation

Authors: *L. KAPAS, E. SZENTIRMAI
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Abstract: Introduction Our previous work identified brown adipose tissue (BAT) as a source of peripheral sleep-inducing signals (1). Pharmacological activation of BAT enhances sleep while sleep loss results in the thermogenic activation of the organ. The heat-producing property of BAT is conferred by the tissue-specific presence of uncoupling protein 1 (UCP-1). Recovery sleep after sleep loss is attenuated in UCP-1 knockout (KO) mice as well as in mice with the sensory denervation of BAT. Since BAT plays a central role in regulating metabolism and sleep and systemic inflammation greatly affects sleep, metabolism and the function of adipose tissue, we hypothesized that sleep responses to inflammation are mediated by BAT-derived signals. To

test this, we determined the effects of systemic inflammation on sleep and body temperature in UCP-1 KO and WT mice. Intraperitoneal (ip) injections of lipopolysaccharide (LPS) and tumor necrosis factor-α (TNFα) were used as a model for acute, systemic inflammation. Methods Male WT and UCP-1 KO mice (n = 7, each) were instrumented for sleep and body temperature recordings. The animals were kept at 30 C, on a 12:12 h light-dark cycle. At dark onset, mice were injected ip with saline on the control day and with 0.3 or 1 µg TNFα or 100 µg/kg LPS on the experimental day. Sleep, body temperature and motor activity was recorded for 24 h after each treatment. Results In WT mice, NREMS increased by 85% after LPS injection for 12 h. Administration of TNFα lead to dose-dependent increases in NREMS . All these sleep responses were completely abolished in UCP-1 KO animals. LPS and 1 µg TNFα elicited biphasic changes in body temperature; after an initial hypothermia, temperature increased for ~12 h in WT mice. In KOs, the hypothermic phases were abolished but increases in body temperature were not affected. Both LPS and TNFα suppressed motor activity. There were no significant differences in the activity responses of WT and UCP-1 KO mice. Conclusions Present results indicate that BAT plays an essential role in generating sleep responses that accompany systemic inflammatory conditions. The febrile responses, however, are independent of the thermogenic activity of BAT. References 1. Szentirmai É and Kapás L, Eur J Neurosci, 2014, 39:984-998.

Disclosures: L. Kapas: None. **E. Szentirmai:** None.

Poster

548. Sleep: Mechanisms and Molecules I

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Topic: E.08. Biological Rhythms and Sleep

Support: NIH Grant NS075545

Title: Homeostatic sleep need is mediated by adenosine via a glial-neuronal circuit

Authors: *T. E. BJORNESS[1], A. SUZUKI[2], N. DALE[3], G. METTLACH[2], J. A. BIBB[2], M. YANAGISAWA[2], A. FIENBERG[4], R. W. GREENE[2]
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Abstract: Sleep need progressively increases with prolonged waking and dissipates following consequent sleep. Sleep need is most often assessed by measuring slow wave activity (SWA, 0.5-4.5 Hz) power within the EEG; SWA power is considered the best indicator of sleep need because of its strong correlation with previous waking duration. Additionally, sleep becomes more consolidated with increased sleep need. Here we show a novel indicator of sleep need, the time constant of SWA decay within a sleep episode, can be employed together with rebound SWA and sleep consolidation to demonstrate that a neuronal-glial circuit mediated by adenosine regulates sleep need. We determined the effect of adenosine modulation on the three parameters of sleep need under baseline and sleep deprivation conditions in adult, male wildtype and genetically modified mice. Sleep/waking activity was measured using EEG and EMG electrodes. SWA power was assess by a Fast Fourier Transform, the time constant of SWA decay was determined by fitting the normalized and averaged SWA decay with a single phase exponential, and sleep consolidation was measured using a cumulative distribution of episode length. Baseline (undisturbed) activity was recorded for 24-48 h, followed by 48 h of a 6 h sleep deprivation (SD) plus recovery cycle in which waking was enforced using a slowly moving treadmill for 4 h followed by 2 h of treadmill off for recovery (chronic SD; 8x 6 h cycles of sleep deprivation plus recovery) or for acute SD: 4 h SD (for 7 consecutive days) or 6 h SD (for 4 consecutive days). Following chronic SD, control groups showed increased SWA power, increased (slowed) time constant of SWA decay during SWS, and increased sleep consolidation compared with baseline conditions. Furthermore, the time constant of SWA decay progressively increased from baseline to acute 4 h SD to acute 6 h SD to chronic SD. In mice lacking neuronal adenosine A1 receptors, which do not show rebound SWA following sleep deprivation as previously reported by this lab, sleep was fragmented and there was a lack of SWA decay during sleep under baseline conditions. Using an inducible, tamoxifen-based conditional knockdown, adenosine kinase was conditionally knocked down in glial cells. Adenosine kinase converts ATP+adenosine into ADP+AMP and is expressed primarily in glial cells in adult animals. The adenosine kinase knockdown mutants showed increased SWA power, slowed SWA decay during SWS (with a time constant similar to that of control mice following chronic sleep deprivation), and increased sleep consolidation compared to controls under baseline conditions with further increases in SWA power and sleep consolidation following chronic SD.

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Poster

548. Sleep: Mechanisms and Molecules I

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Title: Selective activation of cholinergic basal forebrain neurons induces immediate sleep-wake transitions

Authors: *Y. HAN[1], Y.-F. SHI[1], W. XI[1], R. ZHOU[1], Z.-B. TAN[1], H. WANG[1], X.-M. LI[1], Z. CHEN[1], G. FENG[2], M. LUO[3], Z.-L. HUANG[4], S. DUAN[1], Y.-Q. YU[1]
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Abstract: The basal forebrain (BF) plays a crucial role in cortical activation. However, the exact role of cholinergic BF (ch-BF) neurons in the sleep-wake cycle remains unclear.We demonstrated that photostimulation of ch-BF neurons genetically targeted with channelrhodopsin 2 (ChR2) was sufficient to induce an immediate transition to waking or rapid eye movement (REM) sleep from slow-wave sleep (SWS).Light stimulation was most likely to induce behavioral arousal during SWS,but not during REM sleep,a result in contrast to the previously reported photostimulation of noradrenergic or hypocretin neurons that induces wake transitions from both SWS and REM sleep.Furthermore,the ratio of light-induced transitions from SWS to wakefulness or to REM sleep did not significantly differ from that of natural transitions,suggesting that activation of ch-BF neurons facilitates the transition from SWS but does not change the direction of the transition. Excitation of ch-BF neurons during wakefulness or REM sleep sustained the cortical activation.Stimulation of these neurons for 1 hr induced a delayed increase in the duration of wakefulness in the subsequent inactive period.Our results suggest that activation of ch-BF neurons alone is sufficient to suppress SWS and promote wakefulness and REM sleep.

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Poster

548. Sleep: Mechanisms and Molecules I

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Title: Nigrostriatal dopamine acting on globus pallidus regulates sleep

Authors: M. H. QIU[1], Q. L. YAO[2], R. VETRIVELAN[2], M. C. CHEN[2], *J. LU[2]
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Abstract: Cell body lesions of the globus pallidus externa (GPe) of the basal ganglia (BG) produce a profound sleep loss (~45%) in rats, suggesting that GPe neurons promote sleep. As GPe neuronal activity is enhanced by dopamine (DA) from the substantia nigra pars compacta (SNc), by acting on D2 receptors in the striatopallidal neurons, we hypothesized that SNc DA via the GPe promotes sleep. To test this hypothesis, we selectively destroyed the DA afferents to the dorsal striatum (caudoputamen, CPu) using 6-hydroxydopamine (6-OHDA) and examined changes in sleep-wake profiles in rats. Rats with 80-90% loss of DA terminals in the CPu displayed a significant 33.7% increase in wakefulness. This increase significantly correlated with the extent of SNc DA neuron loss. Furthermore, these animals also exhibited sleep-wake fragmentation and reduced diurnal variability in sleep-wake cycle. We then photostimulated SNc DA terminals in the CPu to build up local DA levels and found that 20Hz stimulation during 9-10 PM increased total sleep by 69% and a tendency of increase in NREM sleep EEG delta power in 1-3Hz range. We finally examined sleep effects of directly exciting GPe neurons with optogenetics stimulation. Similar to SNc DA terminal stimulation, 20Hz stimulation of the GPe from 9 to 10 PM increased total sleep by 66% and significantly increased EEG delta power in the 0.5-3Hz range. These findings elucidate a novel circuit for DA control of sleep, and help to make clear the mechanisms of abnormal sleep seen in BG disorders such as Parkinson's disease, Huntington's disease and obsessive-compulsive disorder.

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Poster

548. Sleep: Mechanisms and Molecules I

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Topic: E.08. Biological Rhythms and Sleep

Support: NINDS/R01 HL095491

T32HL007901

T32AG000222-22

Title: The role of GABAergic neurons of the central amygdala in emotion-triggered cataplexy

Authors: *C. E. MAHONEY[1,3], L. VONG[2], B. B. LOWELL[4,2], T. SCAMMELL[1,5]
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Abstract: Narcolepsy is a common sleep disorder characterized by chronic sleepiness and cataplexy – sudden muscle weakness triggered by strong, usually positive, emotions. We recently found that lesions of the amygdala reduce cataplexy, and that GABAergic neurons of the central amygdala (CeA) target brainstem regions known to suppress muscle atonia. We hypothesize that GABAergic neurons of the CeA are a major inhibitory output of the CeA that may promote emotion-triggered cataplexy. To test the role of the CeA GABAergic neurons in cataplexy, we used Designer Receptors Exclusively Activated by Designer Drugs (DREADD) technology in orexin knock out x vGAT-Cre mice (OXKO x vGAT-Cre). The M3 DREADD receptor is an engineered muscarinic receptors that depolarizes neurons in response to clozapine-N-oxide (CNO) but not to acetylcholine. We injected an adeno-associated viral vector coding for Cre-dependent DREADD (AAV8-hsyn-hM3-mCherry; 20nL) into the CeA of OXKO x vGAT-Cre mice and implanted EEG/EMG electrodes. Vehicle (Saline) or CNO (0.3mg/kg, ip) was injected just before lights off in one of three conditions, with no stimulus or with chocolate (Hershey's chocolate) or running wheel (RW) access. EEG/EMG and infrared video were acquired for 48hours after injection. The EEG/EMG traces were digitally filtered and scored using SleepSign. Mice were perfused and brains were sectioned and processed for mCherry immunohistochemistry to assess injection sites. Analysis of the EEG/EMG traces adhered to a consensus definition of cataplexy (Scammell et. al. (2009) *Sleep*). CNO increased the amount of cataplexy for the first 3 hours after dosing (bilateral and unilateral AAV injections; CNO 10.3$\pm$1.7% vs. Saline 5.9$\pm$1.6% $p<0.05$). CNO produced no additional increase in cataplexy in the presence of chocolate (CNO 13.2$\pm$1.9% vs. Saline 9.9$\pm$1.2% $p>0.05$) or running-wheels

(CNO 9.9±1.3% vs. Saline 8.3±1.0% p>0.05). The effect of CNO on cataplexy may be limited in the presence of these motivating stimuli due to the recruitment of a common network. These results demonstrate that GABAergic CeA neurons are sufficient to trigger cataplexy. These findings should improve our understanding of the neurobiology of positive emotions and motor control, and ultimately, this should lead to better therapies for cataplexy.

Disclosures: **C.E. Mahoney:** None. **B.B. Lowell:** None. **T. Scammell:** None. **L. Vong:** None.

Poster

548. Sleep: Mechanisms and Molecules I

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Topic: E.08. Biological Rhythms and Sleep

Support: NIH R01 NS077408

Title: Cortical nNOS/NK1 neurons are regulated by cholinergic inputs

Authors: *R. H. WILLIAMS, J. VAZQUEZ-DEROSE, A. NGUYEN, T. S. KILDUFF
SRI Intl., Menlo Park, CA

Abstract: Cortical GABAergic interneurons shown to co-express neuronal nitric oxide synthase (nNOS) and the neurokinin-1 (NK1) receptor are activated during sleep (Dittrich et al., 2012, Front. Neural Cir). These neurons have been proposed to play a role in sleep homeostasis (Gerashchenko et al., 2008, PNAS)as the proportion of this population expressing cFOS correlates with homeostatic sleep drive (Morairty et al 2013, PNAS). As these cells appear to "sense" sleep need, we investigated whether cholinergic inputs affect the electrophysiological properties of cortical nNOS/NK1 neurons. We prepared coronal mouse brain slices (250µm thick) for whole-cell patch-clamp recording in both voltage clamp and current clamp modes. After a brief application of the fluorescent NK1 agonist tetramethylrhodamine (TMR), cortical nNOS/NK1 neurons were readily identifiable. Following a 60min wash-out period to eliminate any residual TMR-mediated response, carbachol (CCh, a cholinergic mimetic) was applied. All cells tested responded to CCh (50µM; n=34). CCh had biphasic effects: an initial membrane hyperpolarization (-2.24±0.57mV, n=6) and outward current (+3.74±0.66pA, n=12) was followed by a large membrane depolarization (+9.18±1.87mV, n=8) and inward current (-5.59±2.22pA, n=12). In addition, CCh predominantly increased spontaneous excitatory postsynaptic currents (sEPSCs; +805.8±62.68%, n=15). In the presence of tetrodotoxin (TTX),

both outward (+3.18±0.74pA, n=9), and inward (-11.96±1.88pA, n=15) currents remained, indicating responses mediated by postsynaptic receptors. We tested whether the excitatory response mediated by CCh was attributable to activation of the Gq-coupled muscarinic type 1 receptors (M1R). Application of CCh in the presence of the M1R antagonist VU0235535 (5μM) reduced the membrane depolarization by 47.9±16.9% and inward current by 32.0±17.7% in 3/5 cells tested. The 2 remaining cells showed no change in their excitatory response but an increase in the outward current (+5.76±2.11pA, n=2). In contrast to sEPSCs, CCh decreased miniature EPSCs (mEPSCs) by -45.6±2.2% (n=6). The presence of VU0235535 did little to alter the reduction in mEPSCs seen following CCh application (after vs baseline: -53.1±2.8%, n=4), suggesting that M1Rs are largely postsynaptic. In summary, CCh has a network effect on excitability within the cortex, as both presynaptic and postsynaptic effects result in an oscillating pattern of nNOS/NK1 neuron excitability. When all inputs are blocked, the predominant response is excitation. We are currently attempting to determine the source of cholinergic inputs to cortical nNOS/NK1 neurons.

Disclosures: R.H. Williams: None. **J. Vazquez-DeRose:** None. **A. Nguyen:** None. **T.S. Kilduff:** None.

Poster

548. Sleep: Mechanisms and Molecules I

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Program#/Poster#: 548.15/RR1

Topic: E.08. Biological Rhythms and Sleep

Support: Supported by a Pelotonia Grant to ACD

Title: Cytotoxic chemotherapy increases NREM and REM sleep with concurrent sleep fragmentation

Authors: *J. C. BORNIGER, M. M. GAUDIER-DIAZ, N. ZHANG, R. J. NELSON, A. C. DEVRIES
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Abstract: Chronic fatigue and sleep disruption are among the most common complaints of cancer patients undergoing chemotherapy and are primary reasons for discontinuing treatment. Indeed, hypnotics are the most frequently prescribed drugs to combat chemotherapy-related side effects. Because of the complex interactions among cancer, treatment regimens, and life-history

traits, investigations into a causal link between chemotherapy and sleep disruption have been sparse. To investigate how chemotherapy acutely influences sleep, we ovariectomized adult (8-9 wks) female c57bl/6 mice and implanted wireless biotelemetry units. We subsequently collected EEG/EMG biopotentials over the course of 3 days pre- and post-injection (at ZT 7; 14:10 light/dark cycle) of 18 mg/kg doxorubicin and 180 mg/kg cyclophosphamide in saline vehicle or vehicle alone. Because previous research has implicated cytokine signaling in sleep disruption and chronic fatigue, we predicted that IV administration of cyclophosphamide + doxorubicin would disrupt sleep and increase central pro-inflammatory cytokine expression in brain areas known to govern vigilance states (i.e., hypothalamus and brainstem). The results largely support these predictions; a single chemotherapy injection dramatically increased NREM and REM sleep during subsequent active (dark) phases and this induced sleep was fragmented and of low quality. Mice displayed marked increases in low theta (5-7Hz) to high theta (7-10 Hz) ratios following chemotherapy treatment, indicating elevated sleep propensity (i.e., fatigue). The effect was strongest on the first night following injection, but mice displayed disrupted sleep for the entire post-injection recording time (3 days). qPCR analysis revealed that sleep disruptions were accompanied by increased IL-6, but not TNF-α or IL-1β, mRNA expression in the hypothalamus, but not the brainstem. Vigilance state timing was not influenced by treatment, potentially indicating that chemotherapy administered at this time alters sleep homeostasis without altering circadian output. Our study is the first to provide a causal link between chemotherapy and acute sleep disruption, and our data support previous research implicating hypothalamic inflammation in sleep disruption.

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Poster

548. Sleep: Mechanisms and Molecules I

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Topic: E.08. Biological Rhythms and Sleep

Support: Dept. of Veterans Affairs (VA merit, RWM)

MH039683 (RWM)

HL095491

Title: Role of thalamic reticular nucleus in sleep spindle generation: An optogenetic investigation in the mouse with implications for schizophrenia

Authors: *S. THANKACHAN, J. M. MCNALLY, R. E. STRECKER, J. T. MCKENNA, R. E. BROWN, R. W. MCCARLEY
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Abstract: Alterations in human cortical sleep spindles have been recently identified as a reliable biological EEG marker of schizophrenia (Sz). Human sleep spindles (12 - 16 Hz EEG oscillations) are thought to originate in the thalamic reticular nucleus (TRN), a structure composed primarily of inhibitory GABAergic neurons containing the calcium binding protein parvalbumin (PV). The interaction of rhythmically bursting PV TRN neurons and thalamocortical (TC) relay neurons initiates spindle oscillations during NREM sleep, and these oscillations are postulated to be self-maintained by reciprocal interactions between cortical, TC relay, and TRN neurons, although the role of specific TRN neurotransmitter phenotypes in spindle generation is not clear. We hypothesized that direct, selective stimulation of PV TRN neurons would promote spindle activity, and inhibition would produce attenuated spindle activity, consistent with evidence of reduced PV expression and abnormal spindle activity in both animal models and clinical studies of SZ. To investigate for the first time, the specific role of PV-positive TRN neurons, we performed optogenetic experiments in mice expressing Cre recombinase in PV neurons (PV Cre mice). In our first experiment to test the role of PV neurons in spindle control, adeno-associated virus-ChR2 was first bilaterally injected into TRN in the PV Cre mouse to specifically transduce PV neurons of TRN, and blue laser-light (473nm) was then applied three weeks later for optogenetic excitation. Optogenetic excitation was tested (n = 5 mice) at varying frequencies (2-60 Hz). This produced the largest cortical EEG response at ~10 Hz, suggesting activation of an intrinsic oscillator tuned to the mouse spindle frequency (10-12 Hz). 10 Hz excitation elicited both a significant increase of cortical EEG power at the stimulation frequency, and regularly produced a cortical EEG spindle. In addition, 5 hrs of optogenetic stimulation at 10Hz (1 sec/min) led to a consistent increase in NREM sleep (n=2) compared to control experiments (no stimulation). Our experiments show that PV-positive TRN neurons have the ability to facilitate cortical spindles and NREM sleep. Ultimately, an increased knowledge of the pathophysiology of spindle deficits may provide potential pharmacological targets for treatment of spindle deficits such as those seen in schizophrenia.

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Poster

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Topic: E.08. Biological Rhythms and Sleep

Title: Chronic administration of glycine modify sleep pattern and metabolic parameters in rats

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Abstract: Sleep is one of the physiological needs which is characterized by a reduction in awareness level and specific metabolic activities. Various neurotransmitters regulate sleep, including the glycine (Gly), which has a facilitating effect in humans, improving quality and reducing daytime sleepiness. Gly also is an effective adjuvant in the treatment and prevention of type 2 diabetes mellitus (T2DM), preventing cell injury by inhibiting the synthesis of proinflammatory cytokines. To determine the effect of chronic administration of Gly on various metabolic parameters and sleep pattern, we used 10 male rats (250-300 g), quantifying the levels of: cholesterol, triglycerides, uric acid, glucose, and various proteins in liver. Subsequently, electrodes were implanted for conventional sleep recordings and grouped under the following conditions: Control, normal water consumed (n=5) and water with Gly (10 g/1000 ml) for 30 days (n=5). Every week we realized polysomnographic recordings. At the end, they were again quantified metabolic parameters. Gly administration during the first week increased slow wave sleep II in relation to the control group, decreasing REM sleep. In the last three weeks, the Gly caused a gradual decrease in REM sleep at the expense of an increase in wakefulness. In the metabolic parameters, the chronic administration of Gly showed a trend to decrease glucose levels and increase levels of various transaminases. From the obtain results we suggest a dual effect of Gly on sleep-dependent on the time administration and possible effective adjunct in the treatment and prevention of various clinical manifestations of DM2.

Disclosures: A. Jimenez-Anguiano: None. **J.C. Rodriguez-Aguilar:** None. **I. Jasso-Villagomez:** None. **G. Blancas-Flores:** None. **F.J. Alarcon-Aguilar:** None. **J. Velazquez-Moctezuma:** None.

Poster

548. Sleep: Mechanisms and Molecules I

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 548.18/RR4

Topic: E.08. Biological Rhythms and Sleep

Support: NIH Grant R01GM104948

Title: Optogenetic activation of cholinergic neurons in the LDT increases REM sleep

Authors: *C. J. VAN DORT[1,3], J. KENNY[1,3], D. ZACHS[2,3], R. GOLDBLUM[4], M. WILSON[3], E. BROWN[2,3]
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Abstract: Rapid eye movement (REM) sleep is tightly regulated, yet the mechanisms that control REM sleep remain incompletely understood. Early pharmacological and unit recording studies suggested that acetylcholine was important for REM sleep regulation. For example, injection of cholinergic drugs into the dorsal mesopontine tegmentum reliably induced a state very similar to natural REM sleep in cats. Unit recordings from the cholinergic areas of the mesopontine tegmentum revealed cells that were active during wakefulness and REM sleep as well as neurons active only during REM sleep. Electrical stimulation of the laterodorsal tegmentum (LDT) in cat increased the percentage of time spent in REM sleep and activation of the pedunculopontine tegmentum (PPT) in rat induced wakefulness and REM sleep. In cat, lesions of the PPT and LDT do disrupt REM sleep but lesions in rodents have had little effect on REM sleep or increased REM sleep. Additionally, c-fos studies have found very few cholinergic cells activated under high REM sleep conditions leading to alternative theories of REM sleep regulation where cholinergic neurons do not play a key role. Therefore, we aimed to determine the role of cholinergic neurons in the LDT on REM sleep regulation using optogenetics. Adult male ChAT-ChR2+ transgenic mice (n=5) and wildtype littermates (n=5) were implanted with bilateral fiber optics aimed for the PPT and EEG and EMG electrodes. Experiments were conducted in the morning with 4 conditions in randomized order, baseline sleep and 3 stimulations (5ms pulses at 5Hz) of varying duration 60, 80, or 180 s. EEG, EMG, and video were recorded and used to score sleep. After completion of the experiments, mice were perfused and identification of the fibers above the LDT was confirmed. Optogenetic activation of cholinergic neurons in the LDT during NREM sleep increased REM sleep from 2 s in ChAT-ChR2- mice to 4.5 s in ChAT-ChR2+ mice for 60 s stimulations, from 2.7 to 8.1 s for 80 s stimulations, and from 12.4 to 22.7 s in 180 s stimulations. The probability of REM sleep

increased over the time course of the stimulation. The increase in REM occurred by increasing the number of REM sleep episodes but not REM sleep episode duration. The induced REM sleep state was electrophysiologically similar to natural REM sleep by power spectra analysis. Inferences were made by constructing 95% Bootstrap confidence intervals where differences are considered significant if the confidence intervals between groups do not overlap. These findings suggest that LDT cholinergic neurons are key modulators of REM sleep onset but not REM sleep maintenance.

Disclosures: C.J. Van Dort: None. **J. Kenny:** None. **D. Zachs:** None. **R. Goldblum:** None. **M. Wilson:** None. **E. Brown:** None.

Poster

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Topic: E.08. Biological Rhythms and Sleep

Support: NIH/NHLBI Grant #T32 HL 007901

Title: Pharmacogenetic activation of cholinergic, glutamatergic and GABAergic neurons in the PPT and their roles in sleep/wake behavior

Authors: *D. KROEGER, L. L. FERRARI, L. J. AGOSTINELLI, E. ARRIGONI, T. E. SCAMMELL
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Abstract: A variety of studies on different species have suggested that the pedunculopontine tegmental (PPT) region in the brainstem is a key site for the regulation of sleep/wake states - specifically REM sleep regulation. However, lesion studies, electrical stimulation, or local infusions of either GABA, glutamate or carbachol into the PPT region cannot determine which of the intermingled neurons in this region is responsible for the observed effect. We chose a genetically targeted approach to selectively and separately activate PPT neurons expressing acetylcholine, glutamate or GABA using excitatory Designer-Receptors-Activated-Exclusively-by-Designer-Drugs (DREADDs). Using three different cre mouse lines (Chat-cre, vGlut2-cre and vGat-cre mice), we transfected neurons in the PPT region with a viral vector coding for DREADD M3 receptors tagged with fluorescent mCherry. Two weeks later, we administered i.p. clozapine-N-oxide (CNO) or vehicle during the light and dark periods. Activation of DREADD-

expressing cholinergic neurons 2.5 hours after lights-on reduced EEG delta amplitude during NREM sleep by over 27% for a 3 h period with rebound delta sleep for the subsequent 6 h. CNO activation of vGlut2 neurons in the PPT potently induced wakefulness for 5-8h with animals showing the normal range of behaviors such as nesting, feeding, grooming, wheel running, etc. In contrast, activation of vGat neurons had little effect on sleep/wake behavior. We conclude that each neurochemical type of PPT neuron plays a distinct role in modulating sleep/wake states.

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Poster

548. Sleep: Mechanisms and Molecules I

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Topic: E.08. Biological Rhythms and Sleep

Support: NIH 1R01AG047671

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Title: Optogenetic interrogation reveals a causal role for VTA dopamine neurons in the regulation of sleep and wakefulness

Authors: *A. D. EBAN-ROTHSCHILD, W. J. GIARDINO, L. DE LECEA
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Abstract: Dopamine (DA) neurons play a central role in many wake-related processes including motivation, reward, and learning. While these functions are associated with heightened arousal, the role of DA neurons in generating and maintaining arousal is largely unknown. In some psychiatric disorders, such as Schizophrenia and substance abuse, there are alterations in DA transmission as well as in arousal and sleep-wake architecture. Studies using knock-out animals and pharmacological manipulations further suggest that DA signaling participates in the

regulation of sleep and wakefulness. Nevertheless, previous studies lacked the spatial and temporal resolutions that are relevant for studying natural sleep/wake events. To decipher the precise role of DA in sleep-wake regulation, we combined optogenetic tools with EEG and EMG recordings in mice. We examined the causal role of DA neuronal activity in the ventral tegmental area (VTA) on cortical activation and sleep-wake dynamics. We focused on DA neurons in the VTA since they send widespread projections throughout the brain, making them particularly suitable for regulating sleep-wake states. To selectively target VTA-DA neurons, we stereotactically injected a Cre-recombinase-dependent adeno-associated virus (AAV) expressing channelrhodopsin 2 (ChR2) into the VTA of mice expressing Cre under the control of the tyrosine hydroxylase (TH) promoter (TH:Cre). As a control, we transduced TH:Cre littermate mice with a Cre-dependent AAV expressing only the fluorescent protein (eYFP). We also implanted the mice with a custom-made EEG-EMG device and a fiber optic cannula. We assessed the effect of optogenetic stimulation (at 1 and 25 Hz) of DA cell bodies and principle projections during the inactive phase on sleep-wake transitions, sleep-wake architecture and EEG power spectra. Our findings show that phasic activation (at 25 Hz) of VTA-DA neurons reliably produced immediate sleep-to-wake transitions and a decrease in slow-wave activity in ChR2 animals, but not in eYFP control mice. These effects were not dependent on sleep pressure. We also found that semi-chronic photostimulation of VTA-DA neurons induced long-term wakefulness. Our results demonstrate a causal role for VTA-DA neurons in promoting wakefulness and identify this neuronal population as a key node in the sleep-wake circuitry.

Disclosures: **A.D. Eban-Rothschild:** None. **W.J. Giardino:** None. **L. de Lecea:** None.

Poster

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Topic: E.08. Biological Rhythms and Sleep

Support: NIDA RO1 Grant DA31367 to D.A.P.

NINDS RO1 Grant NS70911 to D.A.P.

Title: Regulation of sleep by Neuropeptide Y in zebrafish larvae

Authors: *C. SINGH, C. N. CHIU, V. SAPIN, D. A. PROBER
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Abstract: More than 40 million Americans are affected by chronic sleep disorders costing the nation $100 billion annually. Despite the impact of sleep disorders, the mechanisms that regulate sleep remain poorly understood. Zebrafish is emerging as a powerful model system to study genetic and neural mechanisms that regulate sleep. It offers the advantages of having simple yet conserved neuronal circuits, optical transparency during larval stages and sleep and arousal behaviors that can be monitored using high throughput assays. Neuropeptide Y (NPY) is a 36-amino-acid peptide that is widely distributed in the brain and peripheral sympathetic nervous system. Studies from rodents and humans have shown multiple effects of NPY on sleep ranging from inducing sleep to increasing wakefulness. These discrepancies in the observed effects of NPY may be due to the complex neuronal systems and experimental methods used. We have tested the effects of genetic perturbation of NPY on sleep-wake behaviors in 4-7 days old zebrafish larvae. Overexpression of NPY using a heat shock inducible promoter decreased locomotor activity and increased sleep. To further characterize the NPY neural circuit that mediates this effect, we generated a transgenic line expressing citrine in NPY neurons. We found that NPY is expressed in discreet nuclei in the telencephalon, hypothalamus, tegmentum of the mesencephalon, olfactory pit, rhombencephalon and retina. The optical transparency of zebrafish larvae allows imaging of the entire NPY neural circuit and will aid in investigating interactions between NPY neurons and other sleep-wake regulatory regions. The use of a simple diurnal model system has the potential to clarify the role of NPY in regulating vertebrate sleep/wake states.

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Poster

548. Sleep: Mechanisms and Molecules I

Location: Halls A-C

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Program#/Poster#: 548.22/RR8

Topic: E.08. Biological Rhythms and Sleep

Support: Willis-Ekbom Disease Foundation

NIH Grant NS14610

the Dept Affairs, VAGLAHS Sepulveda

Title: Glutamate release in the cortex of the inferior colliculus is correlated with phasic motor activity in sleep

Authors: T. KODAMA[1], K.-C. HSIEH[2], J. M. SIEGEL[3], *Y.-Y. LAI[4]
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Abstract: The external cortex of the inferior colliculus (ICX) receives afferent projections not only from the auditory pathway, but also from somatic sensory structures including the dorsal column nuclei. The majority of the somatic sensory projections are from the hindlimb (Aitkin et al., 1978). It has been postulated that the ICX plays a role in the integration of auditory and sensory-motor activities. Periodic leg movements (PLMs) have been observed in slow wave sleep in rats with either AMPA or NMDA infusion into the ICX (Hsieh et al., 2013). The PLMs in sleep induced by glutamate agonists infused into the ICX resemble the key behavioral symptom of Willis-Ekbom disease (WED or restless legs syndrome). In this study, we examined whether glutamate release in the ICX correlated with motor activity in sleep in the chronic rat using *in vivo* microdialysis and HPLC analysis techniques. Normal rats and humans show low levels of sleep PLMs. Sprague-Dawley rats weighing 250-500g were implanted with EEGs, nuchal and hindlimb EMGs, and a guide cannula aimed at the ICX. The animals were individually housed in sound-proof chambers with 12-12 light-dark cycles. A microdialysis probe (CMA/11) was inserted into the ICX 12 hours before the start of the experiment. On the day of the experiment, artificial cerebrospinal fluid at a flow rate of 2 µl/min was infused through the probe from ZT2 to ZT11. Twenty microliters of dialysate was collected every 10 min from ZT4 to ZT11. The next day, ICX-glutamate agonist infusion was also performed in all rats after dialysates collection. A total of 96 dialysates was collected from 3 rats, where ICX infusion of glutamate induced PLMs. We found that glutamate levels in the ICX are positively correlated with hindlimb phasic motor activity ($R=0.391$, $p<0.001$) in sleep. A small but significant correlation was also found between glutamate levels in the ICX and neck muscle activity in sleep ($R=0.22$, $p<0.05$). Histological examination showed that all dialysates were collected from the ICX. Conclusion: Glutamate levels in the ICX strongly correlate with hindlimb phasic motor activity in sleep in the normal rat. This correlation may account for the pathological PLM phenomenon seen in rats infused with AMPA or NMDA into the ICX. Abnormal release of glutamate, from peripheral sensory inputs, into the ICX may regulate the pathogenesis of WED.

Disclosures: T. Kodama: None. **Y. Lai:** None. **K. Hsieh:** None. **J.M. Siegel:** None.

Poster

548. Sleep: Mechanisms and Molecules I

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 548.23/RR9

Topic: E.08. Biological Rhythms and Sleep

Support: Merck & Co., Inc.

Title: Orexin receptor antagonists promote both non-rem and rem sleep similar to physiological sleep onset in pre-clinical species

Authors: ***S. V. FOX**[1], P. L. TANNENBAUM[2], A. L. GOTTER[3], S. L. GARSON[3], A. T. SAVITZ[2], J. STEVENS[2], S. D. KUDUK[4], P. J. COLEMAN[4], C. J. WINROW[3], J. J. RENGER[3]
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Abstract: INTRODUCTION: Orexin receptor antagonists provide a novel treatment for insomnia by reducing wakefulness and enabling sleep. In clinical trials, dual orexin receptor 1/2 antagonists (DORAs) decrease latency to persistent sleep and promote sleep maintenance in insomnia patients while promoting both non-REM (NREM) and REM sleep. Since insomnia patients exhibit reduced NREM and REM sleep, increasing both NREM and REM is important. Assessing structurally distinct DORAs and selective OX2 receptor antagonists (SORAs) in pre-clinical species is a translatable method for evaluating insomnia therapeutics. Sleep architecture and qEEG effects of DORAs, SORAs and GABAA receptor modulators were compared to that of natural sleep in rats, dogs and monkeys. METHODS: Wireless bio-physiological devices in rats, dogs and monkeys assessed electroencephalogram, electromyogram, electrooculogram (dog & monkey) and locomotor activity 24hr/day in multi-day crossover studies. Sleep architecture parameters were compared between vehicle treatment during the inactive period and novel DORA, SORA or GABAA receptor modulator treatment during the active period (insomnia model). A series of structurally distinct compounds were evaluated. RESULTS: In baseline recordings, both NREM and REM sleep were significantly increased at sleep onset in rats, dogs and monkeys. All compounds promoted NREM sleep when dosed in the active phase, however unlike GABA-A modulators, DORAs and SORAs also increased REM similar to that of baseline sleep onset; there were no generalizable characteristic NREM/REM differences between DORA and SORA sleep. By comparison, benzodiazepine and non-benzodiazepines significantly reduced REM sleep and disrupted qEEG spectral power relative to baseline sleep. CONCLUSION: In nonclinical studies both DORAs and SORAs increase NREM and REM sleep across preclinical species. Given that insomnia patients exhibit decreases in both NREM and REM sleep, therapeutics which restore NREM and REM sleep may be appropriate, and further clinical evaluations would be informative.

Disclosures: **S.V. Fox:** A. Employment/Salary (full or part-time):; Merck & Co., Inc. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Stock and/or Stock Options. **P.L. Tannenbaum:** A. Employment/Salary (full or part-time):; Merck & Co., Inc.. E. Ownership Interest (stock, stock

options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Stock and/or Stock Options. **A.L. Gotter:** A. Employment/Salary (full or part-time):; Merck & Co., Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Stock and/or Stock Options. **S.L. Garson:** A. Employment/Salary (full or part-time):; Merck & Co., Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Stock and/or Stock Options. **A.T. Savitz:** A. Employment/Salary (full or part-time):; Merck & Co., Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Stock and/or Stock Options. **J. Stevens:** A. Employment/Salary (full or part-time):; Merck & Co., Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Stock and/or Stock Options. **S.D. Kuduk:** A. Employment/Salary (full or part-time):; Merck & Co., Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Stock and/or Stock Options. **P.J. Coleman:** A. Employment/Salary (full or part-time):; Merck & Co., Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Stock and/or Stock Options. **C.J. Winrow:** A. Employment/Salary (full or part-time):; Merck & Co., Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Stock and/or Stock Options. **J.J. Renger:** A. Employment/Salary (full or part-time):; Merck & Co., Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Stock and/or Stock Options.

Poster

548. Sleep: Mechanisms and Molecules I

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 548.24/RR10

Topic: E.08. Biological Rhythms and Sleep

Support: KAKENHI 24621014

Title: Trib2-immunization induces hypocretin/orexin changes

Authors: S. TANAKA, Y. HONDA, K. HONDA, M. HONDA, M. WATANABE, *T. KODAMA
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Abstract: Introduction: Autoimmune process is suggested to be involved in narcolepsy. Recent findings showed that 16-26% of narcolepsy patients were positive for anti-TRIB2 antibody and icv administration of IgG purified from sera of narcolepsy patients positive for anti-TRIB2 autoantibody caused marked hypocretin (also called as orexin) producing cell loss. We investigated pathophysiological roles of anti-TRIB2 antibody against hypocretin cells using TRIB2-immunized rats and hypocretin-ataxin3 transgenic mice, which show gradual denature of hypocretin cells. Method: Female Sprague Dawley rats were given every other week the subcutaneous injection of one of the following antigens; keyhole limpet hemocyanin(KLH), KLH conjugated-TRIB2 peptide, or saline. Sera, cerebrospinal fluid(CSF), and hypothalamic tissues were collected to investigate the change of anti-TRIB2 titers, hypocretin contents, mRNA expressions, and histologically determined hypocretin cell counts. The sera from hypocretin-ataxin3 transgenic mice were also used to examine whether anti-TRIB2 antibody increases in the sera during the denature of hypocretin producing cells. Results and Discussion: All the TRIB2-immunized rats, but none of KLH or saline-immunized rats were positive for anti-TRIB2 antibody. TRIB2 antibody titers were slightly increased in CSF of TRIB2-immunized rats (compared with CSF of intact rats). Both chronic TRIB2 and KLH-immunization reduced the hypocretin mRNA synthesis in hypothalamic tissue and hypocretin content in CSF. However, no differences were found in hypothalamic hypocretin contents, hypocretin cell number, and the levels of pro-melanin-concentrating hormone mRNA between TRIB2 and KLH immunized rats. There were no significant correlations between anti-TRIB2 antibody index and above mentioned parameters. Some hypothalamic hypocretin cells were positive for rat IgG in TRIB2-immunized rats. The sera from hypocretin-ataxin3 mice over 24 week-old showed positive reactions against TRIB2 antigen. Our results show that immunization caused the decrease of hypocretin mRNA and CSF hypocretin content regardless of the presence or absence of TRIB2 antigen, suggesting the general immune activation has the effect on the function of hypocretin neurons. Since no change in hypocretin cell number was found even in the immunized-rats showing high titer of anti-TRIB2 antibody in CSF, we assume that factors other than anti-TRIB2 antibody play a part in the loss of hypocretin-producig cells observed in narcolepsy.

Disclosures: S. Tanaka: None. **Y. Honda:** None. **K. Honda:** None. **M. Honda:** None. **M. Watanabe:** None. **T. Kodama:** None.

Poster

548. Sleep: Mechanisms and Molecules I

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Topic: E.08. Biological Rhythms and Sleep

Support: NIH Grant HL095491

Title: Optogenetic dissection of the neural circuitry underlying hypercapnia induced-arousals

Authors: *S. KAUR, D. KROEGER, P. FULLER, C. SAPER
Neurol., Beth Israel Deaconess Med. Ctr. and Harvard M, Boston, MA

Abstract: We have previously reported that glutamatergic signaling from the external lateral parabrachial subnucleus (PBel) regulates cortical arousals to hypercapnia. Anterograde projection patterns of the PBel suggest that these arousals may be mediated through forebrain structures such as the lateral hypothalamic area (LH), central nucleus of the amygdala (CEA), or basal forebrain (BF). However, the specific functions of these pathways in hypercapnic arousals remain unclear. To model cyclic hypercapnia as seen during sleep apnea, we investigated repeated EEG arousals to 10% CO2 given for 30s every 300s (RCA). We used optogenetic inhibition of glutamatergic neurons in the PBel area in one experiment and inhibition of the fiber terminals from PBel projecting to CEA in another, to investigate the role of this projection in regulating hypercapnic arousals. In Vglut2-Cre mice, we injected the PBel area on one side of the brain with Cre dependent adeno-associated virus (AAV) containing archaerhodopsin T (AAV-ArchT) and AAV containing diphtheria toxin subunit A (DTA) on the other side (EXP-1). In a separate set of Vglut2-Cre mice, we injected AAV-ArchT in the PBel area bilaterally and lesioned the CEA on one side using 30nl of 5% ibotenate (IBO)(EXP-2). Both cohorts of mice were also instrumented for sleep recording and implanted with a glass fiber targeting the PBel injected with ArchT in EXP-1 or targeting the CEA on the side contralateral to the IBO lesion in EXP-2. We compared the cortical arousals to RCA in these two cohorts, with and without the 593nm laser light to hyperpolarize the PBel or its terminal field in the CEA. The 60s of laser stimulation started 20s before the onset of 10% CO2 and lasted for 10s after the stimulus. Mice showed normal responses to CO2 (arousal latency: 10±2s) at baseline (with no laser), and they aroused within 30s on every trial (0% failure to arouse). When the 593nm laser light was applied to the PBel injected with ArchT in EXP-1, the mean latency of the response to CO2 increased to 38±10s and in three mice with accurate placement of injection and fiber on one side and deletion of PBel neurons on other, animals failed to arouse in 38% of the trials. In EXP-2, laser illumination of PBel terminals in the CEA containing ArchT (n=2) also delayed arousal to CO2 (arousal latency- 36±0.5s) compared to baseline (16±2s), and animals failed to arouse to CO2 on 26% of trials (compared to 0% with no laser). These results suggest that PBel projections to CEA mediate cortical EEG arousals to hypercapnia. CEA may mediate such arousals via projections to the BF and LH, which then project to the cerebral cortex. However, the roles of PBel projections to LH and BF need further investigation.

Disclosures: **S. Kaur:** None. **P. Fuller:** None. **D. Kroeger:** None. **C. Saper:** None.

Poster

548. Sleep: Mechanisms and Molecules I

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 548.26/RR12

Topic: E.08. Biological Rhythms and Sleep

Title: Modifying sleep continuity with transcranial direct current stimulation

Authors: ***F. JAHN**[1], S. ZITTEL[1], L. KRONE[1], P. SELHAUSEN[1], L. FRASE[1], H. PIOSCZYK[1], B. FEIGE[1], D. RIEMANN[1], M. NITSCHE[2], C. NISSEN[1]
[1]Univ. Med. Ctr., Freiburg, Germany; [2]Univ. Med. Ctr., Göttingen, Germany

Abstract: Objective The tDCS (transcranial direct current stimulation) has proven to induce local changes in cortical activity and the local frontal cortex areas are substantially involved in the top-down sleep regulation. It therefore seems logically consistent that excitability dependent sleep parameters can be modified by tDCS. The underlying hypothesis was that periodical bilateral prefrontal anodal stimulation leads to a boost in cerebral excitability and arousals and disturbs sleep continuity whereas cathodal stimulation in the same region causes the reverse effect. Furthermore, it is evident that sleep contributes to the long-term consolidation of new memories. In addition to the sleep parameters, we observed how tDCS provoked modifications affect the declarative and the procedural memory. Methods In our randomized controlled study, we examined 18 healthy subjects (7 men, aged 40-65 years) for a total of 5 nights each in a sleep laboratory. After one night of adaption and one baseline night, the individuals were stimulated with tDCS immediately prior to polysomnographically monitored sleep. The stimulation protocol was composed of anodal-activating, cathodal-deactivating and placebo stimulation in a counterbalanced order. Each intervention was separated by an interval of 7 days. We performed the stimulation with 2mA current strength for 2x 9 minutes (cathodal) respectively 13 minutes (anodal) and an interstimulus interval of 20 minutes. Previous studies have shown that this protocol induces long-lasting aftereffects lasting up to several hours after the stimulation. Before undergoing the stimulation protocol, the subjects had to do one declarative and one procedural memory task. These were then repeated the next morning. We examined how diverse sleep parameters and memory consolidation changed according to the different stimulation conditions. Results The analysis of our data indicated significant polarity dependent effects of the stimulation. In essence, we observed important diminutions in total sleep time (27.6 minutes on

average) and sleep efficiency (5.6 percent on average) in the anodal condition (activation) compared to the cathodal (deactivation) condition. In contrast, we did not find any significant differences between the conditions concerning the memory tasks. Conclusion The results of this study provide a proof-of-concept that the change of prefrontal cortical activity has strong effects on regulatory circuits affecting sleep continuity. These results might be substantial for a better understanding of sleep regulation and for the development of new treatments for patients with sleep disturbances.

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Poster

548. Sleep: Mechanisms and Molecules I

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Program#/Poster#: 548.27/RR13

Topic: E.08. Biological Rhythms and Sleep

Title: Modulating sleep in a human model for cortical hyperarousal by transcranial direct current stimulation

Authors: *P. SELHAUSEN[1], S. ZITTEL[1], F. JAHN[1], L. KRONE[1], L. FRASE[1], H. PIOSCZYK[1], B. FEIGE[1], M. NITSCHE[2], D. RIEMANN[1], C. NISSEN[1]
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Abstract: Objective The regulation of sleep onset and maintenance is a complex process. Several regulatory brain circuits have been identified, including prefrontal corticothalamic feedback loops. Dysfunctions in one or more of those circuits might lead to an insomnia spectrum disorder. The ‘hyperarousal model of insomnia’ postulates an increased state of arousal leading to a disturbance of sleep onset and continuity. This arousal has been demonstrated to go along with an increased cerebral metabolism and accelerated rhythms in electroencephalography. In accordance with the ‘hyperarousal model of insomnia’ patients with primary insomnia (PI) can be seen as a human model for cortical hyperarousal. Transcranial direct current stimulation (tDCS) has been shown to be able to modify excitability in the prefrontal cortex and to modulate resting-state activity. The aim of this study is to answer the question whether modifying the cortical excitability in the prefrontal cortex via bilateral tDCS in patients with PI as a human model for cortical hyperarousal leads to a change in sleep parameters. The hypothesis is that

prefrontal anodal stimulation leads to an increase in excitability and arousal thereby disturbing sleep continuity whereas cathodal stimulation over the same region might improve the patients' disturbed sleep. **Methods** 19 patients with PI (13 females, aged 20-61 yrs) are being included. After an adaptation night, each subject undergoes a baseline polysomnography and three experimental sleep laboratory nights with a tDCS protocol immediately prior to polysomnographically monitored sleep. Three different conditions, anodal, cathodal and sham stimulation, are being applied in a counterbalanced order. **Results** A first analysis of our pilot data of N=6 subjects has not shown significant differences in our main outcome parameters sleep latency, total sleep time or sleep efficiency between the three different stimulation-conditions. The explorative data analysis revealed a significant effect in the rapid eye movement (REM) sleep-density. This has shown to be significantly denser after cathodal stimulation compared to anodal or sham stimulation. **Conclusion** The lack of significant results in our main outcome parameters so far might be a problem of low statistical power (N=6). Another possible explanation is that the hyperarousal overlies the modulating effects of tDCS. However, the significant effect in REM sleep-density shows that modulation of sleep by tDCS might be possible even in the state of cortical hyperarousal. The study will be completed in May 2014. At the annual meeting in November, a full analysis of the data of N=19 subjects will be presented.

Disclosures: P. Selhausen: None. **S. Zittel:** None. **F. Jahn:** None. **L. Krone:** None. **L. Frase:** None. **H. Piosczyk:** None. **B. Feige:** None. **M. Nitsche:** None. **D. Riemann:** None. **C. Nissen:** None.

Poster

548. Sleep: Mechanisms and Molecules I

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Program#/Poster#: 548.28/RR14

Topic: E.08. Biological Rhythms and Sleep

Title: Modulation of sleep by transcranial direct current stimulation as an area-specific effect

Authors: *L. KRONE[1], S. ZITTEL[1], P. SELHAUSEN[1], F. JAHN[1], L. FRASE[1], H. PIOSCZYK[1], B. FEIGE[1], M. NITSCHE[2], D. RIEMANN[1], C. NISSEN[1]
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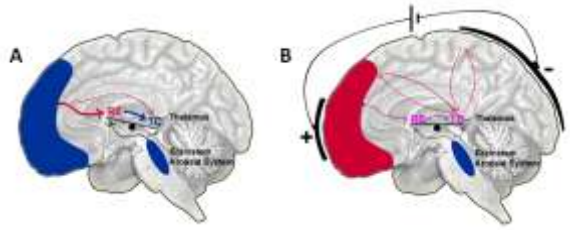
Abstract: Objective Corticothalamic feedback loops are supposed to be substantially involved in the initiation and maintenance of non-rapid eye movement (NREM) sleep, representing a 'top-

down' pathway of sleep regulation, which interacts with the 'bottom-up' pathway of the brainstem arousal system. In a pilot study, periodical application of transcranial direct current stimulation (tDCS) to prefrontal cortical areas resulted in a difference of 27.6 minutes total sleep time ($p < 0.01$) when anodal (activating) stimulation was compared to cathodal (deactivating) stimulation. In this study we investigated whether the effect of tDCS on sleep regulation is location-specific for stimulation of the prefrontal cortex. **Methods** We applied the standardized stimulation protocol (2mA, 2 x 9 min. cathodal, 2 x 13 min. anodal) to the parietal cortex of 10 healthy subjects in a randomized crossover design and compared the results to our initial group, which had undergone prefrontal stimulation. **Results** In contrast to prefrontal stimulation, parietal stimulation did not yield any significant effects. **Conclusion** This result strongly supports the hypothesis that sleep-modifying effects of tDCS are based on a modulation of specific corticothalamic feedback loops between the prefrontal cortex and thalamus. **Figure 1: Modulation of corticothalamic sleep regulation by transcranial direct current stimulation** [A] During NREM sleep prefrontal areas promote the functional disconnection of thalamus and cortex. Reticular thalamic (RE) neurons are activated and suppress transduction of information in thalamocortical (TC) neurons. [B] Anodal (activating) transcranial direct current stimulation of prefrontal areas disturbs physiological sleep regulation and enhances cortical activation via TC neurons.



Disclosures: **L. Krone:** None. **S. Zittel:** None. **P. Selhausen:** None. **F. Jahn:** None. **L. Frase:** None. **H. Piosczyk:** None. **B. Feige:** None. **M. Nitsche:** None. **D. Riemann:** None. **C. Nissen:** None.

Poster

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Topic: E.08. Biological Rhythms and Sleep

Support: NHLBI (HL095491)

NINDS (NS082854)

Title: Orexin projections control locus coeruleus activity

Authors: *L. FERRARI, D. PARK, L. J. AGOSTINELLI, T. E. SCAMMELL, E. ARRIGONI
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Abstract: The orexin neurons play an essential role in driving arousal and maintaining normal wakefulness. Lack of orexin neurotransmission results in narcolepsy, with excessive sleepiness, frequent transitions between wakefulness and sleep, and episodes of cataplexy. Orexin neurons innervate and excite all the major arousal centers including the locus coeruleus (LC) neurons. Orexin neurons produce orexin-A and B, glutamate and dynorphin but the dynamics of their release are still unclear. We used channelrhodopsin-2 (ChR2)-assisted-circuit-mapping (CRACM) in *in vitro* brain slices to examine the input from orexin neurons to the LC. To express ChR2 in orexin neurons, we used an adeno-associated viral (AAV) vector coding for cre-dependent ChR2-YFP (AAV-Flex-ChR2(H134R)-YFP) in Dyn-ires-cre mice. We microinjected the AAV-ChR2-YFP into the perifornical region where the only neurons expressing dynorphin are the orexin population. Four to six weeks after these injections, we recorded LC neurons in whole-cell configuration. We identified LC neurons based on their anatomical location and electrophysiological fingerprint. We stimulated ChR2-expressing axons and terminals using 5-ms pulses of blue-light (473 nm). Photostimulation of orexin terminals evoked short latency (6.84 ± 0.6 ms) excitatory postsynaptic currents (EPSCs) in noradrenergic LC neurons. These photo-evoked EPSCs were abolished by DNQX, indicating that they were mediated by postsynaptic AMPA receptors. Photostimulation with a 10 sec train (10Hz) of 5 ms pulses, rapidly depressed the photoevoked EPSCs and was accompanied by a slow inward current compatible with orexin signaling. The frequency of asynchronous EPSCs was increased during and after the photostimulation train, indicating short-term synaptic facilitation. These results suggest that the orexin neurons directly activate the LC neurons through the release of glutamate, and in addition, orexin peptides can elevate LC excitability by increasing heterosynaptic glutamatergic afferent input to the LC neurons. These responses may provide a synergistic mechanism through which orexin neurons act upon the LC to promote wakefulness and improve cognitive performance.

Disclosures: **L. Ferrari:** A. Employment/Salary (full or part-time):; Jazz Pharmaceuticals. **D. Park:** None. **L.J. Agostinelli:** None. **T.E. Scammell:** None. **E. Arrigoni:** None.

Poster

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Topic: E.08. Biological Rhythms and Sleep

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NSF REU-CRIB Program Grant 1156810

Title: Pumilio regulates the sleep homeostatic response to chronic sleep deprivation in *Drosophila* melanogaster

Authors: *L. DE JESUS[1], N. RODRIGUEZ[2], C. PACHECO[2], J. ORTEGA[2], H. RODRIGUEZ[2], G. DIAZ[2], F. RIVERA[2], M. REYES[2], E. RIVERA[2], J. ALEMAN-RIOS[2], A. AVALOS[2], J. L. AGOSTO-RIVERA[2]
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Abstract: *Drosophila melanogaster* has been extensively used as a model system for sleep studies. Although much is known about the circadian regulation of sleep, the mechanisms underlying sleep homeostasis and its relationship with the circadian system remains largely unknown. Previously, our lab has generated data indicating that during chronic sleep deprivation, *Drosophila* exhibits a progressive increase in sleep over time, despite the continuous presence of a sleep depriving stimulus. RNAi knockdown of the neuronal homeostasis gene *pumilio*, using the timeless-Gal 4 driver, resulted in a reduction of the sleep recovery rate. Conversely, *pumilio* overexpression increases the sleep recovery rate. Furthermore, the sleep rebound normally observed after deprivation is abolished by *pumilio* knockdown. These findings suggest that *pumilio* is involved in promoting sleep drive. Given the role of *pumilio* in cellular homeostasis processes, our data suggests that mechanisms of cellular homeostasis in the circadian system, are linked to sleep homeostasis.

Disclosures: L. De Jesus: None. **N. Rodriguez:** None. **C. Pacheco:** None. **J. Ortega:** None. **H. Rodriguez:** None. **G. Diaz:** None. **F. Rivera:** None. **M. Reyes:** None. **E. Rivera:** None. **J. Aleman-Rios:** None. **A. Avalos:** None. **J.L. Agosto-Rivera:** None.

Poster

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Topic: E.08. Biological Rhythms and Sleep

Support: NIH NINDS

NIH NIDA

Title: Discovery of a neuropeptide signaling pathway that regulates sleep/wake behavior in zebrafish

Authors: *C. N. CHIU[1], J. RIHEL[2], E. A. MOSSER[1], D. A. LEE[1], C. SINGH[1], S. CHAKRAVARTHY[1], A. F. SCHIER[3], D. A. PROBER[1]
[1]Caltech, Pasadena, CA; [2]Univ. Col. London, London, United Kingdom; [3]Harvard, Cambridge, MA

Abstract: The discovery of the neuropeptide hypocretin's role in sleep and sleep disorders was a major breakthrough in sleep research. This finding underscored the potential advances that can be made by using genetics to discover new regulators of sleep. However, few molecular regulators of sleep have been discovered since. To identify genes that regulate vertebrate sleep, we performed a genetic overexpression screen using a high-throughput larval zebrafish locomotor activity assay. We found that overexpression of neuromedin U (NMU) dramatically increased locomotor activity and decreased sleep during both the day and night. To identify the signaling pathways that mediate this phenotype, we mutated the zebrafish orthologs of the two mammalian NMU receptors. We found that NMUR2, which is primarily expressed in the central nervous system, and not NMUR1, which is predominantly expressed peripherally, is required for NMU-induced arousal. We also found that inhibiting NMUR2 using a small molecule antagonist disrupts sleep/wake architecture by causing fragmented waking and frequent transitions to sleep. Previous studies hypothesized that NMU promotes behaviors associated with stress via the hypothalamic-pituitary-adrenal (HPA) axis, which is initiated by hypothalamic corticotropin-releasing hormone (CRH) signaling and ultimately stimulates adrenal production of glucocorticoids. We found that the NMU overexpression phenotype persists in zebrafish glucocorticoid receptor mutant larvae, suggesting that the HPA axis does not mediate NMU-induced arousal. Instead, we found that NMU overexpressions activates crh-expressing neurons in the rostral brainstem. These cells may be analogous to mammalian brainstem CRH populations in the locus coeruleus (LC) and pedunculopontine tegmental nucleus (PPT) which play an important role in sleep/wake regulation. Taken together, our data identify a role for NMU in regulating sleep/wake behaviors and suggest that the relevant effectors are arousal systems in the brainstem rather than classical HPA axis in the periphery.

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Poster

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Program#/Poster#: 549.03/RR18

Topic: E.08. Biological Rhythms and Sleep

Title: The effects of combined lithium and ethanol treatment on the behavioral circadian activity rhythm

Authors: *J. A. SEGGIO, K. CARLSON, N. NASCIMENTO, D. AMARAL, D. PYNE, G. NASH
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Abstract: Lithium has been traditionally used to treat Bipolar disorder among other psychiatric disorders that have been linked to disruptions in the circadian rhythm. Numerous studies have shown that lithium treatment produces period lengthening and amplifies expression of per2. Conversely, ethanol drinking has been shown to produce period shortening and reductions in per2 expression. In addition, ethanol drinking also blunts the phase-shifting effects of light pulses. Thus, this investigation aims to uncover the effects of combined ethanol and lithium treatment on the period and phase of the circadian rhythm. All B6 mice were placed into DD for 3-weeks with water only; after the initial 3-weeks, mice were given one of four drinking solutions: water, 10 mM LiCL, 10%-ethanol, or a combination of lithium and ethanol, and were allowed additional 3-weeks in DD. In addition, light pulses at ZT 15 and ZT 21 were conducted to determine if lithium can prevent ethanol's blunting of photic phase responses. As expected, lithium significantly increased the period, while alcohol produced a small but non-significant decrease in the period. When combined ethanol/lithium solution was given, it produced similar free-running periods to control mice. Additionally, ethanol produced reductions in photic phase shifting, but when lithium was given with ethanol, the combined effect produced phase shifts similar to water controls. The combined lithium/ethanol group drank significantly less fluid than the lithium, ethanol, and water groups, indicating that lithium treatment can be used as a mechanism for decreased ethanol intake, and thus, reducing the effects of both drugs on the circadian clock. These results may also indicate that lithium and ethanol may be affecting the same clock mechanisms and having opposite effects.

Disclosures: **J.A. Seggio:** None. **K. Carlson:** None. **N. Nascimento:** None. **D. Amaral:** None. **D. Pyne:** None. **G. Nash:** None.

Poster

549. Sleep: Mechanisms and Molecules II

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Program#/Poster#: 549.04/RR19

Topic: E.08. Biological Rhythms and Sleep

Title: Rapid eye movement sleep induction and maintenance by gabaergic neurons in the ventral medulla

Authors: ***F. WEBER**[1], S. CHUNG[1], M. XU[1], Y. DAN[1,2]
[1]Dept. of Mol. and Cell Biol., UC Berkeley, Berkeley, CA; [2]Howard Hughes Med. Inst., Berkeley, CA

Abstract: Rapid eye movement (REM) sleep is characterized by activation of the cortical and hippocampal electroencephalogram (EEG) and concomitant muscle atonia. Here, we demonstrate the role of the ventral medulla GABAergic neurons in inducing and maintaining REM sleep. To test how the activity of these neurons influences sleep, we recorded the EEG and nuchal electromyogram (EMG) in freely moving mice, while optogenetically activating the ventral medulla. Optogenetic stimulation of GABAergic neurons in the ventral medulla during Non-REM sleep reliably induces REM sleep and strongly increases the duration of REM sleep periods. Using optetrodes (tetrodes coupled to an optical fiber) we recorded GABAergic medullary neurons in freely moving mice. Most GABAergic neurons exhibited highest firing rates during REM sleep. Finally, to unravel regions providing input to the REM-inducing GABAergic neurons in the ventral medulla, we applied a monosynaptically restricted, trans-synaptic rabies virus tool. Our experiments combining optogenetics and electrophysiology in freely moving mice provide strong evidence that GABAergic neurons in the ventral medulla are causally involved in the induction and maintenance of REM sleep.

Disclosures: **F. Weber:** None. **S. Chung:** None. **M. Xu:** None. **Y. Dan:** None.

Poster

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Topic: E.08. Biological Rhythms and Sleep

Support: NIH grant R01 NS073613

Title: Gabaergic parafacial zone neurons are necessary for the expression of normal slow-wave-sleep

Authors: *C. ANACLET, L. FERRARI, E. ARRIGONI, P. FULLER
BIDMC Harvard, BOSTON, MA

Abstract: We have previously reported that acute and selective activation of GABAergic parafacial zone (PZ) neurons is sufficient to produce slow wave sleep (SWS) and cortical slow wave activity (SWA), independent of the time of day. Using optogenetic-based mapping we have further established the presence of a functional synaptic PZ◊parabrachial nucleus◊basal forebrain◊cortex pathway through which GABAergic PZ neurons can potently drive SWS and modulate the cortical EEG. It however remains unclear if GABAergic PZ neurons are necessary for the expression of normal SWS and cortical SWA or, rather, are functionally redundant to the forebrain sleep-promoting ventrolateral preoptic nucleus (VLPO). To investigate whether GABAergic PZ neurons are necessary for the expression of normal SWS and cortical SWA, we placed small bilateral injections of an adeno-associated viral (AAV) vector containing a cre-enabled inhibitory receptor system [hM4Di-AAV10] into the PZ of Vgat-IRES-cre mice. Ligand (CNO, 0.3mg/kg, IP), but not vehicle, injections at ZT3 - a time of high sleep drive in the mouse - produced a significant increase in wake in mice expressing inhibitory hM4Di receptor in PZ GABAergic neurons. The waking response, observed during acute and selective inhibition of PZ GABAergic neurons, is the opposite of what we observed following acute activation of GABAergic PZ neurons. CNO injections were without effect on the waking cortical EEG power spectrum. These results recapitulate the effects produced by genetic-driven PZ lesions in mice and provide convincing evidence that GABAergic PZ neurons are necessary for the expression of normal SWS amounts in behaving mice and are not functionally redundant in their sleep-promoting properties to forebrain VLPO neurons. Supported by R01 NS073613

Disclosures: **C. Anaclet:** None. **L. Ferrari:** None. **E. Arrigoni:** None. **P. Fuller:** None.

Poster

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Program#/Poster#: 549.06/RR21

Topic: E.08. Biological Rhythms and Sleep

Support: NIH Grant F31AG043329

Title: The sleep promoting effect of DORA-12 is sex dependent in rats

Authors: *J. A. MONG, D. M. CUSMANO
Univ. Maryland, Sch. Med., BALTIMORE, MD

Abstract: Sleep disruptions are more commonly reported in women and typically coincide with periods of hormonal fluctuation. We have previously shown in rats that estradiol (E2) suppresses sleep in females and that sex differences in sleep are due to activational effects of E2 on sexually differentiated circuitry. Masculinization of the brain renders sleep behavior in males and masculinized females insensitive to the suppressive effects of E2. We have identified the median preoptic nucleus (MnPN) as a key site of E2 action. The MnPN is a sleep-promoting region that sends inhibitory projections to arousal nuclei like the lateral hypothalamus (LH). E2 reduces the activation of sleep-associated MnPN neuron and antagonism of estrogen receptors within the MnPN attenuates the E2-mediated suppression of sleep. Orexinergic neurons in the LH are regulated by MnPN activity and orexin is a key neuropeptide involved in arousal. Prepro-orexin mRNA expression is sexually dimorphic in the hypothalamus; females have higher levels compared to males. Circulating levels of gonadal steroids also modulate expression of orexin and its receptors. Thus, we hypothesize that orexin is the mediator of E2's suppressive effects on sleep. To test, we administered a dual orexin receptor antagonist (DORA-12, gift from Merck) in the presence or absence of E2. If orexin mediates E2's suppression of sleep, then we predict that antagonism of orexin receptors will attenuate E2's effect. Additionally, due to the sex difference in prepro-orexin mRNA and steroidal modulation of receptor expression, we predict that sleep promotion by DORA-12 will be sex and E2 dependent. Gonadectomized female and intact male rats were randomly assigned to DORA-12 or vehicle (VEH) groups. DORA-12 (30mg/kg) or VEH was given orally prior to lights out (ZT12) each day. Females received an oil injection and then two doses (5µg and 10µg) of E2 benzoate 24hrs apart at ZT21, while males received only oil injections. In females, DORA-12 significantly reduced wake and promoted sleep, but it did not block the suppression of total sleep by E2. The percent change in each vigilance state induced by E2 is not significantly different between VEH and DORA-12 treated females. DORA-12's sleep-promoting effect in females is greater than in males. In males, DORA-12 significantly suppressed wake 3-4hr post-administration. Wake suppression in females was steady across the 12hr dark phase following DORA-12 administration. Sex differences in sleep circuitry and/or drug metabolism may contribute to increased sleep promotion in females. We are

currently exploring this hypothesis by testing the metabolism of DORA-12 in males and oil/E2 treated females.

Disclosures: J.A. Mong: None. **D.M. Cusmano:** None.

Poster

549. Sleep: Mechanisms and Molecules II

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NSF-CREST Grant HRD 1036494

NASA Grant NNX12AH22A

Title: Real-time sensing of glutamate in the basolateral amygdala (BLA) in the rat brain across sleep-wake states

Authors: *L. D. SANFORD[1], M. H. KIM[2], L. L. WELLMAN[1], H. YOON[2]
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Abstract: Evolving research demonstrates that the BLA can be a significant modulator of sleep and that it regulates the association between fear behavior, fear memories and sleep. Eighty percent or more of the neurons in BLA are phenotypically glutamatergic and, not surprisingly, glutamate has been a focus in studies of the amygdala and its role in the acquisition and storage of fear memory as well as in regulating sleep. As part of an effort to understand the role of BLA in regulating sleep, we used biosensors to examine glutamate release during spontaneous sleep and wakefulness. Four rats were implanted with cannula aimed into BLA and with telemetry transmitters to allow assessment of arousal state and allowed to recover. For recording, a glutamate biosensor (Pinnacle Technology, #7011) was slowly inserted into the pre-implanted cannula. These are glutamate oxidase (GluOx)-coated biosensors that allow virtual real-time detection of changes in extracellular glutamate levels. The biosensor was calibrated prior to insertion and after recording using glutamate and ascorbic acid in temperature controlled PBS. After a minimum of two hours to allow the signal to stabilize, recordings were made concurrently with spontaneous changes in sleep and wakefulness. All glutamate recordings were

made with a CH Instruments 760D potentiostat connected to the biosensor via a custom cable and PlasticsOne commutator to allow the rats free movement. The amplitude variation of measured currents across changes in arousal and sleep ranged between 0.04 nA to 0.07 nA for wakefulness, non-rapid eye movement sleep (NREM) and rapid eye movement sleep (REM), which corresponds to 51 nM ~ 89 nM concentration changes relative to the pre-calibration data. There was a clear association between changes in glutamate level in the BLA and changes in EEG associated with wake and sleep stages based on current increases during wakefulness and REM relative to levels in NREM. Thus, this work demonstrates changes in extracellular glutamate activity that are linked to changes in arousal states. Future work will be required to determine whether these changes are important for initiating changes in sleep and wakefulness.

Disclosures: L.D. Sanford: None. **L.L. Wellman:** None. **M.H. Kim:** None. **H. Yoon:** None.

Poster

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Topic: E.08. Biological Rhythms and Sleep

Support: NSF Grant DMS 1121361

Colorado School of Mines Undergraduate Research Fellowship

Title: Analyzing sleep/wake architecture in mice with progressive orexin/hypocretin cell loss

Authors: A. F. BRANCH[1], W. NAVIDI[1], S. TABUCHI[2], A. YAMANAKA[2], T. E. SCAMMELL[3], *C. DINIZ BEHN[1]
[1]Dept. of Applied Math and Statistics, Colorado Sch. of Mines, Golden, CO; [2]Res. Inst. of Envrn. Med., Nagoya Univ., Nagoya, Japan; [3]Dept. of Neurol., Beth Israel Deaconess Med. Ctr., Boston, MA

Abstract: The orexin (also known as hypocretin) neurons are important for consolidating sleep/wake behavior, and loss of these cells is associated with the sleep disorder narcolepsy. The recent development of a rodent model utilizing the controlled expression of diphtheria toxin A in orexin neurons provides an opportunity to assess changes in sleep/wake architecture during progressive orexin neurodegeneration over the course of weeks. Disrupted sleep/wake architecture has been reported in these animals; however, the specifics of this disruption have not

been described. We analyzed scored sleep/wake data in these mice to quantify the changes in sleep/wake architecture associated with orexin cell loss and to investigate the implication of these changes for orexin function. Survival analysis of bouts of wake, rapid eye movement (REM) sleep, and non-REM (NREM) sleep durations showed a dose-dependent progression to the narcolepsy phenotype with putative orexin cell loss. Analysis of the hazard rates for wake bouts suggests a role for orexin in sustaining long periods of wakefulness. However, for wake bouts less than ~1.5 min, the hazard rate was lower in animals with orexin neurodegeneration, indicating that orexin may not contribute to sustaining brief wake bouts. For NREM sleep bouts, the hazard rate was consistently higher in animals with orexin neurodegeneration. In addition, progressive orexin cell loss was associated with dose-dependent attenuation in several measures of light/dark differences, thus supporting a role for orexin neurons in relaying circadian modulation of sleep/wake behavior.

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Poster

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Program#/Poster#: 549.09/RR24

Topic: E.08. Biological Rhythms and Sleep

Support: NHLBI HL095491

Title: Selective tracing of descending basal forebrain projections

Authors: ***L. J. AGOSTINELLI**[1], T. E. SCAMMELL[2,3]
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Abstract: Neurons of the basal forebrain (BF) project to the cortex to promote cortical activation and behavioral arousal, and this ascending pathway has been heavily studied. However, the BF also innervates many subcortical regions. Additionally, the BF is neurochemically heterogenous, and conventional tracing methods have limited our ability to map neurochemically specific projections. To further characterize and map these descending projections, we used conditional anterograde tracing of BF neurons producing acetylcholine, GABA, or glutamate to examine BF projections to subcortical regions. Using mice that express Cre recombinase selectively in GABA (vGAT-Cre), glutamate (vGlutT2-Cre), or acetylcholine (ChAT-Cre) neurons, we

microinjected the BF with an adeno-associated viral vector (AAV) coding for a Cre-dependent red fluorescent protein (mCherry). To examine how projections varied across the BF, we targeted several parts of the BF, including the substantia innominata, diagonal band, etc. Four weeks later, we double immunolabeled sections for mCherry and ChAT, vGlutT2 or vGAT and mapped the innervation of specific descending targets. This method produced robust and selective anterograde labeling in GABA, glutamate, or ACh BF neurons. *In situ* hybridization and immunolabeling confirmed that mCherry expression was limited to the correct cell types. GABAergic and glutamatergic BF neurons robustly innervated many similar targets such as the medial amygdala, lateral hypothalamus, supramammillary area, parasubthalamic nucleus, ventral tegmental area, substantia nigra pars compacta, dorsal and median raphe, lateral periaqueductal gray, and midbrain reticular formation. The lateral habenula was exclusively and intensely innervated by glutamatergic BF neurons, while the central medial amydgala was exclusively innervated by the GABAergic BF neurons. Cholinergic BF neurons heavily innervated the basolateral amygdala, but otherwise their descending projections were more limited than the GABAergic and glutamatergic projections. In addition, more caudal regions of the BF such as the substantia innominata had stronger descending projections than more rostral parts of the BF. These descending projections from the BF may play important roles in regulating and coordinating the activity of arousal networks.

Disclosures: L.J. Agostinelli: None. **T.E. Scammell:** None.

Poster

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Topic: E.08. Biological Rhythms and Sleep

Support: HL-047600 and HL-116508.

Title: Distribution of terminals originating in pontine noradrenergic A6 (locus coeruleus), A7 and subcoeruleus neurons in the medullary viscerosensory nucleus of the solitary tract and hypoglossal motor nucleus

Authors: *L. KUBIN, K. BENINCASA HERR, C. D. HESKETH, D. V. VOLGIN, G. L. MANN
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Abstract: Pontine noradrenergic neurons (PNN) have widespread and divergent projections and characteristically high activity during wakefulness, reduced activity during slow-wave sleep and no activity during rapid eye movement sleep. Although PPN aggregate into distinct groups, these features suggest that all PNN belong to one system whose purpose is to modulate activity throughout the CNS in a state-dependent manner. However, noradrenergic terminal density varies greatly among different brain regions, and retrograde tracing studies indicate that different groups of PNN project to different targets, suggesting some heterogeneity. We previously reported that the hypoglossal motor nucleus (XII) receives axonal projections from the pontine A5, A7 and subcoeruleus (subC) neurons, whereas projections to the adjacent viscerosensory nucleus of the solitary tract (NTS) originate in the A5 and A6 groups and to a much lesser extent in A7 or subC (Rukhadze & Kubin, J. Chem. Neuroanat. 2007). To further elucidate the anatomical basis of these differences, we now investigate the termination patterns of PNN in the XII and NTS using anterograde tracing in Long-Evans rats designed to express Cre element in tyrosine hydroxylase (TH)-synthesizing neurons. In four rats, we injected 100-400 nl of adeno-associated viral vector containing Cre-dependent, double-floxed sequences for channel rhodopsin-2 and enhanced yellow fluorescent protein (EYFP) into the dorsolateral pons. After 29-64 days, the animals were perfused and fluorescent double-labeling revealed strong expression of EYFP in TH-positive A7 and subC neurons in all animals, with additional labeling of A6 neurons in one animal. EYFP was then revealed in medullary sections using Ni-enhanced horseradish peroxidase reaction to visualize fine noradrenergic terminals in the XII and NTS. When all noradrenergic terminals are labeled regardless of their cellular origin, their density is higher in the NTS than in XII and within XII they are most dense in its ventromedial quadrant. In contrast, following selective labeling of terminals originating in A7 and subC only, we observed a similar noradrenergic terminal density in NTS and all parts of XII. This suggests that the characteristic differences in noradrenergic terminal density between the NTS and XII and within the XII are caused by noradrenergic projections from other noradrenergic groups whereas A7 and subC contribute a relatively uniform density of noradrenergic innervation to both nuclei. This is consistent with A7 and subC mediating diffusely distributed, state-dependent noradrenergic modulation in functionally distinct regions.

Disclosures: L. Kubin: None. **K. Benincasa Herr:** None. **C.D. Hesketh:** None. **D.V. Volgin:** None. **G.L. Mann:** None.

Poster

549. Sleep: Mechanisms and Molecules II

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 549.11/RR26

Topic: E.08. Biological Rhythms and Sleep

Support: NIH TR01 GM104948

Title: Electrical stimulation of the parabrachial nucleus induces reanimation from general anesthesia

Authors: *F. MUINDI[1], C. J. VAN DORT[1,2], J. KENNY[2], N. TAYLOR[2], K. SOLT[2], M. WILSON[1], E. N. BROWN[1,2]
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Abstract: Background: The parabrachial nucleus (PBN) has been shown to play an important role in promoting arousal. Here, we tested whether electrical stimulation of the PBN is sufficient to induce reanimation (active emergence) from isoflurane general anesthesia. **Methods:** Mice were implanted with electroencephalogram/electromyogram (EEG/EMG) electrodes and also with a single bipolar insulated stainless steel electrode into the PBN (n=8) or the central inferior colliculus (n=7) (control area). After a minimum 7-day recovery period, a dose (0.9% ± 0.1 vol%) sufficient to maintain loss of righting was used. Electrical stimulation (30 seconds in length) was initiated from 30 μA and increased in intensity every 30 μA until the righting reflex was restored. All stimulations occurred during continuous isoflurane anesthesia. **Results:** In 5 of 8 mice, electrical stimulation of the PBN at 60 μA at 100 Hz rapidly induced behavioral arousal (kicking, clawing and lifting the head) and ultimately caused the restoration of the righting reflex during continuous isoflurance general anesthesia. In contrast, the same stimulation in the central inferior colliculus did not cause restoration of the righting reflex. Spectral analysis of EEG recordings revealed that the stimulation produced a significant decrease in EEG power in the delta (<4 Hz) range during PBN stimulations. **Conclusions**: Electrical stimulation of the PBN, but not the central inferior colliculus, is sufficient to induce reanimation during general anesthesia with isoflurane. These results suggest that the PBN may be used to accelerate recovery from general anesthesia and ameliorate anesthesia-related cognitive dysfunction.

Disclosures: F. Muindi: None. **C.J. Van Dort:** None. **J. Kenny:** None. **N. Taylor:** None. **K. Solt:** None. **M. Wilson:** None. **E.N. Brown:** None.

Poster

550. Visual Perception: Neural Mechanisms

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Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 550.01/RR27

Topic: F.01. Human Cognition and Behavior

Support: SRPBS, MEXT,

Ministry of Internal Affairs and Communications entitled, 'Novel and innovative R&D making use of brain structures'

KAKENHI 22830136

Title: Common brain activity patterns during perception, imagery, and dreaming

Authors: *T. HORIKAWA[1], Y. HOSOKAWA[2,1], Y. KAMITANI[1]
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Abstract: Dreaming often accompanies visual experience, which appears to be phenomenally similar to stimulus-induced perception, and also to top-down mental imagery. It has been debated whether the neural mechanism underlying dreaming is perception-like or imagery-like. Our previous study (Horikawa et al., 2013) has shown that visual dream contents (object categories) can be predicted from fMRI activity patterns in the visual cortex during sleep by machine-learning decoders trained on brain activity induced by stimulus images containing objects. This “perception-to-dream” decoding indicates that perception and dreaming share neural representations of visual contents. Here we extend this approach to investigate the commonality and difference of neural representations between perception, imagery, and dreaming in brain areas known to be responsive to perception and/or imagery tasks. In addition to decoders trained on stimulus-induced brain activity (perception-trained decoders), we used decoders trained on brain activity during a task in which subjects visually imagined objects (imagery-trained decoders), to predict dreamed objects from brain activity during sleep. We found that the overall accuracies for the decoding of dreamed objects were comparable between the perception-trained decoders and the imagery-trained decoders. Interestingly, the perception-trained decoders outperformed the imagery-trained decoders in relatively lower-level areas, the ventral visual cortex and inferior parietal cortex, while the imagery-trained decoders outperformed the perception-trained decoders in higher-level areas, the dorsolateral prefrontal cortex and hippocampus. These results suggest that dreaming shares neural representations with perception and imagery in multiple brain areas with greater similarity to perception and imagery in lower and higher areas, respectively. Thus, the neural representation of dreaming may not be unilaterally perception-like or imagery-like, but consist of a unique mixture of perception-like and imagery-like brain states.

Disclosures: T. Horikawa: None. **Y. Hosokawa:** None. **Y. Kamitani:** None.

Poster

550. Visual Perception: Neural Mechanisms

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 550.02/RR28

Topic: F.01. Human Cognition and Behavior

Support: NIH Grant R21EY023067

Title: Recovery of high-level visual functions in patients with lobectomy or hemispherectomy

Authors: *A. NESTOR[1], T. LIU[2], D. C. PLAUT[2], C. PATTERSON[3], M. BEHRMANN[2]
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Abstract: The recovery of perceptual functions that occur following cortical damage can offer key insights into the nature and plasticity of brain organization. In this respect, studies of individuals post-lobectomy/hemispherectomy offer a unique window into the nature and extent of cortical plasticity. First, in contrast with more common lesions, the extent of the damage in such patients can be extreme (i.e. an entire hemisphere in some cases) yet, at the same time, very well controlled - both cortical and subcortical structures of the remaining hemisphere are typically intact. Second, the extent of the recovery is often disproportionate relative to the extent of the damage - many compromised functions are regained partly or even completely. Our present work examines the neural basis of visual recovery in a population of children who have undergone surgical lobectomy or hemispherectomy of ventral cortex in either hemisphere. An fMRI investigation of high-level visual functions focused on cortical mapping of face, object and word form recognition. This investigation revealed more restricted activation to objects in the spared hemisphere of our patients relative to matched controls. In contrast, faces tended to elicit more extensive activation in the spared hemisphere of the patients relative to their controls. A further examination of language processing, including visual word forms, produced comparable selectivity maps across patients and controls. Overall, the current results suggest that basic compensatory mechanisms can underlie visual recovery (e.g., for the purpose of face recognition) but also that such mechanisms do not account for the full extent of the recovery (e.g., for language processing).

Disclosures: A. Nestor: None. **T. Liu:** None. **D.C. Plaut:** None. **C. Patterson:** None. **M. Behrmann:** None.

Poster

550. Visual Perception: Neural Mechanisms

Location: Halls A-C

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Program#/Poster#: 550.03/RR29

Topic: F.01. Human Cognition and Behavior

Support: The Wellcome Trust

Japan Society for Promotion of Science

NeuroCreative Lab

CREST, Japan Science and Technology Agency

Title: Energy landscape of human brain activity during bistable perception

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Abstract: Inter-individual variability in the structure of parietal and prefrontal cortex is highly correlated with the stability of bistable visual perception. Despite this well-known observation, the mechanisms bridging the gap between the brain structures and behaviour remain elusive. In the present study, we found such a link by characterising the energy landscapes of brain activity during bistable perception. By applying a pairwise maximum entropy model to fMRI signals recorded from human seeing a structure-from-motion stimulus, we found that the dynamics of brain activity during bistable perception could be described as fluctuating between three spatially distributed energy minima: visual-area dominant, frontal-area dominant, and intermediate states. For each participant, the transitions between these energy states well predicted behaviour: participants whose brain activity tended to be in the visual-area dominant state experienced more stable perception, whereas those whose activity transited to the frontal-area-dominant state reported more frequent perceptual switches. Moreover, these tendencies of brain activity dynamics were well correlated with inter-individual variability in grey matter volume of the focal cortical areas. These findings suggest that the dynamics of brain activity determined by the features of the energy landscape link individual differences in brain anatomy and subjective visual experience.

Disclosures: **T. Watanabe:** None. **N. Masuda:** None. **F. Megumi:** None. **R. Kanai:** None. **G. Rees:** None.

Poster

550. Visual Perception: Neural Mechanisms

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 550.04/RR30

Topic: F.01. Human Cognition and Behavior

Support: National Research Foundation of Korea(NRF) Grant (2006-2005137)

Title: Functional differences in processing preferred and non-preferred stimuli at object selective areas

Authors: ***U.-S. CHOI**[1], Y.-W. SUNG[2], Y.-B. KIM[1], Z.-H. CHO[1], H. YOON[3], S. OGAWA[2]
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Abstract: Some high-level visual areas show category-selectivity. Fusiform face area (FFA) is known specialized for face processing and Parahippocampal place area (PPA) for scene/building processing. In this study we investigated how a non-preferred stimulus affects to the response to a preferred stimulus through functional MRI (fMRI) responses in FFA and PPA evoked by a composite picture. The composite picture was made of a face picture imbedded into the center of a scene picture. The face in the composite picture was made sufficiently small (less than four degrees of visual angle) so that the perception of the scene was not disturbed by the face. The scene picture was large enough to cover the visual field of twenty eight by twenty one degrees. Three kinds of stimuli, composite, face-only and whole scene (scene-only) pictures, were used. The response in FFA to the composite picture was not different from that to the face-only picture. But the response in PPA to the composite picture was smaller than that to the whole scene picture. The response to composite picture in V1-peripheral site was smaller than that to the whole scene picture even though the composite picture in V1-peripheral site was exactly the same as that to the whole scene picture. When we replaced the face in the composite picture with an object picture, the responses to the composite picture in both PPA and V1-peripheral site were not different from those to the whole scene. These indicate that the effects of non-preferred stimuli on the responses to the preferred stimuli are different between FFA and PPA. The composite picture seems to be interpreted as the face at FFA but the face and scene in the composite picture seem to be separately processed at PPA.

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Poster

550. Visual Perception: Neural Mechanisms

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 550.05/RR31

Topic: F.01. Human Cognition and Behavior

Title: Preserved expert object recognition in a case of unilateral visual agnosia

Authors: ***J. RENNIG**[1], H.-O. KARNATH[1,2], M. HIMMELBACH[1,3]
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Abstract: We examined a stroke patient (HWS) with a unilateral lesion of the right medial ventral visual stream. A high resolution MR scan showed a severe involvement of the fusiform and parahippocampal gyri sparing big parts of the lingual gyrus. In a number of object recognition tests with lateralized presentations of target stimuli, HWS showed remarkable deficits for contralesional presentations only. His performance on the ipsilesional side was unaffected. We further explored his residual capabilities in object recognition confronting him with objects he was an expert for. These were items he knew from his job as a trained car mechanic that were occupationally and personally relevant for him. Surprisingly, HWS was able to identify these complex and specific objects on the contralesional side while he failed in recognizing even highly familiar everyday objects. This observation of preserved expert object recognition in visual agnosia gives room for several explanations. At first, these results may be caused by enhanced information processing of the ventral system in the intact hemisphere that is exclusively available for expert objects. On the other hand, expert knowledge could also trigger top-down mechanisms supporting object recognition despite of impaired basic functions of object processing. Finally, a more efficient stimulus processing for expert objects might simply not require complete resources of an intact ventral stream.

Disclosures: **J. Rennig:** None. **H. Karnath:** None. **M. Himmelbach:** None.

Poster

550. Visual Perception: Neural Mechanisms

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Program#/Poster#: 550.06/RR32

Topic: F.01. Human Cognition and Behavior

Support: NWO Brain and Cognition grant 433-09-248

Title: Tracking dynamic mental imagery in early visual cortex

Authors: *A. ALBERS, P. KOK, R. BOYACIOğLU, I. TONI, F. P. DE LANGE
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Abstract: Previous studies suggest that the early visual cortex can hold information about visual content that is kept in working memory (Serences et al, 2009; Harrison & Tong, 2009), or generated through imagery (Albers et al, 2013). These findings are in line with the notion that the primary visual cortex can function as a blackboard that represents both bottom-up stimulus input and top-down mentally generated content. However, it is an open question what the role of early visual cortex is during mental transformations of visual content such as during mental rotation, where an imagined stimulus is rotated from a starting orientation into a final orientation (Shepard & Metzler, 1973). Here we asked whether the early visual cortex is also continuously involved in this dynamic process, or only stores the outcome of the mental transformation. To address this question we measured neural activity in early visual cortex with fMRI at high temporal resolution (TR = 210 ms) using a multiband MRI sequence, while participants (N=7) imagined and mentally rotated an oriented stimulus grating by 60, 120 or 180 degrees. After this mental transformation subjects were required to keep the rotated image in mind for 6 seconds and subsequently compare it to a probe that appeared on the screen. Importantly, the grating to be imagined and rotation angle were both symbolically cued, to ensure that all imagery-related activity was independent from bottom-up stimulation. In a separate localizer session, we measured the brain response to unattended perception of gratings and retinotopically mapped V1, V2 and V3. This approach allowed us to dynamically track stimulus representations in early visual cortex over time. Using Multivariate Pattern Analysis (MVPA) methods we could successfully track which of 6 different orientations was perceived by participants during the localizer. During mental imagery trials, neural activity patterns in early visual cortex changed over time and eventually reflected the orientation of the imagined stimulus after the mental transformation. Furthermore, preliminary results show that neural activity patterns in a temporal window between the start cue and before the end of the transformation period appeared to reflect

intermediate representations of the starting stimulus and subsequent rotation process, suggesting that early visual cortex is involved in the transformation process itself. These findings extend the role of early visual cortex to the dynamical generation of mental content.

Disclosures: **A. Albers:** None. **P. Kok:** None. **I. Toni:** None. **R. Boyacioğlu:** None. **F.P. de Lange:** None.

Poster

550. Visual Perception: Neural Mechanisms

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 550.07/RR33

Topic: F.01. Human Cognition and Behavior

Title: Neural representation for properties determining the navigability of a scene

Authors: J. KANG, H. I. SAIR, *S. PARK
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Abstract: Navigating in a natural environment is fundamental to everyday functions and interactions of an individual. Although the importance of the navigability in a scene has been mentioned in previous studies, exact features of natural scenes that determine the navigability are not well defined. In this study, we investigate which features in a scene drive differences in the navigability judgment of the scene, and how these features are represented neutrally to guide our action in a natural environment. In an fMRI scanner, participants (N=7) were presented with 80 different scene images that depict natural closed environments (e.g., pictures of various views of forests). In a separate behavioral session using Amazon Mechanical Turk, each of these scenes was independently rated on 13 navigationally relevant properties by asking participants to answer questions such as “How clear of a path is in the scene?” While participants were in the scanner, we observed multi-voxel patterns of neural activity in the following regions of interests: the parahippocampal place area (PPA), the restroplenial cortex (RSC), the transverse occipital sulcus (TOS) and V1. Using an iterative variant (Chan *et al*., 2010) of split-half correlation analysis (Haxby *et al*., 2001), we computed similarity matrices of multi-voxel pattern activity for all possible binary pairs of 6 functional runs for each ROI. From the similarity matrices, we produced multi-dimensional scale (MDS) plots and investigated grouping of natural scenes based on the multi-voxel pattern activity. The similarity matrices and MDS plots were re-sorted based on behavioral ratings of each scene properties asked in the 13 questions, as well as groups of principal scene properties that were determined by principal component analysis of the 13

questions. Correlation values between behavioral similarity matrices and neural similarity matrices are computed. We hypothesized that different regions of interests will show different patterns of correlation depending on the nature of navigational features that the matrix is sorted with: similarity patterns of multi-voxel data in LOC will highly correlate with behavioral ratings about object-based property, such as the size, moveability and permanence of objects in a scene. Similarity patterns of multi-voxel data in the PPA will correlate highly with behavioral ratings about the spatial layout in a scene, such as the clearness and straightness of a path. RSC and TOS patterns of multi-voxel data will highly correlate with both the questions about the structure and functional affordance of a scene, such as the clearness of a path, enjoyability or easiness to continue forward or back.

Disclosures: J. Kang: None. **H.I. Sair:** None. **S. Park:** None.

Poster

550. Visual Perception: Neural Mechanisms

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Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 550.08/RR34

Topic: F.01. Human Cognition and Behavior

Title: Temporospatial entropy- and phase-based connectivity in the tilt illusion: The effects of context in perception

Authors: *F. J. PARADA[1,2], L. MORAVEC[1], B. EMERICK[1], O. SPORNS[1], T. BUSEY[1]
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Abstract: A tilt illusion perception task was used in order to measure the effect of context on neural oscillations elicited by visual perception. In the tilt illusion, perception of line orientation is influenced by the orientation of surrounding lines. If the angle difference between stimulus and surround is <50º, the center lines are repelled and appear turn or tilt further away from the surround (Wenderoth & Johnstone, 1988). In our experiment, half of the trials presented the illusion by having the center stimulus flush with the background while the other half had a 1.0-degree gap between the stimuli and background, not presenting the illusion. We collected 32-channel electroencephalographic (EEG) signals from 6 participants (3 males). Data were sampled at 500Hz/channel and filtered from 0.1 to 100 Hz. Each participant ran 2 sessions in different days, later merged into a single data matrix per subject. Independent components analysis was used to remove artifacts (Delorme & Makeig, 2004). Data were Morlet-transformed

into analytic signal (34 frequencies, 4-70 Hz). Cross-spectral density was used to compute debiased weighted phase-lag index connectivity (dwPLI, Vinck et al. 2011). The amount of information measured by entropy of dwPLI for illusion-present and illusion-absent trials was used in order to obtain dwPLI-based mutual information (MI) connectivity in the time-frequency domain. dwPLI-based MI connectivity was then converted into a standard Z-score (MIz) using non-parametric permutation testing. MIz and dwPLI connectivity were analyzed to determine oscillatory connectivity patterns elicited by the tilt illusion. Subject- and group-level robust statistics were performed on both connectivity measures using non-parametric permutation testing framework. The success of our manipulation was reflected by behavioral responses showing an inverse relationship between annulus presence and perception of tilt illusion. MIz connectivity results suggest that information carried by the phase of beta oscillations significantly differs between illusion-present and illusion-absent trials (~170 to ~280 ms). dwPLI connectivity results show that the phase of gamma (~350 ms) and alpha oscillations (~170 ms) is more consistent across illusion-present trials when compared to illusion-absent trials in frontal and occipital scalp. In contrast, phase consistency in the beta range (~200 to ~250 ms) is greater for illusion-absent trials in parietal scalp when compared to illusion-present trials. Our data suggests that phase scattering in the beta range between 200 and 300 ms is crucial for large-scale perceptual information integration in the tilt illusion.

Disclosures: **F.J. Parada:** None. **L. Moravec:** None. **B. Emerick:** None. **O. Sporns:** None. **T. Busey:** None.

Poster

550. Visual Perception: Neural Mechanisms

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Topic: F.01. Human Cognition and Behavior

Support: NS060887

Title: Cortical neuronal oscillations modulated by respiration

Authors: *D. H. HECK[1], A. BABAJANI-FEREMI[2,6], R. REZAIE[3], Y. LIU[4], A. PAPANICOLAOU[5,6,2]
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MEMPHIS, TN; [5]Anat. & Neurobio., Univ. of Tennessee Hlth. Sci. Ctr., Memphis, TN; [6]Neurosci. Institute–, Le Bonheur Children's Hosp., Memphis, TN

Abstract: Oscillations of neocortical neuronal activity in the theta (4-8Hz), beta (12-30 Hz) and gamma (30 - 80 Hz) frequency ranges have been implicated in sensory perception, attention, decision-making, problem solving and memory formation. Patterns of cortical oscillations change during early brain development and with normal aging and are abnormal in patients afflicted with mild cognitive impairment, age related dementia, Alzheimer's disease and cognitive disorders such as autism or schizophrenia. However, our understanding of the origins and neuronal mechanisms controlling cortical oscillatory activity is still fragmented. Here we report new findings showing that respiration serves as a rhythm generator for the modulation of neuronal oscillatory activity in sensory, motor and association cortical areas in humans and mice. Specifically, magnetoencephalographic (MEG) recordings of cortical activity in human subjects and extracellular recordings of cortical spike and local field potential activity in awake mice showed respiration-locked power-modulations of neuronal oscillations in the theta, alpha and beta frequency bands in both species and respiration-locked modulations of single unit spike firing in mice. In mice oscillations in the gamma frequency band also showed modulation of power phase locked to respiration. The average respiratory rates of mice and humans at rest are around 3 and 0.3 Hz, respectively. Mouse resting respiration is thus within the delta (1-4 Hz) frequency band, within which we found strong phase coupling of spike and LFP activity to breathing. Time-frequency analyses of visually evoked responses showed that visual-cortex response amplitudes were modulated as a function of respiratory phase, suggesting a role of respiration in modulating sensory processing. In imaging studies cycle-by-cycle respiration-related signal fluctuations are typically considered to be of non-neuronal origin, and are disregarded and eliminated from the signal. However, our new findings suggest that respiration does modify spontaneous and sensory evoked cortical neuronal activity at the single unit and population level and must be taken into account when analyzing neuronal processes in the neocortex. In mice we have identified olfactory bulb activity as the main driving force behind respiration-locked cortical delta-band oscillations. Sine the olfactory system is relatively less prominent in humans. We suggest that the sensory drive behind respiration-locked cortical activity in humans is more likely the result of summed contributions from mechanical and chemical sensors in the airways, lungs, chest and abdomen.

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Poster

550. Visual Perception: Neural Mechanisms

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Program#/Poster#: 550.10/RR36

Topic: F.01. Human Cognition and Behavior

Title: Fronto-occipital beta band phase synchronization underlying perceptual closure process in two-tone visual image presentation

Authors: *Y. KAKIMOTO, A. OKI, H. SAGAWA, H. TAGAWA, O. ARAKI
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Abstract: “Perceptual closure”, which is the recognition of fragmentary visual stimuli as a complete one, is an important ability to identify visual objects from insufficient inputs. To induce the perceptual closure, two-tone (TT) images, which are made from the monotone images by binarizing with appropriate threshold values, were generally used. Although the TT images appear to be meaningless at first glance, the subjects can gradually recognize the contents with appropriate interpretation. Most of the previous studies compared the neural activities before and after viewing gray scale version of the TT images and revealed that widespread cortical regions (the occipital and the temporal visual cortex, parietal cortex and the prefrontal cortex) correlated with visual disambiguation process. In addition, recent researches reported the enhancement of functional interaction between these brain regions in the perceptual closure. However, it remains unclear about the spatiotemporal brain dynamics in the spontaneous perceptual changes from the state to perceive incoherent visual image to the state to achieve the perceptual closure. To address this issue, we recorded electroencephalography in the 10 subjects during a TT image task. The EEG data was performed time-frequency analyses by using wavelet transformation and Phase locking value (PLV) was used for the index of the functional interaction. In this study, the TT images were presented intermittently. The degraded level of the presented images were gradually changes from hard to easy. Subjects were instructed to respond if they recognized the contents of the presented images by pressing the button as quickly as possible. The subjects responded at 50.0 % and mean response times were 623 ms. EEG results showed that high PLV of the beta frequency was observed in 50 ms after the onset of the TT image in the presentation block in which the subject pressed the button. However, this beta band PLV enhancement was not seen in the previous presentation block. These results suggest that the beta phase synchronizations between the frontal and the occipital area contributes to the perceptual closure process and functional coupling between the frontal and the occipital areas reflects completion of conscious object recognition.

Disclosures: Y. Kakimoto: None. **A. Oki:** None. **H. Sagawa:** None. **H. Tagawa:** None. **O. Araki:** None.

Poster

550. Visual Perception: Neural Mechanisms

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 550.11/RR37

Topic: F.01. Human Cognition and Behavior

Title: Prefrontal-parietal EEG correlation during motor imagery in expert video game players

Authors: *M. L. ALMANZA, J. LLAMAS, M. A. GUEVARA, M. HERNÁNDEZ-GONZÁLEZ
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Abstract: The aim of this study was to characterize the prefrontal-parietal EEG correlation in experienced video game players (VGPs) in relationship to individuals with little to no videogame experience (NVGPs) during a motor imagery condition for an action video game. The participants from both groups viewed a first person shooter (FPS) gameplay of Halo Reach, during five minutes. None of the participants was notified about the content of the video before watching it. Only the VGPs showed an increased right intrahemispheric prefrontal-parietal correlation (F4-P4) in the gamma band (31-50 Hz) during the observation of the gameplay. This data provide novel information about the participation of the gamma band during motor imagery for an action video game. It is probable that this major degree of coupling between prefrontal and parietal cortices could represent a characteristic pattern of the brain functionality in VGPs when they make a motor representation.

Disclosures: M.L. Almanza: None. **J. Llamas:** None. **M.A. Guevara:** None. **M. Hernández-González:** None.

Poster

550. Visual Perception: Neural Mechanisms

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 550.12/RR38

Topic: F.01. Human Cognition and Behavior

Support: The intramural Research Program of the NIH/NIDDK

Title: Variations on the quartet illusion

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Abstract: Motion perception is not simply encoded by single neurons but by networks of neurons that transform spatiotemporal data to sense motion events. A consequence of this is that our brains can be fooled into perceiving motion in the absence of actual movement. This is called apparent motion. The quartet illusion produces this phenomenon by alternating dots set at opposing corners of a square. It is also observed that the perceived trajectories alternate between horizontal and vertical motion despite a constant stimulus; a form of multistable perception. We present new manipulations to the quartet illusion to gain insight into motion detection and perceptual rivalry. For example we show how changing the interval between dot transpositions affects the dominant percept duration.

Disclosures: S. Vattikuti: None. **C.C. Chow:** None.

Poster

550. Visual Perception: Neural Mechanisms

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Topic: F.01. Human Cognition and Behavior

Support: Fund for Scientific Research - Flanders (to J.B.)

IDO Project of KU Leuven (IDO/10/003)

Title: Visual number beats abstract numerosity: Format-dependent representations of Arabic digits and dot patterns in the human parietal cortex

Authors: *J. BULTHE, B. DE SMEDT, H. OP DE BEECK
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Abstract: In numerical cognition there is a well-known but contested hypothesis that proposes an abstract representation of numerosity in the human intraparietal sulcus (IPS) as the explanation for the common activation in this brain region by different numerical formats. On

the other hand, researchers of object cognition have suggested a very different type of representation in IPS, namely that this activity simply correlates with the number of visual objects or units that are perceived. We contrasted these two hypotheses by analyzing multivoxel activity patterns elicited by dot patterns and Arabic digits of different magnitudes while 12 participants were explicitly processing the represented numerosity in a fMRI experiment. Correlational multivoxel activity patterns analysis showed that the activity pattern elicited by the digit '8' was more similar to the activity pattern elicited by one dot, with which the digit shares the number of visual units, than to the activity pattern elicited by eight dots, with which the digit shares the represented abstract numerosity. Also, a multivoxel pattern classifier trained on one format (e.g. dots) and tested on the other format (e.g. Arabic digits) sharing the same numerosities (e.g. 2 dots versus 4 dots to digit 2 versus digit 4) showed no overlapping activity patterns between the two formats in the IPS or any other brain regions. Even more, that same multivoxel pattern classifier trained to differentiate one dot from eight dots, classified all Arabic digits in the one dot pattern category, irrespective of the numerosity symbolized by the digit. These results were consistently obtained in the IPS and its sub-regions, and in many other brain regions. As predicted from object cognition theories, the number of presented visual units forms the link between the parietal activation elicited by symbolic and non-symbolic numbers, meaning that activity in the IPS during processing numbers reflects object individuation and identification. The current study is difficult to reconcile with the hypothesis that parietal or IPS activation elicited by numbers would reflect a format-independent representation of number, what was previously thought in numerical cognition.

Disclosures: J. Bulthe: None. **B. De Smedt:** None. **H. Op de Beeck:** None.

Poster

550. Visual Perception: Neural Mechanisms

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Topic: F.01. Human Cognition and Behavior

Title: What is likely to happen next? Neural correlates of probabilities of future events

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Abstract: For adaptive behavior and decision-making, it is necessary to take into account the relative likelihood of future input and scenarios. In the context of reinforcement learning, a number of studies have shown that the BOLD response is sensitive to the probability that a reward is given. However, the regions identified with those studies (e.g., striatum, mesial prefrontal cortex) are considered to execute more generic evaluation processes and are not directly related to the representation of perceptual information. As of yet, the question of whether and how the brain codes for the occurrence probability of perceptual information in stimulus-specific areas remains to be addressed. Here, we used event-related functional magnetic resonance imaging to address this issue. Eighteen subjects (mean age: 26.3, 10 females) were trained in two behavioral sessions before scanning to learn the correct order of pictures within a sequence. The task was the detection of a rare deviant of this learned order. The sequences consisted of three consecutive pictures; the type of one of these pictures was either a face or a house, with either a high or a low probability of occurrence. Each sequence was preceded by a cue that gave participants probabilistic information about which picture type to expect next. After training, subjects repeated the task in the scanner. This allowed us to examine probability-related, anticipatory modulation of activity in stimulus-specific areas (fusiform face area; FFA, and parahippocampal place area; PPA). A region-of-interest analysis showed that activity in FFA was higher for high expectation as compared to low expectation of faces. The data demonstrate that the brain flexibly distributes vascular resources according to the relative likelihood of future perceptual input. These data are in line with the hypothesis of a “Bayesian brain”, which states that the brain codes information probabilistically.

Disclosures: **S. Trapp:** None. **J. Lepsien:** None. **S. Kotz:** None. **M. Bar:** None.

Poster

550. Visual Perception: Neural Mechanisms

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Topic: F.01. Human Cognition and Behavior

Support: NSF Graduate Research Fellowship

Title: Neural representation of visual fractals

Authors: *T. P. O'CONNELL[1], M. M. CHUN[1,2]
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Abstract: Visual fractals are complex, self-repeating visual patterns generated through recursive application of simple mathematical rules. The hallmark feature of visual fractals is the presence of identical or nearly identical visual structure present at multiple scales. Visual fractal structures are present throughout the natural world, including river networks, tree branches, and blood vessels. The ubiquitous presence of fractals throughout the natural world suggests that abstract, artificially generated fractals may preserve visual features necessary for representing natural visual stimuli (e.g., scenes, objects, faces). While fractals have previously been used as visual stimuli in cognitive neuroscience research, the exact nature of their neural representation in humans is not known. Here, we investigated the sensitivity of different category-selective visual brain regions to the visual features preserved in artificial fractals. We addressed this question using functional Magnetic Resonance Imaging (fMRI) and multi-voxel pattern analysis of blood-oxygen-level-dependent signal elicited during viewing of visual fractals. A factorial set of visual fractals was generated using three structures and three color-schemes. Neural activity was assessed in eight subjects during a 3 alternative-forced-choice fractal structure discrimination task using fMRI. We restricted our analyses to five regions of interest known to be specifically sensitive to natural visual categories: scene-selective parahippocampal place area (PPA), retrosplenial cortex (RSC), and occipital place area (OPA), object-selective lateral occipital cortex (LOC), and face-selective fusiform face area (FFA). Spatial patterns of BOLD signal extracted from each region of interest were used as features to predict fractal structure and color using support-vector machine classification. We found that fractal structure could be reliably predicted from patterns of BOLD activity in the scene-selective OPA ($p < 0.0008$) and the object-selective LOC ($p < 0.0005$). This suggests that artificial visual fractal structures preserve visual features necessary for the neural representation of scenes and objects. We will discuss the potential application of visual fractals as substitutes for natural scenes and objects in vision research.

Disclosures: T.P. O'Connell: None. **M.M. Chun:** None.

Poster

550. Visual Perception: Neural Mechanisms

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Methusalem program (METH/08/02)

ERC (ERC-2011-Stg-284101)

IUAP (IUAP-P7/11)

Intramural Research Program of the NIMH

Title: Influence of lexical status, orthographic similarity and semantics on the multi-voxel response of the visual word form area

Authors: *A. BAECK[1], D. KRAVITZ[2], C. BAKER[3], H. OP DE BEECK[1]
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Abstract: Previous studies demonstrated that a region in the left fusiform gyrus, often referred to as the "visual word form area" (VWFA), is responsive to written words, but the precise functional role of VWFA remains unclear. In the present study, we investigated the influence of orthographic similarity, lexical factors and semantics on the multivoxel response patterns to written stimuli. Using high-resolution fMRI at 7 Tesla, we scanned 16 participants to compare the organization of visual word representations in VWFA to the organization in early visual cortex and a language region in the superior temporal gyrus. Sets of four letter words and pseudowords were presented, in which orthographic similarity was parametrically manipulated. Further, real word stimuli with no orthographic overlap were semantically related (example real word stimulus set: beat-feat-flat-flag-flog). We found that VWFA is responsive to the lexical status of a stimulus, but both real words and pseudowords were further processed in terms of orthographic similarity. No evidence was found for a role of semantics in VWFA. In contrast, the early visual cortex was only responsive to the visual aspects of the stimuli. In the left superior temporal gyrus an interaction was found between lexical status and orthography: real words, when not semantically related, are organized according to orthographic similarity, while no functional organization was found for pseudowords. These findings indicate that VWFA represents the word/non-word status of letter strings as well as their orthographic similarity.

Disclosures: A. Baeck: None. **D. Kravitz:** None. **C. Baker:** None. **H. Op de Beeck:** None.

Poster

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Support: NIH Grant R01 EY018923

Title: Perception of sound-encoded faces selectively activates the left fusiform face area in congenitally blind humans

Authors: P. L. PLAZA[1], L. A. RENIER[2], A. G. DE VOLDER[2], *J. P. RAUSCHECKER[1]
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Abstract: Currently, little is known about the role of sensory experience in the development of functionally specialized cortical modules. Sensory substitution devices (SSD) offer a unique opportunity to investigate the functional specialization of "visual" cortex in congenitally blind (CB) subjects by conveying "visual" information via a preserved (substituting) sensory modality (e.g. hearing). The purpose of the present study was to test to what extent face- and house-sensitive visual brain areas develop and maintain their functional role in CB subjects and can be recruited using an SSD. We used functional magnetic resonance imaging and an SSD that converts visual images into sounds to identify the brain areas recruited during the perception of faces and houses in CB and sighted control (SC) subjects. Brain activity was monitored in these subjects while they were discriminating faces and houses encoded into sounds or perceived visually (in SC subjects only). The fusiform face area (FFA), the parahippocampal place area (PPA) and the lateral occipital complex (LOC) were identified in sighted subjects under visual conditions using a functional localizer consisting of pictures of famous persons and real houses. Then, region-of-interest analyses were performed on the data acquired in both CB and SC subjects when the SSD was used to discriminate schematic drawings of faces and houses. Results indicate that the left LOC was activated under both of these conditions in both groups, while the left FFA was activated during the auditory face discrimination condition in CB subjects only. No significant brain activity was found in the PPA in CB or SC subjects at the group level. The specific recruitment of the FFA during the perception of sound-encoded faces in CB subjects represents new evidence about the development of functional specialization of this region in the absence of visual inputs.

Disclosures: P.L. Plaza: None. **L.A. Renier:** None. **A.G. De Volder:** None. **J.P. Rauschecker:** None.

Poster

550. Visual Perception: Neural Mechanisms

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Program#/Poster#: 550.18/RR44

Topic: F.01. Human Cognition and Behavior

Title: Human brain mapping metamodel

Authors: *C. C. LEITH[1], J. ROSENGARTEN[2], P. EPSTEIN[3], L. ROBBINS[4], L. SIEGEL[5], H. SUN[1]
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Abstract: **Introduction:** In all human brain mapping work the fundamental assumption is that perception, awareness and preparation for response of a subject can be inferred from the brain activation map. It relies on a convoluted and obscure map from the understanding, cognition and freewill of the mind to neural impulses in the brain. A different theoretic frame is needed to clarify what to expect from such a map. **Method:** We examined our neuroimaging data acquired from a few groups of patients (38 brain lesion preoperative, 90 chronic migraine, 56 fibromyalgia, 4 Asperger and 150 healthy controls). All subjects were scanned with our Adapted Diffusion Tensor Imaging method (aDTI) (the preoperative 38 scanned with BOLD fMRI). We checked the protocols for image acquisition and analysis, focusing on the typical work flow as followed by most labs. The metamodel was developed under this situation. **Result:** We found that the subjectivity of the patient is not avoidable in any experiment. It simply arose from the subject's understanding, memorizing and executing of the experimental task. Choices or freewill were always present. In attempting to capture brain activity the BOLD fMRI took the local blood flow as the surrogate of it. The aDTI imaged water diffusion anisotropy change as modulated by neural impulses in the axonal fiber tracts. From the brain activation map we inferred the probable firing of neurons under a few marked areas, but we were never sure about the unmarked areas as inactive. The metamodel was formulated as shown in the Figure. **Conclusion:** The metamodel indicates that oI, the true mind is the prime mover and that sI, the apparent mind is only endowed with the feelings of awareness and freewill. It implies an inconvenient truth that sI is an illusory scratch pad or simulator for oI to interact with others. The one self "I", the owner, cannot escape from responsibilities. Clearly, oI has always evolved regardless of sI's knowledge. The inverse transform forewarns us taking precautions in conceptualizing findings in oI with our sI habit, and in claiming centers, streams or netwoks in the highly distributed CNS.

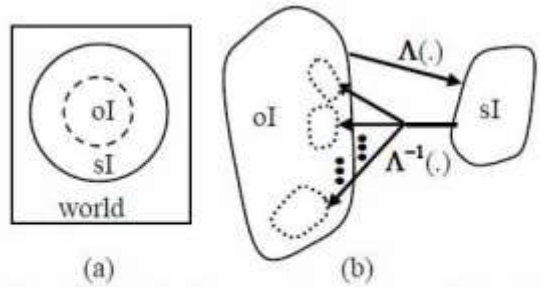

Fig. Human brain mapping metamodel. (a) To probe the mind of a self identity "I", we must first realize its objective representation "oI", the ever-evolving ensemble of neural impulse trains. The interaction with the world was left to the subjective representation "sI", the subject matter of psychological inquires, or, the apparent mind as we all are familiar with. In contrast oI was the rather unfamiliar true mind and the subject matter of neuroscience. (b) In essence sI served as the "broker" for oI in dealing with the world, and the brokerage called for a conversion or transform Λ(oI)→sI in mathematical terms. Knowing oI fully implied knowing sI completely. However, the inverse transform Λ^{-1}(sI) was different, discrete and patchy, hinting on oI not completely knowable through sI.

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Poster

550. Visual Perception: Neural Mechanisms

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Support: KAKENHI Grant Numbers 22830039, 23135517, 24240041, 25135720

Title: Multiple neural processes underlying binocular rivalry in retinotopic visual areas

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[1]Grad. Sch. of Human and Envrn. Studies, Kyoto Univ., Kyoto, Japan; [2]Dept. of Med. Engin., Aino Univ., Ibaraki, Osaka, Japan; [3]Ctr. for Information and Neural Networks, Osaka Univ., Suita, Osaka, Japan; [4]Dept. of Med. Informatics, [5]Dept. of Neurosurg., Meiji Univ. of Integrative Med., Nantan, Kyoto, Japan

Abstract: Binocular rivalry has been extensively investigated by neuroscientists exploring the neural substrates of visual awareness. Many fMRI and electrophysiological studies have revealed that multiple stages of visual processing, from the thalamus to the visual cortex and fronto-parietal regions, are involved in subjective visual awareness. However, these studies have yielded inconsistent results regarding the relationship between visual awareness and visual processing hierarchy. Human and monkey fMRI studies have repeatedly shown that activity in V1 as well as extrastriate visual areas correlates with modulations of visual awareness during binocular rivalry. In contrast, several monkey electrophysiological studies have reported that only a small fraction of neurons in early visual areas exhibit spiking activity correlated with visual awareness, whereas a large proportion of neurons in high-level visual areas showed correlated activity. In the present human fMRI study, we found two distinct patterns of activity in retinotopic visual areas during binocular rivalry, each of which were consistent with previous fMRI or electrophysiology. We measured brain activity while subjects viewed a novel binocular rivalry stimulus, which consisted of a flashing mask presented to subjects' dominant eye and a rotating checkerboard probe presented to the other eye. Subjects' visual awareness of the probe fluctuated due to strong interocular suppression, called continuous flash suppression. The rotating probe allowed us to measure not only the response modulations around the time of perceptual transitions but also the responses evoked by the physical onset of the probe whether it was suppressed or dominant during binocular rivalry. The onset responses were larger when it was visible than invisible in all of the retinotopic visual areas we measured (V1, V2, V3 and V4v). This elevation of the responses increased along the visual processing hierarchy, consistent with previous electrophysiology. On the other hand, the response modulations around the time of perceptual transitions were equally large across in all of the areas measured, which is consistent with previous fMRI studies. Our results suggest that the discrepancy between fMRI and electrophysiology may not be an artifact due to the intrinsic signal difference, but may arise from two distinct processes in retinotopic visual areas underlying binocular rivalry.

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Poster

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Support: Fund of the Ministry of Education through the National Research Foundation of Korea (NRF-2013R1A1A2007247)

Title: Effects of temporoparietal tDCS on EEG relative powers and cybersickness in virtual navigation

Authors: *H. JEON, Y. CHUN, C. PARK, T. W. WENDIMAGEGN, J. JEONG, H. KIM
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Abstract: Cybersickness refers an uncomfortable state in virtual navigation. Although a number of studies have tried to find ways to eliminate cybersickness, an optimal method of avoiding cybersickness has not been established. Recently, studies using transcranial direct current stimulation (tDCS) demonstrated modulations of sensation and perception. Kyriakareli et al. (2013) reported that temporoparietal tDCS could manipulate the thresholds of the vestibulo-ocular reflex (VOR) and motion perception, which might be important factors that modulate cybersickness. Thus, we investigated whether temporoparietal tDCS can mitigate cybersickness in virtual navigation. Thirteen participants were randomly assigned into a tDCS (CP6-anodal/CP5-cathodal stimulation, 1.0 mA, 15 min) group or a sham-control group (same site as tDCS group, 1.0 mA, 30 s). A static image from a movie clip for virtual navigation was presented to participants for five minutes before tDCS as a baseline, and the movie clip for virtual navigation was presented to participants for ten minutes after tDCS to induce cybersickness. Electroencephalograms (EEGs) were recorded on twelve electrode sites (F3, Fz, F4, C3, Cz, C4, P3, Pz, P4, O1, Oz, and O2) on the scalp corresponding to the international 10/20 system before and after tDCS, and relative power analysis was examined. Our results indicated that the relative beta power in the tDCS group was significantly higher than that in the sham-control group on the Oz site. Additionally, the relative theta power in the tDCS group was marginally lower than that in the sham control group on the Cz site. Based on previous observations (Chen et al., 2010; Kim et al., 2005) demonstrating a decrease in beta power and an increase in theta power in cybersickness, the results of the present study suggest that the application temporoparietal tDCS could be an effective method to reduce cybersickness.

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Poster

550. Visual Perception: Neural Mechanisms

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Topic: F.01. Human Cognition and Behavior

Support: EpilepsyAction

Title: The dissociation of different measures of cortical inhibition in the visual system, and their use for non-invasive monitoring of epilepsy susceptibility

Authors: *P. YAZDANI[1], J. READ[1], R. WHITTAKER[3], R. GEORGIOU[2], A. TREVELYAN[2]
[1]Newcastle Univ., Newcastle Upon Tyne, United Kingdom; [2]Newcastle Univ., Newcastle upon Tyne, United Kingdom; [3]Royal Victoria Infirmary, Newcastle upon Tyne, United Kingdom

Abstract: Accurate seizure prediction algorithms would transform the lives of people living with epilepsy. They would also provide information about disease progression. One theory suggests that the timing of seizures reflects fluctuations in the quality of cortical inhibition. Non-invasive assays of cortical inhibition may, therefore, provide clinically useful information. Visual psychophysics assays have been used in schizophrenia (Tadin et al. J.Neurosci, 2006), autism (Koldewyn et al. Brain, 2010) and major depressive disorders (Golomb et al., J.Neurosci., 2009). To investigate whether such tests may also be useful in assessing seizure risk, we investigated the performance in different visual cortical inhibition assays and whether this co-varied between tasks. We have recruited 153 volunteer control subjects with no history of neurological disorder and 39 patients with clinically confirmed diagnosis of epilepsy. The control subjects ranged in age from 17.3 to 69.0 (mean 36.6 +/- 17.3years); patients range, 17.0 to 82.3, (mean 42.7 +/- 18.6 years). Some patients were tested soon after diagnosis before anti-epileptic medication was started. Others had long standing epilepsy, and medication was maintained. The duration of epilepsy ranged from a few months to 34 years. Two different stimulus paradigms were used: (1) a two-choice motion discrimination paradigm (Tadin et al, 2003; left-right choice of movement of sinusoidal gratings“(Gabor patches”); small versus large stimuli, at either high / low contrast) and (2) a four-choice, stationary annulus paradigm (Serrano-Pedraza et al, 2012; identification of location of an oriented sinusoidal, orthogonal or parallel to a background sinusoidal display; high and low contrast). Duration thresholds were derived from fits of psychometric functions, and used to derive surround suppression indices (SSIs) for the two tests. There was a large variance in these measures, but further analysis suggested a clear difference between the two different test paradigms. For instance, age was an important confounding variable in the Tadin SSI, showing a highly significant reduction with increasing age in all groups, but not for the annulus SSI. Also,

the Tadin and annulus SSIs were not correlated across populations, or over repeated measures within single individuals. We will also present analyses examining the longitudinal variance in individual patients and control subjects.

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Poster

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Topic: F.01. Human Cognition and Behavior

Title: Effect of anticipation triggered by prior dyspnea experience on brain activity

Authors: *H. NAKAI[1], K. TSUJIMOTO[2], T. FUCHIGAMI[3], S. OMATSU[4,5], H. NAKANO[6,5], S. MORIOKA[5]
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Abstract: Objective: Prefrontal cortex oxygenated hemoglobin (oxy-Hb) concentration is closely associated with dyspnea. Dyspnea is influenced not only by physical activity, but also by visual stimuli, and several studies suggest that the oxy-Hb concentration changes in response to certain external stimuli. However, the relationship between brain activity, oxy-Hb trends, and the discrepancy between anticipated and actual dyspnea remains unknown. This study explores the influence of anticipation triggered by previous episodes of dyspnea on brain activity. Methods: Fifteen healthy volunteers with a mean age of 25.0 ± 3.0 years and a mean maximal peak inspiratory pressure of 78.0 ± 26.4 cmH2O (Micro-Spirometry) participated in the study. Each experimental trial were viewing representations of 20 s, breathing resistance of 30 s, and 20 s selective Borg scale. Volunteers viewed a random combination of photographs showing pleasant, neutral, and unpleasant representations. The subjects were instructed to expect a breathing resistance identical to the photograph. After viewing the image, breathing resistance was randomly administered to the subjects as identical to, easier, or harder than that shown in the image; the image and resistance were identical 33% of the time and discordant 66% of the time. The breathing resistances (7 mmHg, PI max 15%, PI max 30%) were induced using the Threshold IMT. The subsequent brain and sympathetic nervous system activities were assessed

using the Borg scale for psychophysical, tracers of sympathetic nervous system activity, and a functional near-infrared spectroscopy for brain activity. Results: The dorsolateral prefrontal cortex oxy-Hb concentration increased when unpleasant images were shown significantly ($p < 0.05$). The right medial prefrontal cortex (rMPFC) oxy-Hb concentration increased at PI 30% max intensity in subjects shown a pleasant image significantly compared with subjects shown an unpleasant ($p < 0.01$). Moreover, the activated rMPFC area was significantly correlated with the magnitude of perceived dyspnea ($R = 0.6$; $p < 0.01$). The sympathetic nervous system activity did not significantly change. Conclusion: These results suggest that rMPFC activity is correlated to the magnitude of dyspnea in subjects perceiving an unpleasant intensity regardless of any previous positive expectations.

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Poster

551. Human Long-Term Memory: Medial Temporal Lobe III

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Topic: F.01. Human Cognition and Behavior

Support: NIH Grant R01 MH 100121

NIH Grant R01 MH 60941

RDF Grant RDF-UOA1001

Title: Dissociable roles of hippocampal subfields in episodic simulation

Authors: *E. M. STEIN[1], V. C. MCLELLAND[2], A. DEVITT[3], D. L. SCHACTER[4], A. R. PRESTON[1], D. ADDIS[3]
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Abstract: Controversy has emerged regarding the role of the hippocampus in episodic simulation tasks that require individuals to imagine future events. While some studies have shown hippocampal lesions impair episodic simulation, others have shown spared performance.

One possibility is that episodic simulation engages multiple processes supported by different hippocampal regions. To resolve conflicting findings from patient research, it is therefore important to determine how individual hippocampal subfields contribute to episodic simulation. In the present study, we used high-resolution fMRI combined with an episodic simulation task that required participants to generate future scenarios using elements from their existing memories. During scanning, participants were presented with recombined sets of person, place, and object details selected from their own memories and were asked to imagine a future event that comprised those details. Participants then rated how much detail was generated during each simulation. After scanning, participants were given a surprise memory test for the imagined events that required recall of the missing person, place or object when cued with two other event details. Our goal was to distinguish hippocampal responses that relate to the successful encoding of imagined events from those that track the amount of detail generated during episodic simulation. We found that left anterior CA1 engagement during episodic simulation was related to later memory for the imagined events, with greater activation for remembered relative to forgotten events. Importantly, the response in this region was not related to the amount of detail generated during simulation. In contrast, activation in left posterior dentate gyrus/CA2,3 increased with the level of detail generated during simulation but was not related to subsequent memory for imagined events. These findings thus suggest dissociable roles for the dentate gyrus/CA2,3 and CA1 in episodic simulation, with dentate gyrus/CA2,3 supporting retrieval of detailed information from existing memories and CA1 supporting encoding of imagined scenarios into memory. Moreover, the localization of successful encoding responses and detail effects to anterior and posterior hippocampus respectively is consistent with theoretical frameworks suggesting that these regions serve distinct memory functions that may extend to episodic simulation. Collectively, our findings implicate multiple hippocampal processes in episodic simulation and thus suggest that the precise nature of hippocampal lesions may determine whether an individual patient is able to imagine the future.

Disclosures: **E.M. Stein:** None. **V.C. McLelland:** None. **A. Devitt:** None. **D.L. Schacter:** None. **A.R. Preston:** None. **D. Addis:** None.

Poster

551. Human Long-Term Memory: Medial Temporal Lobe III

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 551.02/RR50

Topic: F.01. Human Cognition and Behavior

Title: Memory reconsolidation and updating: The role of memory strength and context

Authors: *K. C. NEWMAN-SMITH, R. L. GOMEZ, L. NADEL
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Abstract: Reactivated memories become labile and can be updated with new information in an adaptive process called memory reconsolidation. Previously we showed that spatial context helps determine when memory updating occurs (Hupbach, Gomez, Hardt, & Nadel, 2007). It remains unclear how the nature and strength of new experiences influence the likelihood they will participate in this memory updating process. We used a modified directed-forgetting paradigm to understand the extent to which the strength of novel information interacts with two aspects of context (spatial or item-based) to influence memory reconsolidation. Participants were randomly assigned to two groups, Same-Spatial context (SSC) or Different-Spatial context (DSC). At Session 1, participants learned 20 objects (Set 1) paired with associated sounds (e.g. an image of a train with the sound of a moving train). At Session 2, 48-hours later, participants returned to the same room as Session 1 (SSC) or a different room (DSC). In both conditions half of the Set 1 sounds were played, reactivating Set 1 memory via this item-based context (Newman-Smith, Nadel, Gomez, & Scalf, in prep). Thus participants in SSC experienced two types of context reactivation, spatial and item-based, while those in DSC experienced item-based reactivation alone. Participants then learned Set 2 comprised of 28 new objects. Half were surrounded by a blue border (Remember objects), half were not (Forget objects). Participants were instructed to remember only the bordered objects. At Session 3, 48 hours after Session 2, recognition memory for Set 1 and Set 2 items was tested. Reconsolidation is reflected in intrusions, defined as items from Set 2 recognized as being learnt in Session 1. If the strength of newly presented information influences the likelihood of updating there should be an intrusion difference of Remember items and Forget items between conditions. Remember items intruded into Set 1 memory in the SSC condition only, while Forget items intruded in both SSC and DSC conditions, suggesting an interaction between memory strength and spatial context Strong items (the Remember items) are linked to a specific context, while weak items (the Forget items) are not. The absence of linkage between the Forget items and the Set 2 context in which they were learned allows them to integrate more easily into the Set 1 memory. These results show that the strength of new information interacts with context to determine the likelihood that any particular item will become part of an updated, reconsolidated, memory.

Disclosures: K.C. Newman-Smith: None. **R.L. Gomez:** None. **L. Nadel:** None.

Poster

551. Human Long-Term Memory: Medial Temporal Lobe III

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 551.03/SS1

Topic: F.01. Human Cognition and Behavior

Support: Rutherford Discovery Fellowship RDF-UOA1001

Marsden Fund Grant UOA1210

Title: The Simpson's Paradox and functional connectivity in fMRI

Authors: *R. P. ROBERTS, S. HACH, L. J. TIPPETT, D. ADDIS
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Abstract: **Objective**: Functional connectivity (FC) analyses determine the inter-relationship of brain regions, either during rest or during a cognitive task, by assessing the correlation of activity in one brain region (a seed) with that in other brain regions. Some analyses assess this correlation within subjects while others perform an across-subject correlation. An important phenomenon relevant to the relationship between these two forms of FC analysis is the "Simpson's Paradox". This occurs when an effect present in an across-subject analysis is absent or reversed within the individuals present in the analysis. Using an event-related fMRI dataset (N=16) with two conditions (semantic and episodic memory), we assessed whether regions exhibiting FC as indexed by across-subject correlations (seed partial least squares, PLS) also reliably show FC within the individuals in the dataset. **Methods**: A task-PLS analysis revealed increased activity for episodic memory (relative to sematic memory) in bilateral hippocampal (HC) clusters. The peak voxel of these clusters were chosen as seeds for a seed-PLS analysis that assessed which regions show across-subject correlations with each seed during the semantic and episodic memory tasks. Within-subject FC was assessed by first calculating, for each voxel within each subject, the percent signal change for each trial. These values were then correlated with percent signal change values for the left and right HC seed. From this, an across-trial r-value was calculated for each voxel, within each condition, for each subject. An r-to-z transform was then computed for each r-value. For those regions showing an effect from the seed-PLS analysis, z-scores from the within-subject analysis were analysed to determine if the across-subject effects observed in the seed-PLS analysis was also present within individual subjects. **Results**: The seed-PLS (across-subject FC) analysis revealed a number of regions exhibiting differential FC with the HC seeds during the episodic but not the semantic memory task. While some of these regions showed similar patterns for the within-subject FC analysis, there were also many regions that showed no effect or the opposite trend to the results of the across-subject FC analysis. **Conclusion**: Our results show the Simpson's Paradox is evident in FC research, and researchers should avoid assuming that across-subject correlations between two regions necessarily imply a within-subject correlation between the regions. Further developing our understanding the

functional differences reflected by across- versus within-subject brain correlations will enable more accurate interpretations of FC results.

Disclosures: **R.P. Roberts:** None. **S. Hach:** None. **L.J. Tippett:** None. **D. Addis:** None.

Poster

551. Human Long-Term Memory: Medial Temporal Lobe III

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Topic: F.01. Human Cognition and Behavior

Support: Cedars-Sinai Neurosurgery

Gustavus and Louise Pfeiffer Research Foundation

NIMH

Title: Representation of subjective memory strength and visual category by single neurons in the human medial temporal lobe during memory retrieval

Authors: S. YE[1,4], M. KOROMA-LE BRAS[7,4], O. TUDUSCIUC[2], I. ROSS[8], J. M. CHUNG[5], A. N. MAMELAK[4], *U. RUTISHAUSER[4,3,1,5,6]
[1]Computation and Neural Systems, [2]Humanities and Social Sci., [3]Div. of Biol. and Biol. Engin., Caltech, Pasadena, CA; [4]Neurosurg., [5]Neurol., [6]Biomed. Sci., Cedars-Sinai Med. Ctr., Los Angeles, CA; [7]Dept. de Biologie, École Normale Supérieure, Cachan, France; [8]Neurosurg., Huntington Mem. Hosp., Pasadena, CA

Abstract: Episodic memories allow us to remember not only that we have seen an item before (familiarity) but also provide a subjective impression of how sure we are that we have seen an item before (confidence). The neuronal mechanisms of confidence judgment about one's own memories are poorly understood. The medial temporal lobe (MTL) plays an important role in both the estimation of memory strength as well as visual categorization, but how these processes interact is unclear. We recorded single neurons in the MTL of patients implanted with depth electrodes for the purpose of localizing epileptic seizures. Patients viewed a sequence of 100 images (50 novel, 50 familiar). Images were chosen from five visual categories (people, animals, vehicles, foods, houses). Patients rated each image as new/old on a 1-6 confidence scale. Patients were able to assess the quality of their memories: the average area under the curve (AUC) of the behavioral Receiver Operating Characteristics (ROC) was 0.78±0.06 (all errors are ±s.d.) and

their response was faster when made with high vs. low confidence (1.56±0.80 sec vs. 2.52±1.32 sec, $p < 0.001$). We recorded 1193 neurons from the hippocampus and amygdala from 28 patients in 46 sessions. We quantified the responses using single-neuron ROC analysis. 95 (8%) of units differentiated new from old stimuli (NO neurons) and their AUC was larger for images remembered with high compared to low subjective confidence (0.68±0.05 vs. 0.63±0.05, $p<0.001$). This indicates that the neuronal response contained more information when memories were retrieved with high confidence. A separate group of 134 (11%) neurons differentiated the visual category of the displayed stimuli. There was no significant overlap between the two groups. We performed regression analysis and used the effect size to quantify how much of the neuronal variance could be attributed to visual category and/or familiarity. We found that category neurons only explained variance due to visual category but not familiarity (peak ω^2 was 2.36% vs 0.57%, respectively; $p < 0.001$), and vice versa (peak ω^2 was 0.65% vs. 5.87%, respectively; $p < 0.01$). We analyzed all recorded neurons together to quantify neuronal dynamics. The effect size of visual category peaked ~400ms before that for memory (new/old). Only the familiarity-related effect size was modulated by subjective memory strength. The memory signal was ≈3 times more informative (mutual information) when retrieval occurred with high compared vs. low confidence. We conclude that NO and category neurons are two distinct functional neuronal populations.

Disclosures: S. Ye: None. **M. Koroma-Le Bras:** None. **O. Tudusciuc:** None. **I. Ross:** None. **J.M. Chung:** None. **A.N. Mamelak:** None. **U. Rutishauser:** None.

Poster

551. Human Long-Term Memory: Medial Temporal Lobe III

Location: Halls A-C

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Program#/Poster#: 551.05/SS3

Topic: F.01. Human Cognition and Behavior

Support: NIH Grant R01 MH-061975

Title: Human entorhinal neurons activate at multiple related locations in an environment

Authors: *J. MILLER[1], I. FRIED[2], J. JACOBS[1]
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Abstract: The ability to remember and navigate our environment is critical for everyday life. Our understanding of the neural basis of navigation is based largely on the hippocampal place cell, whose activity represents a specific location in a spatial environment. An open question underlying the nature of place cells is distinguishing which aspects of place cell activity emerge from intrahippocampal computations and which are present outside of the hippocampus. We investigated this topic using human single neuron recordings from neurosurgical patients as they performed a virtual navigation task, traversing similar-looking pathways of a larger virtual environment. We found that many cells in the entorhinal cortex (EC), but not hippocampus, exhibited repetitive patterns of activation at the same relative locations on different paths. This result suggests that the EC codes broad non-specific features of an environment that could be used to represent commonalities between different spatial settings, and that this code may be the building block of more specific neural representations.

Disclosures: J. Miller: None. **I. Fried:** None. **J. Jacobs:** None.

Poster

551. Human Long-Term Memory: Medial Temporal Lobe III

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Topic: F.01. Human Cognition and Behavior

Support: NIH Grant MH-061975

Title: An analysis of human navigation-related theta oscillations using spike triggered field potentials and autocorrelations

Authors: *T. J. COFFEY[1], J. JACOBS[2], N. BURGESS[3]
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Abstract: An issue of theoretical importance is understanding whether hippocampal theta oscillations play a critical role in the neuronal processes underlying mammalian navigation. We analyzed human theta oscillations by examining the oscillatory timing in the spiking of individual neurons with temporal auto-correlation (AC) analyses as well as time-frequency and spike-triggered averaging analyses of oscillations in the local field potential. This two-pronged approach is helpful because previous AC analyses of cross species differences in the theta

rhythmicity of entorhinal grid cells (Yartsev et al., 2012) were hard to interpret because very low firing rates can preclude detection of high frequency modulations (Barry et al., 2012). Our results indicate that humans exhibit oscillations in the hippocampus and entorhinal cortex during navigation at various frequencies, including ~3Hz and ~8Hz. However, AC methods perform relatively poorly above 3 Hz, detecting few oscillatory cells as compared to the LFP method. These findings suggest that methods based on local field potentials can provide a clearer picture of network oscillations, at least in humans, as also seen in studies of spike-field coherence (Rutishauser et al., 2010). We discuss why this may occur, perhaps owing to the intermittent nature of human hippocampal spiking.

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Poster

551. Human Long-Term Memory: Medial Temporal Lobe III

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Topic: F.01. Human Cognition and Behavior

Support: NIH Grant R01 MH-061975

Title: Human hippocampal theta oscillations are traveling waves

Authors: *H. ZHANG[1], J. JACOBS[2]
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Abstract: The hippocampal theta oscillation is widely considered as playing a key role in coordinating neuronal activity to support memory and cognition. Recently it was found in rodents that theta waves travel in a progression along the length of hippocampus (Lubenov & Siapas., 2009). This altered our view of hippocampal computation by suggesting that theta oscillations reveal how different neural assemblies activate sequentially. We know from previous work that hippocampal theta oscillations in humans have distinctive properties compared to rodents, including a slower frequency. Thus here we tested whether human theta oscillations are traveling waves by examined brain recordings from epilepsy patients that had electrodes implanted at multiple locations within the hippocampus. These multisite recordings allowed us to measure how hippocampal theta waves propagate spatially in humans. Our analyses revealed that human theta oscillations move in a posterior-to-anterior direction through the hippocampus, which is directly analogous to the dorsal-ventral traveling waves found in rodents. Traveling

theta waves occurred at a range of frequencies, unlike the ~8-Hz oscillations found in rodents. The temporal frequency of these theta waves correlated with the speed of spatial propagation, such that there is a fixed spatial frequency of ~9 degrees per centimeter. This correlation between theta's frequency and speed of propagation supports models of theta generation based on coupled oscillators.

Disclosures: H. Zhang: None. **J. Jacobs:** None.

Poster

551. Human Long-Term Memory: Medial Temporal Lobe III

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Topic: F.01. Human Cognition and Behavior

Support: ONR MURI N00014-10-1-0936

Title: Structural differences in hippocampal and entorhinal gray matter volume support individual differences in first-person navigational ability

Authors: *K. R. SHERRILL[1,2], E. R. CHRASTIL[1,2], I. ASELCIOGLU[1], M. E. HASSELMO[1], C. E. STERN[1,2]
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Abstract: The ability to successfully navigate in the world varies dramatically across individuals. Recent functional MRI studies from our lab have demonstrated that the hippocampus supports goal-directed navigation (Brown, Hasselmo, and Stern, 2014; Sherrill et al., 2013; Brown and Stern, 2013), and previous studies have linked hippocampal volumetric differences to topographical and spatial abilities (Maguire et al., 2006; Bohbot et al., 2007; Iaria et al., 2008; Schinazi et al., 2013; Brown et al., 2014). The current imaging study related differences in behavioral performance during active first-person perspective and survey-perspective navigation in a landmark-free open field environment to differences in brain morphology using voxel-based morphometry. An fMRI paradigm compared goal-directed navigation in an open field environment from either a first person perspective (FPP) or Survey perspective (Sherrill et al., 2013; Sherrill et al., *in prep*). Participants (N = 57, mean age 21.84 ± 3.56 (SD), 30 males) underwent structural and functional MRI scanning. Behaviorally, participants reached the goal with precision from the FPP in 64.84% of the trials (SEM 2.66) and

from the Survey perspective in 75.89% of the trials (SEM 2.35). We used SPM8 to conduct both functional and voxel-based morphometry (VBM) analyses, analyzing the relationship between brain morphology and navigational accuracy in both the FPP and Survey perspectives. Our analysis included regressors for whole brain volume, age, sex, and FPP and Survey navigational accuracy. Our results demonstrate significant morphometric differences in the right hippocampus (head, body, and tail), left hippocampal tail, right entorhinal cortex, and left thalamus ($p<0.05$, k = 946) related to first-person navigational accuracy. In contrast, successful navigation from the Survey perspective was significantly correlated with morphometric differences in the left supplementary motor region and right angular gyrus but did not demonstrate a correlation with medial temporal lobe regions. Our results support the idea that individual differences in goal-directed navigation relate to underlying structural differences in the hippocampus and entorhinal cortex, regions thought to be critical to human navigation. More specifically, our results suggest this structural-functional relationship depends on the reference frame, with hippocampal and medial temporal lobe regions correlating more with egocentric first-person navigational abilities. These results extend our understanding of the link between brain structure and the diverse cognitive processes involved in spatial navigation.

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Poster

551. Human Long-Term Memory: Medial Temporal Lobe III

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Program#/Poster#: 551.09/SS7

Topic: F.01. Human Cognition and Behavior

Title: Combined working memory exercise and prefrontal transcranial direct current stimulation influences cognitive control

Authors: *B. M. ROBERTS, S.-F. WANG, M. MONTCHAL, B. WANCEWICZ, C. CARTER, C. RANGANATH
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Abstract: Neuroimaging and electrophysiological studies have suggested that activation of the dorsolateral prefrontal cortex (DLPFC) is important for optimal cognitive function, but there is a lack of causal evidence for the role of cortical excitation in human cognitive performance. Studies that manipulate neural activity are necessary in order to provide a better understanding of

the mechanisms involved in cognitive function. In this study, anodal transcranial direct current stimulation (tDCS) was implemented to stimulate the DLPFC during a working memory exercise (visuospatial n-back task), in order to determine the effects of DLPFC activation on cognitive control (Dot Pattern Expectancy (DPX) task) and episodic memory (Relational and Item-Specific Encoding (RiSE) task). 20 healthy young adults received both active anodal and sham stimulation during two separate visits. During each visit, subjects first completed practice blocks of all tasks. After practice, two rubber electrodes encased in saline-soaked sponges were placed on the scalp with the anode over the left DLPFC (electrode site F3) and the cathode over the right supraorbital region. Current was applied at 2mA for 20 minutes for the active condition. For the sham condition, current was applied at 2mA for 30 seconds, and then was turned off for the remainder of the session. Subjects were blind to their condition. During stimulation, subjects completed a working memory exercise, which consisted of blocks of 2-back and 3-back of the visuospatial n-back task. Following stimulation, subjects completed the DPX and RiSE tasks during EEG recording from 32 scalp sites. Behavioral results indicate that performance on the DPX task was dependent upon the stimulation condition, as indicated by a significant interaction between error type and stimulation condition (ANOVA: $F [1, 38] = 4.889$; $p = 0.033$). Specifically, errors on trials in which a non-target cue was followed by a target probe (BX trials) improved for the active stimulation condition relative to sham, while trials in which a target cue was followed by a non-target probe (AY trials) showed increased error rates. Results from the RiSE task also revealed a trend for improved performance on the associative recognition trials, but results were not significant. Further analysis will focus on the EEG correlates of cognitive performance following stimulation. These findings provide causal evidence for DLPFC excitation in cognitive control, as well as provide insight into the potential for using brain stimulation as a therapeutic to optimize cognitive function, particularly in disorders characterized by disrupted prefrontal function, including schizophrenia.

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Poster

551. Human Long-Term Memory: Medial Temporal Lobe III

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Topic: F.01. Human Cognition and Behavior

Support: NIH K99/R00 AG036845

NIH P50 MH094263

Title: Encoding related delay period activity in hippocampal subfields and medial temporal cortex during delayed matching to sample: A high-resolution fMRI study

Authors: *R. K. NAUER[1,2,3], A. S. WHITEMAN[3,2,1], M. F. DUNNE[4,2], C. E. STERN[1,2,3], K. SCHON[4,2]
[2]Ctr. for Memory and Brain, [3]Dept. of Psychological and Brain Sci., [1]Boston Univ., Boston, MA; [4]Dept. of Anat. and Neurobio., Boston Univ. Sch. of Med., Boston, MA

Abstract: The goal of this study was to more precisely elucidate the contributions of medial temporal lobe (MTL) and hippocampal subfields to working memory and long-term memory encoding using a delayed matching to sample (DMS) design. Previous fMRI studies have indicated that MTL cortices and the hippocampus are recruited for stimulus maintenance during a delay period, and that this delay period activity is linked to subsequent recognition memory (Schon et al., 2004, 2005). Single-unit and slice-recording studies and computational models suggest persistent spiking mechanisms in entorhinal and hippocampal neurons may support both working memory maintenance and episodic encoding. Because the entorhinal cortex (EC) relays incoming information to the hippocampus via direct projections to the dentate gyrus (DG), CA1, and CA3 subfields, we hypothesized that these regions would show encoding related BOLD activity during the delay period. Participants underwent high-resolution fMRI optimized for MTL cortex and hippocampal subfield contributions (Philips Achieva, 3.0T, TR=2s, 1.5mm^3 isotropic voxels) while performing a DMS task in which they viewed a series of 144 unfamiliar complex outdoor scenes. For each DMS trial, an initial scene was shown (2 sec), followed by a 10-sec delay period, followed by a second scene (2 sec). Participants were asked to make a judgment of whether the second scene was a match to the initial scene for that trial. Approximately 15 minutes after the scanning session, participants performed a surprise subsequent recognition memory test (SMT) using a confidence rating scale to distinguish all 144 DMS scenes from an equal number of content similar lures. We examined delay period activity predictive of subsequent recognition memory. Regions of interest included MTL cortex (EC, PrC, parahippocampal cortex) and hippocampal subfields (CA1, DG/CA3, subiculum), and were manually traced on T1-MPRAGE images (1mm^3 isotropic voxels). Preliminary group analysis (N = 15; 21.4 ± 3.2 years) of the delay period activity showed that increased activation in DG/CA3, CA1, and on the EC/PrC border, predicted high confidence subsequent recognition memory. Our data support previous work demonstrating a subsequent memory effect in the EC/PrC region, and extend previous findings by showing encoding-related delay period activity in CA1 and DG/CA3 hippocampal subfields.

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Poster

551. Human Long-Term Memory: Medial Temporal Lobe III

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Title: To be, or not to be, remembered: Patterns of memorability in the medial temporal lobe

Authors: *W. A. BAINBRIDGE[1], D. D. DILKS[3], A. OLIVA[2]
[1]Dept. of Brain and Cognitive Sci., [2]CSAIL, MIT, Cambridge, MA; [3]Dept. of Psychology, Emory Univ., Atlanta, GA

Abstract: We are constantly encoding the world around us into memory, but remembering only some images of faces or places, while forgetting others. These images differ in their memorability - a predictive value of whether a novel stimulus will be remembered or forgotten that is highly consistent across observers (Bainbridge et al., 2013; Isola et al., 2011). The current study investigates how memorability is represented in a classic memory-related cortical region, the medial temporal lobe. In an fMRI study, human observers (N=16) viewed images of faces and scenes that were determined a priori as being highly memorable or highly forgettable, and viewed them only once each. While in the scanner, participants performed an orthogonal categorization task (i.e., male/female for faces; indoor/outdoor for scenes). Participants were not aware of any memory-related nature to the study, and completed a post-scan memory test. Stimuli were controlled for both low-level image statistics (i.e., spatial frequency, color), as well as higher-level attribute information (i.e., gender, race, attractiveness, and emotion for faces, and indoor/outdoor, natural/manmade, and no faces or animals for scenes). Despite these tightly controlled visual and semantic attributes, significant differences in BOLD activity were found between memorable versus forgettable images in regions of the medial temporal lobe: the amygdala (for faces only), perirhinal cortex, and parahippocampal cortex. In addition, multivoxel pattern analyses found significantly high pattern classification accuracy for memorable images versus forgettable images in the hippocampus, perirhinal cortex, and parahippocampal cortex. Neither univariate nor multivariate effects of memorability were found in the entorhinal cortex. These results indicate that i) even when an image is first perceived, the medial temporal lobe is distinguishing images that should be later remembered versus forgotten, and ii) image

memorability can be utilized as a general image attribute that is linked to stereotyped cortical activity.

Disclosures: **W.A. Bainbridge:** None. **D.D. Dilks:** None. **A. Oliva:** None.

Poster

551. Human Long-Term Memory: Medial Temporal Lobe III

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Topic: F.01. Human Cognition and Behavior

Support: NIH Grant MH068721

Title: Cortical representation of serial position information in temporal sequences

Authors: ***L.-T. HSIEH**[1,2], C. RANGANATH[1,2]
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Abstract: The ability to remember what happens when is critical for human memory functions. Previous studies in animals have shown that the hippocampus is critical for representing time between sequential events, but little is known about whether or how serial order information about learned sequences is represented by the hippocampus and other brain regions. Our recent fMRI study (Hsieh et al., 2014) showed that multivoxel hippocampal activity patterns specifically carry information about objects in temporal position, indicating conjunctive object-by-position coding in the hippocampus, in contrast to nearby perirhinal and parahippocampal cortices whose activity patterns are sensitive to object and serial position information, respectively. To further examine the role of other cortical regions in temporal sequence representation, we conducted multivoxel pattern analyses on the same data set, this time focusing on cortical regions of interest (ROIs) that have been implicated in episodic retrieval. The experiment consisted of a pre-scan learning phase and a post-learning MRI retrieval session. During the learning phase, participants learned five "Fixed" and one "Random" sequences. Each sequence consisted of five distinct objects and the order of the objects was always constant for the "Fixed" sequences. For the "Random" sequence, it always consisted of the same five objects but the order of the objects was always random. Thus, participants were able to learn about each object in the "Random" sequence, but could not consistently associate an object with any serial position. Next, they were scanned during exposure to multiple repetitions of these sequences while making a series of semantic judgments on each presented object. Pattern analyses were

conducted on data extracted from individual cortical ROIs. Cortical ROIs were identified using an automated parcellation procedure implemented in Freesurfer which defines regions based on individual participant's gyral and sulcal anatomy. Preliminary results show that many ROIs in prefrontal and parietal cortex, exhibited pattern similarity profiles that are indicative of coding of serial position across fixed and random sequences, whereas ventral stream ROIs show profiles that are consistent with object-specific coding. Importantly, none of these cortical ROIs exhibit patterns that are similar to the conjnuctive object by position coding seen in the hippocampus.

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Poster

551. Human Long-Term Memory: Medial Temporal Lobe III

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Topic: F.01. Human Cognition and Behavior

Support: Johns Hopkins Science of Learning Initiative

Title: A high-resolution fmri investigation of temporal memory within the medial temporal lobe

Authors: *J. M. ROBERTS, Z. M. REAGH, E. MURRAY, M. A. YASSA
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Abstract: The encoding of the temporal relationship between previously experienced events is an important aspect of episodic memory. The ability to remember that "A happened before B" is a critical basis for causal inferences, and is therefore a crucial component of an animal's ability to learn to modify behavior in order to increase its odds of survival. Previous studies of memory for temporal sequences and the recent discovery of "time cells" suggest that the medial temporal lobe (MTL) plays a role in encoding time within memory. Further, the discovery that many cells acting as "time cells" within the MTL also act as "place cells" or "grid cells" suggesting that temporal memory and spatial memory may share neural processes within the MTL. We used high-resolution BOLD fMRI techniques to investigate the role of sub-regions of the MTL in processing the temporal aspects of memory. The behavioral task used in the study was designed to examine the effects of temporal interference (operationally defined in terms of the number of intervening items) on temporal order discrimination (operationally defined as correct judgment of "which came first?") Behaviorally, we observed that temporal order discrimination performance increased linearly as we reduced temporal interference, consistent with our prior

work (Roberts et al., in press). This parametric modulation was a necessary pre-condition before using the task to possibly examine a pattern separation process in the temporal domain using high-resolution fMRI. Our fMRI analyses found signals in the CA1 and DG/CA3 regions that were modulated by accuracy and by amount of temporal interference. Our findings are consistent with a role for hippocampal subfields in temporal order memory processes and potentially a pattern separation process.

Disclosures: J.M. Roberts: None. **Z.M. Reagh:** None. **E. Murray:** None. **M.A. Yassa:** None.

Poster

551. Human Long-Term Memory: Medial Temporal Lobe III

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Topic: F.01. Human Cognition and Behavior

Support: NSF GRFP

R01MH068721

R01MH083734

Title: Learning contextual significance in the medial temporal lobe

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Abstract: Learning the contextual significance of environmental cues is critical for navigating everyday life, but relatively little is known about the neural underpinnings of this process. Medial temporal lobe (MTL) regions have been shown to code for contextual regularities, and prefrontal cortex (PFC) function has been associated with the use of explicitly-cued contextual rules, but it is unclear if and how these regions are involved in the learning and deployment of context-guided rules for behavior. To address this question, we tracked the evolution of neural patterns across the learning of a complex set of associative rules. During fMRI scanning, participants were presented with sixteen repeating sequences of three objects. In each sequence, the first two objects (Cue 1 and Cue 2) were drawn from a set of 3D renderings of novel objects; the third object (outcome) was either a hat or a glove. During presentation of Cue 2, participants predicted whether the third object would be a hat or glove, and feedback on their prediction was

provided in concert with the outcome. No single cue was independently predictive of hat or glove outcome; it was only through integration between Cue 1 and Cue 2 that participants could make accurate predictions. Critically, some pairs of Cue 1 objects preceded the same Cue 2-outcome associations such that these Cue 1 pairs provided overlapping contextual information that could guide ensuing prediction responses. We predicted that, over the course of learning, brain regions coding for contextual significance activated by Cue 1 would increasingly show greater neural similarity between pairs of trials with the same Cue 2-outcome associations, compared to different associations. Single-trial parameter estimates corresponding to activity evoked by the Cue 1 phase of each trial were calculated and entered into multivoxel pattern similarity analysis. Searchlight analysis revealed MTL and anterior PFC clusters that coded for Cue 1 contextual significance. A computational modeling approach applied to individual subject outcome prediction responses revealed differences in the relationship between prediction success and context coding in these regions that was dependent upon the shape of the learning curve. Planned analyses will relate model-based metrics of contextual significance learning to voxel activity patterns on a trial-by-trial basis.

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Poster

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Support: ONR MURI Grant N00014-10-1-0936

Title: Tracking location during complex human path integration recruits hippocampus and retrosplenial cortex

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Abstract: Path integration is the constant updating of the navigator's position and orientation within the environment during movement, and it has been studied extensively in animals. In humans, fundamentals for path integration have been localized in the medial temporal lobe

(MTL), and hippocampal activity predicts accuracy in a triangle completion path integration task (Wolbers et al., 2007) and goal-directed navigation tasks (Sherrill et al., 2013; Brown et al, 2014; Brown and Stern, 2013). The retrosplenial cortex (RSC) and posterior parietal cortex (PPC) may play a key role in tracking movement. In addition, specific aspects of movement such as distance traveled, arc degrees traveled, and time traveled could influence processing (Chrastil, 2013). This experiment used fMRI to test the hypothesis that the hippocampus, RSC, and PPC support human path integration. 24 participants performed a location-tracking task (loop closure) while undergoing fMRI scanning. In the Loop Closure task, participants viewed a short video (5-25 sec) of a virtual environment (random poles on a textured ground plane), with movement traveling in a loop pattern. Movement was varied such that it either formed a complete circle to return to the start location, only traversed part of a circle, or overshot the start location. The design included variance in loop radius and travel speeds. After each video, participants indicated whether the loop movement ended at the loop's start location or in a different location. Successful loop closure required participants to track their location throughout the movement relative to the start location. During event-related fMRI scanning, 36 trials were collected across six 10-minute scans (3 T Siemens TrioTim MRI scanner, 3.4x3.4x3.4 mm voxels, TR = 2s, 33 slices, flip-angle = 85o). To examine brain regions recruited for successful loop closure, our univariate analysis contrasted Correct > Incorrect Trials. These results demonstrated significantly greater activity in the right hippocampus, left RSC, bilateral precuneus, and bilateral medial prefrontal lobe, suggesting these areas play a role in accurately tracking location during movement. In addition, parametric analyses were carried out examining distance traveled from start location, arc degrees traveled, time traveled, and distance relative to the goal. Preliminary results show that multiple brain regions track these parameters, including caudate, thalamus, cingulate, superior parietal lobule, and lateral prefrontal cortex. Together, our results suggest that regions within the MTL, RSC, and medial prefrontal cortex support successful location tracking during human path integration.

Disclosures: E.R. Chrastil: None. **K.R. Sherrill:** None. **M.E. Hasselmo:** None. **C.E. Stern:** None.

Poster

551. Human Long-Term Memory: Medial Temporal Lobe III

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Support: NIH Grant K99/R00 AG036845

NIH Grant UL1-TR000157

Title: Encoding related activity in the hippocampus and medial temporal lobe cortex is modulated by aerobic fitness and BDNF in humans

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Abstract: Convergent evidence from human studies and animal models suggest aerobic exercise and fitness may alter brain morphology and improve cognition. In rodents, running is thought to enhance hippocampal dependent memory performance, possibly by increasing expression of the neurotrophin BDNF in hippocampus and upregulating adult neurogenesis in the dentate gyrus (DG). Recent studies in humans suggest aerobic fitness may be associated with cerebral blood volume in the DG (and possibly the entorhinal cortex - EC; Pereira et al., 2007) and with hippocampal activity during virtual navigation (Holzschneider et al., 2012). Here, we sought to examine whether aerobic fitness and BDNF modulate brain activity in specific medial temporal lobe (MTL) cortex and hippocampal subregions in humans. We examined this question by using a standard graded treadmill test of aerobic capacity (VO_2 peak), blood draws for serum BDNF, and high-resolution fMRI (Phillips Achieva, 3.0T, TR = 2s, resolution = 1.5 mm^3) optimized for precise localization of task-related activation within the MTL. We scanned participants as they performed a variant of a delayed match to sample (DMS) task known to recruit the hippocampus and EC/perirhinal cortex (Schon et al., 2004, 2005). Participants learned a set of 144 trial unique but content similar outdoor scenes in the context of a DMS paradigm (Nauer et al., SfN abstract 2014). After fMRI scanning, participants performed a surprise subsequent memory test for all 144 old scenes, randomized with an equal number of content similar lures. We used SPM8 for analysis and ANTs software (Avants et al., 2011) for cross participant alignment of manually traced regions of interest (EC, perirhinal, and parahippocampal cortex; hippocampal subregions CA1, DG/CA3, and subiculum). Consistent with our previous work (Whiteman et al., 2014), preliminary analysis (N = 16) showed that a subsequent memory effect (remembered > forgotten) was modulated by both BDNF and aerobic fitness throughout the MTL cortex and hippocampus. In addition, aerobic fitness was positively associated with volume in the EC. Our study extends findings from rodent models to humans and supports the idea that aerobic fitness impacts structure and function in the entorhinal-hippocampal memory system.

Disclosures: **M.F. Dunne:** None. **A.S. Whiteman:** None. **R.K. Nauer:** None. **C.E. Stern:** None. **K. Schon:** None.

Poster

551. Human Long-Term Memory: Medial Temporal Lobe III

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Topic: F.01. Human Cognition and Behavior

Support: CIHR Grant MOP93644

Title: Are familiarity-based memory representations in human perirhinal cortex distributed

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Abstract: Over the past decade, much neuropsychological and functional imaging research has focused on identifying the brain regions related to familiarity and recollection. One prominent proposal that has emerged from this research stipulates that perirhinal cortex (PrC) supports familiarity-based item recognition, whereas hippocampal contributions are not critical for this process. Relatively little is known about how familiarity signals are coded in human PrC. Here, we started addressing this question using multivoxel pattern analysis to examine right PrC activity associated with the perceived familiarity of faces. A linear classifer allowed us to extract a pattern that successfully distinguished between perceived familiarity and novelty (Martin et al., 2013, Journal of Neuroscience, 33, 10915-23). We next sought to characterize the spatial distribution of this pattern. This issue is important given that recent research has pointed to a functionally defined contiguous region that responds differentially to faces in right PrC (i.e., a 'face patch'; O'Neil et al., 2014, NeuroImage, 92, 349-55). The diagnostic voxels that allowed for successful classification were widely distributed across right PrC; only 18% of voxels in the pattern that allowed us to distinguish between subjectively familiar and novel faces overlapped with the functionally defined face patch that was identified using data from an independent localizer. In line with this finding, attempts to classify familiar and novel faces using the restricted set of voxels that comprise the contiguous face patch were unsuccessful. Critically, the distributed set of voxels that did allow for familiarity-based classification still showed category-specificity (i.e., a strong response to faces than objects and scenes). We next explored the notion of distributed representation at the level of distributed information content. In this set of analyses we found evidence that pattern classifier performance does not merely reflect sensitivity to a spatially distributed mean difference between familiar and novel trials. This latter outcome was found to be related to the fact that the pattern included decreases as well as increases in signal

between familiar and novel trials. Taken together, the current findings offer promising initial evidence that argues in favor of distributed familiarity representations.

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Poster

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Topic: F.01. Human Cognition and Behavior

Support: CIHR44041

Title: Relationship of hippocampal subfields to memory performance changes with age

Authors: ***S. G. TRAVIS**[1], Y. HUANG[1], F. OLSEN[1], R. CARTER[1], E. FUJIWARA[1], P. SERES[2], N. V. MALYKHIN[1]
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Abstract: Background: Whole hippocampal volumes can decline with age and predict episodic memory performance; however it is unclear how volumes of subfields within the hippocampus (HC) decline with age, and how this affects their relationship with episodic memory over an entire lifespan. Most structural MRI studies have examined the HC subfields within a single subregion of the HC and used specialised experimental memory paradigms. The purpose of the present study was to determine the association between volumes of whole HC and HC subfields with performance on a standard neuropsychological assessment of memory. Methods: We recruited 103 healthy participants from the ages of 18 to 85, and used a median split in age to determine young and old groups. Participants were screened neurodegenerative and psychiatric disorders. Ultra high resolution MRI datasets were acquired on a Varian 4.7T scanner. The HC subfields - the cornu ammonis 1-3 (CA), dentate gyrus (DG), and subiculum (SUB) - were segmented manually within three hippocampal subregions - the head, body and tail. Participants were administered the Wechsler Memory Scale, 4th edition (WMS-IV) to assess performance in the Visual Memory Index (VMI), Auditory Memory Index (AMI), Visual Working Memory Index (VWMI), and the Delayed and Immediate Memory Indicies (DMI and IMI). Pearson correlations were used to determine relationships between age, subfield volumes, and memory performance. Results were corrected for multiple comparisons using Bonferroni correction. Results: All memory indexes correlated negatively with age (AMI $r=-.48$, $p<.001$; VMI $r=-.76$,

p<.001; VWMI r=-.74, p<.001; IMI r=-.67, p<.001; and DMI r=-.69, p0.27, p0.28, p.28, p<.008), however not the HC Head or Tail. Conclusions: In general, total HC, total HC body and total DG volumes were the most predictive of memory performance. Over the entire lifespan, the dentate gyrus may play the most central role in aging of all hippocampal subfields. This is true not only in neurodegenerative disease processes, but in a sample of healthy individuals.

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Poster

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Program#/Poster#: 551.19/SS17

Topic: F.01. Human Cognition and Behavior

Title: Oculomotor capture by aversive stimuli in the absence of contingency knowledge

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Abstract: Can stimuli associated with an aversive event via Pavlovian fear conditioning capture attention even when participants cannot explicitly report the association? In a recent eye tracking investigation, we found that individuals look disproportionately at items in a complex scene that predict the presence or absence of shock. Critically, this differential viewing effect was evident before participants were aware of the item's value. Here, we investigate whether or not eye movements are captured involuntarily by a conditional stimulus (CS) when this pattern of viewing runs counter to task demands. To address this question, we developed a novel variant of the irrelevant singleton paradigm that has been used to study attention capture. Subjects performed a two-part task while their eye movements were recorded. During an initial training phase displays consisted of eight red objects located on an imaginary circle surrounding central fixation. One object was either a horizontal or vertical rectangle, and the remainders were circles. Subjects were instructed to fixate the rectangle as quickly as possible because slow performance would elicit a shock. In reality, shock delivery was predetermined. One rectangle (e.g., horizontal) was paired with shock on 80% of the trials (CS80) while the other (e.g., vertical) was paired with shock on 20% of the trials (CS20). During a subsequent test phase, each trial began with the presentation of eight red circles surrounding a central fixation point. Following a 1s exposure to this display, one circle changed from red to gray and subjects were told to fixate the

gray circle as quickly as possible. The CS80 appeared in the display coincident with this change on 1/3 of the trials; the CS20 was presented on another 1/3 of the trials. After the test phase, subjects completed a post-experimental questionnaire designed to evaluate contingency awareness. It was predicted that oculomotor capture would occur most often when the CS80 was present in the test display. During training, saccades to CS80s were faster than to CS20s. This effect seems to reflect a gradual acquisition of contingency knowledge, as faster saccades to CS80s only occurred during the last half of the training session. During test, subjects were slower to fixate the target when a CS was present. As predicted, eye movements were captured more often by the CS80 than the CS20, an effect that did not depend on explicit knowledge of the imposed contingencies Together, these results suggest that eye movements can be captured by an aversive item involuntarily and without awareness of learned associations.

Disclosures: L. Hopkins: None. **F.J. Helmstetter:** None. **D.E. Hannula:** None.

Poster

551. Human Long-Term Memory: Medial Temporal Lobe III

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Topic: F.01. Human Cognition and Behavior

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MH068721

MH083734

Title: Contextual significance shapes item representations in the medial temporal lobe

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Abstract: Models of medial temporal lobe (MTL) function hold that individual MTL regions support the processing of different aspects of memory. Specifically, the perirhinal cortex (PrC) and parahippocampal cortex (PhC) are thought to represent information about items and contexts, respectively, while the hippocampus functions to bind this item and context information. Recent investigations have also implicated the MTL in representing environmental regularities and in biasing behavior based on a prior encoding context. Despite this, it is

unknown whether contextual significance, or predictive validity for future events, can influence item representations. To address this question, neural pattern similarity was assessed before and after participants learned to predict whether 16 fully crossed pairs of 8 novel objects were associated with either a hat or glove outcome. Object pairs and outcome information were presented sequentially during learning; half of the objects were always presented first in a pair (Cue 1), while the remaining half were always presented second (Cue 2). Participants were asked to predict the category outcome associated with the pair during Cue 2 presentation, and were subsequently given feedback on their choice. Critically, object pair-category outcome sequences were designed to create subsets of Cue 1 objects with shared contextual significance, or shared information about possible Cue 2-outcome associations. For example, Cue 1A and Cue 1A' both resulted in a hat outcome when paired with Cue 2C, and a glove outcome when paired with Cue 2D. This manipulation allowed for an assessment of whether item representations in the MTL, as measured by multi-voxel activity patterns, were modulated by learning the contextual significance of each object in the full set of object pair-outcome sequences. Preliminary analyses indicate that regions in PrC and PhC showed learning-related changes in object representations that were linked to behavioral performance at the end of learning. Future analyses will apply computational modeling techniques to relate differences in contextual learning to neural changes in object representations.

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Poster

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NIH Grant R01 MH 60941

Title: Disparateness of episodic details modulates default network activity during future simulation

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Abstract: Objectives: Remembering the past and imagining the future recruit a common network including many regions of the default-mode network. However, previous work has shown that some nodes of this network (e.g. the hippocampus) produce a greater BOLD response when imagining relative to remembering. One interpretation for this increased default network activity is that imagining the future requires the flexible integration of details from disparate memories to produce a coherent imagined scenario. The current study directly tested this hypothesis by manipulating the degree of disparateness of memory details incorporated into an imagined scenario. **Methods**: In a pre-scan session, participants reported 120 people, locations and objects from 3 social spheres (i.e. 40 per sphere) they had encountered in the previous 5 years. These details were recombined to create 90 person/location/object sets where the 3 details all came from the same social sphere (low disparateness) or the 3 details each came from a different sphere (high disparateness). In the fMRI session, participants were presented with 45 low and 45 high disparateness trials in an event-related design; for each trial, they had to silently imagine a future scenario involving all 3 details (10 *s*) and make a button press once they had constructed an event. This was followed by rating the preceding imagined scenario for amount of detail (4 *s*). In addition, a semantic/visuospatial control task was also presented. **Results**: Behavioural results showed that scenarios in the low disparateness condition were constructed more quickly and rated as more detailed than scenarios in the high disparateness condition. A mean-centred partial least squares analysis produced a significant latent variable showing increased activity in default-mode network regions for both simulation conditions relative to the control task. Importantly, analysis of the brain scores demonstrated that this network was more strongly engaged in the low versus high disparateness condition. **Conclusion:** The finding of reduced default-network activity when constructing simulations from highly disparate episodic details is likely due to participants not being able to successfully integrate the details from different social spheres into a coherent scenario. One potential avenue for future research is to adopt an individual differences approach to determine if certain traits (e.g. cognitive flexibility or creativity) are associated with the ability to imagine scenarios involving highly disparate memory details.

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Poster

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Topic: F.01. Human Cognition and Behavior

Support: Wellcome Trust

Title: Hippocampal theta during encoding predicts subsequent attentional deployment

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Abstract: Spatial and contextual associations in long-term and short-term memory are known to depend on a network of areas centred around the hippocampus, and theta-band activity has been implicated in the integration of information across the brain areas involved. Much less well understood is whether and how learned spatial and contextual associations can guide attention when individuals anticipate target events within learned contexts. In this experiment, we used magnetoencephalography (MEG) to explore the neural signatures of item-context encoding, using a paradigm based on contextual cueing developed in our lab. We were interested in how the encoding of spatial locations of objects in complex scenes would develop across learning, and how this would relate to deployment of attention to those spatial locations in a subsequent memory-guided attention paradigm. Using a beamforming technique, we found a theta network of areas involving the left and right hippocampus, right parahippocampal cortex, precuneus, and medial prefrontal cortex, as well as a network of areas in the alpha range, including anterior cingulate cortex, frontal eye-fields and intraparietal sulci. Additionally, the magnitude of hippocampal theta during learning was correlated with subsequent lateralized alpha-band activity when subjects were utilizing their memories to deploy attention. Our results reveal co-activation of traditionally segregated memory and attention networks within different frequency bands, which interact during encoding, as well as predict the strength of memory-based attentional bias.

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Poster

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Topic: F.01. Human Cognition and Behavior

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Title: Human perirhinal cortex supports frequency judgments as well as judgments of cumulative lifetime familiarity

Authors: *D. DUKE[1], B. BOWLES[2], C. B. MARTIN[1], S. R. ROSENBAUM[3,4], K. MCRAE[1], S. KÖHLER[1,4]
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Abstract: Neurophysiological research in non-human primates has revealed differences between recency and cumulative familiarity signals in the anterior temporal lobe, including perirhinal cortex (PrC). These signals are thought to contribute to recognition-memory, independent of successful recollection of episodic detail about a pertinent prior exposure. To date, most studies in humans employing meaningful stimuli to probe recognition memory have addressed recency rather than cumulative lifetime familiarity. While it has been demonstrated that humans can judge the cumulative familiarity of verbal concepts over a lifetime of experiences (e.g., how familiar are you with aardvarks?) in a reliable manner, little is known about the neural mechanisms that support this ability. Here, we asked whether a left anterior temporal-lobe lesion that includes PrC but spares the hippocampus is associated with abnormalities in the assessment of lifetime familiarity in an individual (NB) with previously documented isolated impairments in recency (i.e., familiarity in recognition memory tasks with recent study exposure; Bowles et al., 2007, *PNAS*,16382-7). We also examined whether a selective hippocampal lesion with sparing of perirhinal cortex would be associated with normal performance in a developmental amnesic individual (HC) with documented deficits in the recollection of lifetime episodes (Rosenbaum et al., 2011, *Neurocase*, 394-409). NB demonstrated abnormal and less consistent lifetime familiarity assessment relative to healthy controls over multiple testing sessions. Furthermore, NB's familiarity ratings were abnormal regardless of whether probed with verbal labels or visual images of object concepts. Patient HC, by contrast, provided lifetime familiarity judgments that did not differ from those of controls, despite her established autobiographical memory impairment. In a second study we employed fMRI to determine whether PrC carries both lifetime familiarity signals and signals of recency for the same type of verbal concepts in healthy individuals. In order to probe recency with a task format similar to that used in the assessment of cumulative lifetime familiarity, we asked participants to judge the frequency of exposure to these stimuli in a study phase. An area in left PrC was the only cortical region in which activity tracked heightened impressions of lifetime familiarity (via increases BOLD signal) as well as the perceived frequency of recent exposures (with decreases). Together, these data strongly suggest that PrC is not only crucial for the assessment of recent changes in familiarity, but is also crucial for judging cumulative lifetime familiarity.

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Poster

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Topic: F.01. Human Cognition and Behavior

Support: Arizona Alzheimer's Consortium

Title: Connectivity between the perirhinal cortex and V2 in young and older adults

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Abstract: Prior research showed that the perirhinal cortex (PRC) is sensitive to the familiarity vs. novelty of whole objects, and also of the parts comprising them. Specifically, for objects in the right visual field (RVF), BOLD activity in the PRC was highest for Familiar Configurations, moderate for Control Novel Configurations in which both the configuration and the parts comprising it were novel, and lowest for Part-Rearranged Novel Configurations, created by spatially rearranging the parts of paired familiar configurations (Peterson et al., 2012). Moreover, activation in V2, a low-level visual area that represents object parts, was greater for the same familiar parts when they comprised a familiar configuration rather than a novel configuration. That the V2 activity pattern mimicked that of the PRC suggested that feedback from the PRC modulated responses in V2, but no connectivity analyses were performed. In the current study, we used BOLD fMRI and functional connectivity analyses to directly investigate whether the PRC modulates responses in V2, and whether this modulation changes with age. Young and older adults performed an object decision task on silhouettes presented in the periphery. Young subjects showed the expected linear pattern of BOLD activation in the left hemisphere (LH) PRC for RVF silhouettes: Familiar > Control Novel > Part-Rearranged Novel Configurations, $p < .05$. Older subjects did not show this linear pattern in the LH PRC, $p > .10$, consistent with the hypothesis that PRC functioning declines with age (c.f., Ryan et al., 2012). A functional connectivity analysis using the LH PRC as the seed region of interest revealed a significantly stronger coupling between the LH PRC and LH V2 for RVF presentations in young than in older adults, $p = .05$. Results support the hypothesis that top-down feedback from the PRC modulates responses in V2, consistent with a dynamic, interactive view of object perception. That this

feedback was significantly reduced in older subjects might indicate an age-related decline in the PRC, its functional connections to low levels, or both. This project is the first to use functional connectivity analysis to demonstrate the influence of the PRC on responses of a lower-level brain region.

Disclosures: L. Cacciamani: None. **E. Wager:** None. **M.A. Peterson:** None. **P.E. Scalf:** None.

Poster

(Unable to Attend)

551. Human Long-Term Memory: Medial Temporal Lobe III

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 551.25/SS23

Topic: F.01. Human Cognition and Behavior

Support: DFG Grant (WO733/13-1; 13-2)

Title: Context and memory phase dependent effects of stress on extinction memory in a predictive learning task

Authors: *O. T. WOLF[1], T. C. HAMACHER-DANG[2,3]
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Abstract: Psychotherapeutic treatment of anxiety disorders, e.g. via exposure therapy, typically relies on the principle of extinction. Recovery effects such as spontaneous recovery or renewal indicate that extinction does not lead to an erasure of the memory trace but constitutes a form of new inhibitory learning mediated by the prefrontal cortex. Stress modulates episodic memory in a phase-dependent way, with impairing effects on retrieval and enhancing effects on consolidation. First evidence suggests that this might also be the case for extinction memory. In three studies applying a predictive learning task in the form of a renewal paradigm, we investigated how stress affects extinction memory along the time course of its acquisition, consolidation and retrieval. On three consecutive days, participants learned an association between stimuli and outcome in one context on day 1, underwent extinction in a second context on day 2 and were tested for retrieval in both contexts on day 3. We induced stress via the socially evaluated cold pressor test prior to retrieval testing on day 3 (study 1), directly after

extinction learning on day 2 (study 2) or 20 minutes before extinction learning (study 3). As assessed by salivary cortisol and blood pressure, stress induction proved to be successful in all studies. A renewal effect was present, as reflected by stronger recovery of responding when retrieval was tested in acquisition context trials compared to extinction context trials. In study 1, stressed participants showed impaired retrieval of extinction memory. In contrast, stress directly after extinction learning (study 2) enhanced the consolidation of extinction memory, as indicated by reduced spontaneous recovery on day 3. This effect, however, was restricted to the extinction context. Pre-extinction stress (study 3) in contrast, strengthened extinction memory independent of context. Our results indicate that stress hormones modulate extinction memory in a predictive learning task in a phase dependent manner. Moreover these effects are context dependent most likely reflecting a stress induced modulation of hippocampal plasticity.

Disclosures: O.T. Wolf: None. **T.C. Hamacher-Dang:** None.

Poster

551. Human Long-Term Memory: Medial Temporal Lobe III

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 551.26/SS24

Topic: F.01. Human Cognition and Behavior

Support: KAKENHI 26560465

Title: Functional connectivity from the human entorhinal cortex: A cortico-cortical evoked potential study

Authors: *H. TAKEYAMA[1,2], R. MATSUMOTO[3], K. KOBAYASHI[2], K. USAMI[2], A. SHIMOTAKE[2], T. KIKUCHI[4], T. KUNIEDA[4], S. MIYAMOTO[4], R. TAKAHASHI[2], A. IKEDA[3]
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Abstract: Background: Entorhinal cortex (EC), the important subregion in the medial temporal lobe memory network, has been considered to have rich connections to various cortical regions as well as to the hippocampus and the classical Papez circuit. The knowledge of connectivity mostly came from the non-primate tract-tracing studies, and thus little is known in humans. We aimed to clarify its connectivity by means of an electrical, tract tracing method of cortico-cortical evoked potential (CCEP) (Matsumoto et al., 2004). Methods: We recruited 7 patients with

medically intractable temporal lobe epilepsy, who underwent invasive presurgical evaluations with subdural electrodes (3 men, age 17-52, 56-102 electrodes). Single-pulse electrical stimulation was delivered to a pair of electrodes on EC and that 2 cm lateral to EC (non-EC: mainly on the anterior fusiform gyrus). Evoked responses time-locked to stimuli (CCEPs) were recorded from the rest of the cortices covered by subdural electrodes. Results: In all 7 patients, EC stimulation evoked early positive potentials of similar waveform and latency within the subjects across almost all electrodes in the temporal, parietal and frontal cortices [Latency of the positive potential: average across electrodes within the subjects, mean 17.5 ms (range 9.5-34.9 ms), SD within the subjects, mean 3.1 ms (range 0.8-9.8 ms)]. This sharp positive potential was generally followed by a blunted, rather sustained negative potential. In contrast, in non-EC stimulation, the positive potential of longer latency was observed at significantly fewer electrodes than in EC stimulation ($p<0.05$, Wilcoxon ranking scale test). Remote isolated CCEP fields, which usually reflect direct cortico-cortical connections, were significantly fewer in EC stimulation than in non-EC stimulation ($p<0.05$, Wilcoxon ranking scale test). Discussion: In the present CCEP study using subdural electrodes, uniform sharply contoured positive potentials were broadly recorded from various cortical regions. This far field potential like responses may indicate that EC has rich connections to various cortical regions probably through subcortical structures such as thalamus, or that EC stimulation simply induced widely distributed electric field due to volume conduction. Further complementary studies with different methodologies are needed to establish the EC connectivity in humans.

Disclosures: **H. Takeyama:** None. **R. Matsumoto:** Other; Endowed department (GSK, UCB, Otsuka, Nihon Kohden). **K. Kobayashi:** None. **K. Usami:** None. **A. Shimotake:** None. **T. Kikuchi:** None. **T. Kunieda:** None. **S. Miyamoto:** None. **R. Takahashi:** None. **A. Ikeda:** Other; Endowed department (GSK, UCB, Otsuka, Nihon Kohden).

Poster

551. Human Long-Term Memory: Medial Temporal Lobe III

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 551.27/SS25

Topic: F.01. Human Cognition and Behavior

Support: NINDS Grant NS076856

Title: Properties of spatial contextual representation within the human hippocampus during episodic memory retrieval

Authors: *C. KYLE, J. STOKES, A. EKSTROM
Univ. of California, Davis, Davis, CA

Abstract: Spatial context forms an integral part of our memory for events yet the neural basis in humans remains unclear. While rodent hippocampal place cells code specific spatial environment and remap in response to changes in landmark configuration, how the human hippocampus codes changes in spatial context is not known. To test this issue, nineteen participants learned the configurations of four virtual reality cities of varying similarity. City 1 and City 2 shared landmark identity and spatial geometry but contained conflicting landmark-location pairings; City 3 involved the same landmarks but with a new spatial geometry while City 4 involved novel landmarks in a novel configuration. Following encoding, participants retrieved information about the recently learned cities by making judgments about their relative distances while undergoing high-resolution hippocampal imaging (1.4x1.5x1.9 mm voxels). A one way ANOVA on performance and reaction time revealed a main effect of city, driven by lower performance and higher reaction time in city 3 (repeated landmarks, novel configuration)(Performance: $F(3,19)=20.9$, RT: $F(3,19)=7.8$, $p<.01$). Examining univariate activation patterns, a 4x5 City by subregion ANOVA revealed a main effect of subregion $F(4,19) = 2.8$, $p=.03$, and a marginal city by subregion interaction $F(12,19) = 1.7$, $p= .058$. Multivariate pattern analyses also revealed differences in voxel pattern similarity for retrieving details from the same vs. different cities. Together, these results suggest the importance of the hippocampus to retrieving details of recently learned spatial layouts. They further suggest that neural patterns during retrieval vary for different cities, consistent with the idea of "remapping."

Disclosures: C. Kyle: None. **J. Stokes:** None. **A. Ekstrom:** None.

Poster

551. Human Long-Term Memory: Medial Temporal Lobe III

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 551.28/SS26

Topic: F.01. Human Cognition and Behavior

Support: UCL Grand Challenge PhD studentship

MRC Grant G1002276

CEL CLRN

CHDI

Title: Distinct white matter correlates of intelligence, recall and recognition: Evidence from developmental amnesia

Authors: *A. M. DZIECIOL[1], E. Z. PATAI[1,2], K. K. SEUNARINE[1], C. A. CLARK[1], F. VARGHA-KHADEM[1]
[1]UCL Inst. of Child Hlth., London, United Kingdom; [2]Dept. of Psychiatry, Univ. of Oxford, Oxford, United Kingdom

Abstract: Developmental amnesia (DA) is a selective episodic memory disorder associated with hypoxia-induced bilateral hippocampal atrophy. Previously, we reported evidence of white matter damage in patients with DA (Dzieciol et al., SfN 2012). Here, we examine white matter abnormalities in DA using tract-based spatial statistics (TBSS; Smith et al., 2006), a quantitative method of analysing diffusion tensor properties. Fractional anisotropy (FA) relates to the directionality of water diffusion, whereas mean diffusivity (MD) relates to the magnitude of diffusion regardless of its direction. In the brain's white matter low FA and high MD can both indicate damage. Echo-planar MR images were acquired on a 1.5 T Siemens scanner with an isotropic set of 20 directions at b=1000 s/mm2, repeated 3 times in a single scan session. Fifteen patients with DA (age range 10 - 35 years), 14 patients with hippocampal atrophy and memory impairment (age range 8 - 16 years) and 29 age- and gender-matched controls participated. Results showed that compared to controls, patients had global reductions in FA and widespread increases in MD throughout the core white matter tracts. To follow-up, we carried out a region-of-interest analysis on mean FA in the white matter skeleton, and in memory-relevant white matter tracts within the medial-temporal lobe: the hippocampal cingulum and the fornix. We uncovered two double dissociations between the neural correlates of the patients' intelligence and memory. First, across the entire patient group, mean FA in the white matter skeleton predicted full scale IQ, but not the memory quotient (MQ). Conversely, mean FA in the fornix was related to MQ, but not to IQ. Second, FA in the fornix was the strongest predictor of patients' verbal recall ability, whereas FA in the hippocampal cingulum was the strongest predictor of auditory recognition in a stepwise regression with backward elimination. Neither verbal recall nor recognition scores were correlated with FA in the white matter skeleton. Interestingly, regional estimates of FA were superior to estimates of hippocampal volume as predictors of memory outcomes. In conclusion, these results suggest that diffuse white matter damage does not contribute to the memory deficits of patients exposed to early hypoxic-ischaemic injury, but it does relate to patients' intellectual function, while local damage in the fornix and the cingulum contributes to recall and recognition processes, respectively. These findings provide new evidence of a separation between neural substrates of recall and recognition in the white matter of the medial temporal lobe.

Disclosures: A.M. Dzieciol: None. **E.Z. Patai:** None. **K.K. Seunarine:** None. **C.A. Clark:** None. **F. Vargha-Khadem:** None.

Poster

552. Language: Neuropsychological Approaches

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 552.01/SS27

Topic: F.01. Human Cognition and Behavior

Support: Emory FERN Education Grant

Title: Effects of varying cloze probability on prediction and integration during sentence processing

Authors: *K. P. REVILL
Emory Univ., Atlanta, GA

Abstract: Understanding sentences involves identifying single words, integrating those words into the grammatical and semantic structure of the unfolding sentence, and using situational and background knowledge to further comprehend the intended meaning. The interplay of information across these levels shapes online comprehension during sentence processing. Prior eyetracking work has shown rapid effects of sentence context on sentence-final spoken word recognition, with participants making anticipatory eye movements to potential objects following a constraining verb and showing less competition from phonologically similar words that are semantically inconsistent. In the ERP literature, the N400 component is modulated by prediction error, with smaller N400s for expected words in highly versus moderately constraining sentences and smaller differences between expected and unexpected words in less constraining sentence contexts. Existing fMRI studies of sentence comprehension have focused almost exclusively on activation differences between expected, unexpected, and anomalous words in highly constraining sentence contexts. The goal of this study is to examine the effects of varying sentence constraint strength on brain activation during sentence processing, both during the accumulation of sentential information and upon encountering a sentence-final word that is either expected or unexpected given the prior context. Sixteen subjects performed an anomaly detection task on the final word of 120 sentences varying in cloze probability from 0 to 1. Sentence final words were expected, unexpected but sensible, or anomalous. Sentences were presented word-by-word, with each word presented centrally for 300ms with a 200ms ISI. A 2-4s delay between the penultimate and final word allowed separation of predictive processing from integration of the sentence-final word. Delay period activity was modulated by the cloze probability of the sentence, with a positive relationship between cloze probability and activation in left middle

frontal gyrus (MFG) and a negative relationship in anterior aspects of the left middle and superior temporal gyri (STG). The sentence's cloze probability had no effect on activation for expected words. Activation during the integration of an unexpected word was affected by cloze probability, with increased activity for unexpected endings of high constraint sentences in left posterior STG and left MFG. These results suggest that predictive processing in language comprehension may activate specific representations at more controlled levels of processing while reducing competition or easing integration costs at lower levels.

Disclosures: K.P. Revill: None.

Poster

552. Language: Neuropsychological Approaches

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 552.02/SS28

Topic: F.01. Human Cognition and Behavior

Support: Natural Sciences and Engineering Research Council of Canada

Title: Native bilingualism shapes the structure of Heschl's gyrus

Authors: *J. A. BERKEN[1,2], K. MOK[1], J.-K. CHEN[1], V. GRACCO[2], S. BAUM[2], K. WATKINS[3], D. KLEIN[1,2]
[1]Cognitive Neurosci., Montreal Neurolog. Institute, McGill Univ., Montreal, QC, Canada; [2]Ctr. for Res. on Brain, Language, and Music, Montreal, QC, Canada; [3]Exptl. Psychology, Univ. of Oxford, Oxford, United Kingdom

Abstract: Successful discrimination of novel speech sounds has been shown to correlate with increased volume of Heschl's gyrus (HG). In addition, it appears that early bilinguals have larger HG compared to monolinguals. Previous work from our lab demonstrated alterations in brain structure in early bilinguals, but to date, few studies have investigated brain anatomical differences in individuals who acquire two languages from birth. Here, we used a whole-brain cortical thickness measure to compare three groups of subjects matched for age, intelligence, and years of formal education: (1) highly proficient French-English simultaneous bilinguals (two languages acquired from birth; N = 16), (2) highly proficient French-English sequential bilinguals (second language acquired after age 5 years; N = 17), and (3) English monolinguals (N = 19). We found that simultaneous bilinguals had significantly greater cortical thickness in right HG than both sequential bilinguals and monolinguals. In contrast, sequential bilinguals did not

show a similar cortical thickness increase in HG relative to monolinguals. These findings suggest that an early perceptual bilingual experience is reflected in the structural plasticity of HG.

Disclosures: J.A. Berken: None. **K. Mok:** None. **J. Chen:** None. **V. Gracco:** None. **S. Baum:** None. **K. Watkins:** None. **D. Klein:** None.

Poster

552. Language: Neuropsychological Approaches

Location: Halls A-C

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Topic: F.01. Human Cognition and Behavior

Support: JSPS KAKENHI Gran Number 25770169

Title: Cerebral blood response for speech production in inferior prefrontal cortex: A near-infrared spectroscopy study in stuttering

Authors: *J. OGURA, S. CHU, R. A, K. OCHI, K. MORI
Res. Inst. Natl. Rehabil. Ctr., Saitama, Japan

Abstract: The left inferior frontal cortex is known to be involved in phonological processing and speech. In particular, it has been reported that the Brodmann's Areas (BA) 45 and 46 are associated with phonological processing and that rote rehearsal activates BA44 in the left hemisphere. However, it has been reported that the activation of the broca's area in people who stutter (PWS) was lower than that in people who not stutter (PWNS). This study examined the cerebral blood responses in the left inferior frontal cortex for speech production in PWS and PWNS by means of non-invasive near-infrared spectroscopy (NIRS). All subjects were native Japanese speakers and right-handed. They were instructed first to read silently and to remember a displayed word (visual response) for an interval of random length (0.5 to 3.0 s), and later to pronounced it as soon as the "+" sign was seen on the screen. Four word categories were used as stimuli: Japanese familiar (F), unfamiliar (U), and pseudo- words (P) and 5 control prolonged vowels (C). NIRS recording was done with photomultipliers illuminated through the head tissue by alternating near-infrared lasers at 3 wavelengths (FOIRE 3000, Shimadzu, Japan). The changes in oxygenated and deoxygenated hemoglobin concentrations were calculated with modified Bear-Lumbert approximation. Unlike functional magnetic resonance imaging, the measurement of NIRS was recorded continuously during the task period included speech. The oxygenated hemoglobin data were analyzed with 2-way ANOVA by means of individual beta

values derived from the general linear model analysis. Significant interaction effects were found between the subject groups and word categories in cerebral blood response at the left BA46. The response to P in PWS was significantly higher than that in PWNS. Within PWS, the response to P was significantly higher than that to F. Within PWNS, the response to P was significantly lower than that to F. Whereas the broca's area (BA44 and 45) had not a significant difference between the subject groups. These findings suggest that the left BA46 is associated with word familiarity of the left BA46 was different between PWS and PWNS in speech production.

Disclosures: J. Ogura: None. **S. Chu:** None. **R. A:** None. **K. Ochi:** None. **K. Mori:** None.

Poster

552. Language: Neuropsychological Approaches

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Topic: F.01. Human Cognition and Behavior

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NIH Grant NS044266

NIH Grant AG032953

NIH Grant AG038490

Title: Impairment in sentence comprehension in patients with the behavioral variant of frontotemporal degeneration

Authors: *R. B. WILLIAMS, K. RASCOVSKY, M. GROSSMAN
Univ. of Pennsylvania, Philadelphia, PA

Abstract: Impairment in sentence comprehension, a complex process that requires both grammatical and working memory, has been observed in patients with bvFTD. These patients are known to suffer from executive and social deficits, as well as grammatical expression deficits in a semi-structured speech sample, although they are not aphasic. To elucidate the basis for the comprehension impairment, we analyzed the relationship between grammatical comprehension

and performance on language, working memory, and executive measures. We compared the scores on all measures in patients with bvFTD with the scores of patients with amnestic Mild Cognitive Impairment (aMCI) and healthy elderly controls. Patients with bvFTD (n=23), aMCI (n=13) and controls (20) were evaluated with a two-alternative sentence-picture matching task, in which patients chose the picture best described by a sentence that depends on grammatical interpretation. Semantic memory was also assessed, using Pyramids and Palm Trees (PPT) and Boston Naming Test (BNT); executive functioning using Trails B (TB), FAS Category Fluency (CF), and the Visual Verbal tests; and working memory using Digit Span backwards (DB). We found significant impairment in overall sentence comprehension bvFTD participants compared to controls ($p < .001$), and aMCI ($p=.052$). Within the sentence comprehension task, there was no effect for grammatical structure or working memory. bvFTD patients' sentence comprehension performance correlated with scores on both the semantic measure PPT ($r=.68$, $p<.002$) and the executive measure FAS ($r=.54$, $p<.01$). These findings suggest that bvFTD subjects are impaired in grammatical comprehension, and, although these participants are not aphasic, contributing factors to this impairment appear to include deficits in executive functioning and semantic memory.

Disclosures: R.B. Williams: None. **M. Grossman:** None. **K. Rascovsky:** None.

Poster

552. Language: Neuropsychological Approaches

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

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Topic: F.01. Human Cognition and Behavior

Title: A functional mri study of the relationship between concise chinese aphasic test scoring and resting state functional connectivity in post-stroke aphasia

Authors: *P.-C. TSAI[1], C.-E. TSENG[2], F.-P. YANG[2], C.-M. LIN[3], B.-S. YIP[1], S.-H. LIN[1], T.-M. CHIANG[4], L.-Y. TSENG[1], L.-Y. HUNG[1], L.-W. KUO[5]
[1]Neurol., Natl. Taiwan Univ. Hosp. Hsin-Chu Branc, Hsinchu City, Taiwan; [2]Dept. of Foreign Languages and Literature, Natl. Tsing Hua Univ., Hsin-Chu, Taiwan; [3]Changhua Christian Hosp., Changhua, Taiwan; [4]Hsinchu Cathay Gen. Hosp., Hsinchu, Taiwan; [5]Natl. Hlth. Res. Inst., Miaoli, Taiwan

Abstract: Resting-state studies organized with aphasic stroke patients are limited. We explored whether patients with better speech ability showed differences in resting-state functional

connectivity. Five right-handed stroke patients with aphasia and left middle cerebral artery territory infarction were included in the study. Stroke patients received Concise Chinese Aphasia Test and functional MRI scan 9-12 weeks after stroke. ROI-wise functional connectivity (FC) analysis was performed using the REST toolkit (Song et al., 2011). The analysis included six networks: the language network (LN), left central executive network (LCEN), right central executive network (RCEN), dorsal default mode network (dDMN), and ventral default mode network (vDMN). We explored the FC between these five networks and correlated the results with CCAT scores. Significant correlations between the CCAT scores and the LN-dDMN FC were observed. When the patients showed worse capability at Figure-Word Imitation subscale, the FC of these five networks were all augmented significantly except LN-LCEN and vDMN-LCEN. The Figure-Substance Pairing scoring showed a significant negative correlation with diffuse FC except dDMN-vDMN, LN-LCEN and vDMN-LCEN. The FC of LN-RCEN and dDMN-RCEN correlated negatively with Auditory Comprehension subscale scoring. We also observed significant negative correlation between the RCEN-LCEN FC and performance in Spontaneous Writing subscale. These preliminary results suggest that increased FC in these five networks is related with the severity of aphasia and consequently may aid in understanding of the neural mechanisms underlying the post-stroke aphasia. References: SONG, X. W., DONG, Z. Y., LONG, X. Y., LI, S. F., ZUO, X. N., ZHU, C. Z., HE, Y., YAN, C. G. & ZANG, Y. F. 2011. REST: a toolkit for resting-state functional magnetic resonance imaging data processing. PLoS One, 6, e25031.

Disclosures: **P. Tsai:** None. **C. Tseng:** None. **F. Yang:** None. **B. Yip:** None. **C. Lin:** None. **S. Lin:** None. **L. Tseng:** None. **T. Chiang:** None. **L. Hung:** None. **L. Kuo:** None.

Poster

552. Language: Neuropsychological Approaches

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Program#/Poster#: 552.06/SS32

Topic: F.01. Human Cognition and Behavior

Support: CIHR Operating Grant to ISJ

NSERC Operating Grant to ISJ

Title: Perception of degraded low-context speech in early psychosis

Authors: D. LADOWSKI[1], E. ABDELMOTAAL[2], *I. S. JOHNSRUDE[1]
[1]Psychology, [2]Psychiatry, Queen's Univ., Kingston, ON, Canada

Abstract: Individuals who experience a shorter duration of untreated schizophrenia have more favourable mental health outcomes (Hegelstad et al., 2012). In order to identify suitable candidates for early intervention, sensitive screening techniques must be developed. Previous research has identified cognitive distortions that can appear in the early or prodromal stages of psychosis and may underlie or predict the presence of psychotic symptoms. For example, reality-testing deficits can lead to misidentification of internal thought as external speech, and the inappropriate assignment of meaning or salience to otherwise meaningless or unimportant stimuli (Bentall, 1990). Individuals with schizophrenia are thought to be driven to make sense of these anomalous perceptionsl; and this may explain delusions and hallucinations (Kapur, 2003). In the present study, participants (14 recruited from an early psychosis clinic, 17 demographically matched controls) listened to 180 sentences degraded by noise (with signal-to-noise ratios of 0, -2, or -4dB) and were asked to report as many words from each sentence as they could. Sentences either had strong semantic context (e.g., “Her new skirt was made of denim”) or were syntactically matched but with weak context (e.g., “Her good slope was done in carrot”). Both groups reported fewer words as noise level increased, and more words from the high-context than the low-context condition ($p < .001$). These two factors also interacted such that context had a bigger effect on word report in low noise than in high noise. The results also indicate a tendency for the clinical group to produce more intrusion errors (words not in the original sentence), particularly in low context materials, which may be reflective of early reality-testing impairments. These findings are promising, and encourage the further investigation of tasks involving perceptual ambiguity for the detection of early cognitive markers of psychosis. Bentall, R. P. (1990). *Psychol Bull*, *107*(1), 82-95.Hegelstad, W. ten V., et al. (2012). *Am J Psychiat, 169*(4), 374-80.Kapur, S. (2003). *Am J Psychiatry, 160*(1), 13-23.

Disclosures: D. Ladowski: None. **E. Abdelmotaal:** None. **I.S. Johnsrude:** None.

Poster

552. Language: Neuropsychological Approaches

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Program#/Poster#: 552.07/SS33

Topic: F.01. Human Cognition and Behavior

Support: CONACYT 167900 Mecanismos en la formación y modulación de redes semánticas durante la infancia

PAPIIT RG300313 Desarrollo de lenguaje en niños con síndrome de Down: la comprensión temprana

Title: Down syndrome children's ability to infer a referent

Authors: *N. ARIAS-TREJO, J. B. BARRÓN-MARTÍNEZ, T. JASSO LÓPEZ, E. A. ALVA CANTO
Facultad de Psicología, UNAM, Mexico, Mexico

Abstract: Typically developing children (TD) learn hundreds of words during their first years of life. Different strategies such as word-object constraints, social cues and fast-mapping enable them to do this not only at a rapid speed but also with a low-error range. Besides learning words, children need to learn grammatical rules too; although learning formal linguistic rules such as grammatical gender and number may be challenging, these rules in fact can accelerate language processing. In Spanish the understanding of the information related to gender (words ending in 'a' tend to be feminine and words ending in 'o' masculine) and number ('s' or 'es' ending for plural) results in a more efficient and rapid language processing. Recent investigations have established that young TD children are sensitive to gender grammatical agreement between articles and nouns. Nonetheless, it remains to be explored whether children with mental disabilities, such as Down syndrome (DS) also benefit from grammatical gender cues to anticipate a familiar referent. We explored in a preferential looking task whether both DS and TD children with 30-month-old mental age use singular indefinite articles (un-una in Spanish) to find a target before it was labeled. In each trial, children saw during 2000 ms two pictures (target-distracter), during this period the carrier phrase 'mira' was heard. At 2000 ms children heard a masculine ('un') or a feminine ('una') article; finally, at 4000 ms the target name was heard. In half of the trials, the nouns had a regular ending matching its gender (e.g., 'o' for masculine and 'a' for feminine). The other half of the trials introduced nouns with an irregular ending (e.g., 'e' for masculine or feminine). The results indicated that both groups, DS and TD children with a 30-month-old mental age, anticipated the target referent by using the information contained in the indefinite article (un-una). Importantly, children were able to correctly anticipate the target when facing regular (ending in 'a' or 'o') or irregular nouns (other ending). The outcome of this research demonstrates that DS children, as well as their TD peers, can use gender information embedded in an article to anticipate a referent. This use influences a more rapid and accurate online language processing by allowing disambiguation of familiar items irrespectively of hearing their names. This is the first demonstration of sophisticated comprehension abilities in children with DS. We will discuss the results in light of a paradoxical deficient speech production in these children.

Disclosures: **N. Arias-Trejo:** None. **J.B. Barrón-Martínez:** None. **T. Jasso López:** None. **E.A. Alva Canto:** None.

Poster

552. Language: Neuropsychological Approaches

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Title: Abbreviated pyramids and palm trees test effectively discriminates semantic-variant progressive aphasia from other variants of primary progressive aphasia

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Abstract: Primary progressive aphasia (PPA) is a pathologically heterogeneous neurodegenerative disease that affects language. PPA is classified into three variants: semantic (svPPA), logopenic (lvPPA), and nonfluent/agrammatic (naPPA), which are diagnosed on the basis of converging clinical, imaging, and genetic/neuropathological evidence. Although clearer diagnostic criteria for PPA variants have been defined (Gorno-Tempini et al., 2011), objective clinical tests are needed to better distinguish language deficits between variants so more reliable diagnoses can be made. Gorno-Tempini et al. observed that while all variants of PPA show impairment with object naming, svPPA has pronounced single-word comprehension deficits

which can make diagnosis of this variant more straightforward than diagnosis of lvPPA or naPPA. Moreover, single-word comprehension deficits in svPPA are more commonly seen with concrete object concepts, compared to abstract concepts, and are most frequently associated with larger semantic memory impairments. We used a modified nonverbal semantic task, Pyramids and Palm Trees (PPT) (Howard & Patterson, 1992), to examine semantic memory of concrete object concepts between groups of each variant. The task is administered across two modalities, pictures and words, and includes 14 cross-culturally validated items. Pathology-confirmed PPA patients, svPPA (n=22), lvPPA (n=20), and naPPA (n=8), completed the task. Patients were instructed to point to which of the two objects on the bottom best go with the target object above. Because some participants completed all 14-items across trials that included a combination of both modalities, or only one modality, the scores used for the analysis were prioritized: (1) pictures, (2) words, or (3) mixed - a combination of picture and word items, if other data was not available. A Kruskal-Wallis analysis across all groups was significant ($X2=11.57$; $p<0.003$). svPPA patients were significantly impaired in the abbreviated 14-item PPT compared to lvPPA ($U=107$; $p=0.003$) and naPPA ($U=34.5$; $p=0.01$) patients, while lvPPA patients do not differ from naPPA ($U=75$; $p=0.77$). Additionally, svPPA patients had significant semantic memory impairments compared to lvPPA and naPPA patients. The abbreviated 14-item PPT task provides an efficient and objective clinical test to discriminate svPPA patients from other PPA variants.

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Poster

552. Language: Neuropsychological Approaches

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NIH Grant AG038490

Title: Dysgraphia in patients with the behavioral variant of frontotemporal degeneration and primary progressive aphasia

Authors: *E. MORAN, R. WILLIAMS, S. ASH, K. RASCOVSKY, M. GROSSMAN
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Abstract: Patients with primary progressive aphasia (PPA) demonstrate language production difficulties in spoken communication, including errors of grammatical structure as well as speech-sound errors. Patients with behavioral variant frontotemporal degeneration (bvFTD) also have speech production deficits. However, studies of written production in PPA and bvFTD have been rare. We analyzed production of a written sentence about the weather in 65 patients, including nonfluent/agrammatic PPA (naPPA, n=8), logopenic variant PPA (lvPPA, n=16), semantic variant PPA (svPPA, n=6) and bvFTD (n=35). Patient groups were matched for MMSE and education ($p>0.05$). Two independent raters, blind to patient diagnosis, analyzed written sentences for grammatical errors. We also analyzed other writing errors, including: Real word errors, subdivided into phonologically-related and semantically-related substitution errors; and Non-word errors, subdivided into phonologically-plausible and phonologically-implausible substitution errors. Groups were matched for the number of words they attempted to write ($p=0.432$). A chi-square test showed a significant group difference for grammatical errors, [$X2(4)=10.59$, $p=0.032$]. Relative to one control subject (6.3%) who made a grammatical error, 5 naPPA patients (62.5%), and 4 svPPA patients (66.7%), 8 lvPPA patients (50%), and 12 bvFTD patients (34.3%) produced grammatical errors in written sentences. We also observed a trend for producing other writing errors [$X2(4)=9.16$, $p=0.057$]. Although no control subjects committed other writing errors, 8 bvFTD patients (22.9%), 2 svPPA patients (33.3%), 2 naPPA patients (25%), and 6 lvPPA patients (37.5%) committed other writing errors. The majority of other writing errors were phonologically plausible non-words (46.9%), followed by phonologically implausible non-words (25%). Errors in written expression are common among patients with bvFTD, svPPA, naPPA, and lvPPA. The presence of grammatical and other writing errors occurred despite the presence of a written record and the opportunity to correct production. Grammatical errors thus cannot be attributed entirely to a short-term memory deficit, and letter substitutions cannot be easily attributed to motor speech abnormalities.

Disclosures: **E. Moran:** None. **R. Williams:** None. **S. Ash:** None. **K. Rascovsky:** None. **M. Grossman:** A. Employment/Salary (full or part-time):; University of Pennsylvania full time employee, NIH support.

Poster

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Topic: F.01. Human Cognition and Behavior

Title: Self-monitoring after stroke: A case study

Authors: *S. MEEKINGS[1], S. EVANS[2], F. GERANMAYEH[3], R. WISE[3], S. K. SCOTT[2]
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Abstract: Many aphasic patients show an impaired ability to monitor and repair errors in their own speech (Wepman, 1958). Puzzlingly, this deficit occurs even in patients with relatively intact comprehension abilities (Schlenck et al, 1987) and seems to apply only to on-line monitoring, since patients are able to detect errors in a recording of their own voice (Maher, Rothi & Heilman, 1994). In a unique variant of this problem, we report the case of a patient experiencing difficulties hearing his own voice following stroke. This patient, a 48-year-old man, presented with a frontal lesion caused by a left middle cerebral artery infarct. His language abilities were assessed using the Comprehensive Aphasia Test (Howard, Swinburn & Porter, 2004). He displayed difficulties with fluent and syntactically correct speech production, contrasted with relatively intact comprehension abilities. Although there was no evidence of hearing loss, the patient reported experiencing his own voice as either inaudible or occurring at a delay of up to half a second. We attempted to determine whether this was a general sound or speech perception deficit by administering a task that required him to hear and make fine temporal distinctions between sounds. In this task, we asked him to determine whether two sounds played in succession (with the delay between them adaptively determined by a staircase procedure) occurred at the same time or not. He was able to accurately complete the task with nonspeech sounds, another person's voice, and a recording of his own voice, confirming that his perception of externally generated sounds was in the normal range. When given delayed auditory feedback of a live speaker, he described the mismatch between seeing the person speak and hearing their voice as similar to his experience of his own voice. He reported an inability to perform tasks normally dependent on self-monitoring, such as singing and mimicry. In particular, his performance on a task involving speaking over noise is discussed and compared with Oomen, Postma & Kolk's (2001) finding that patients with Broca's aphasia find noise masking less disruptive than healthy controls.

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Poster

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Topic: F.01. Human Cognition and Behavior

Support: NIDCD HD 331133-14

Title: Affective prosody in adults with william syndrome

Authors: ***N. WOO-VONHOOGENSTYN**[1], P. LAI[1,2,3], J. REILLY[2], U. BELLUGI[1]
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Abstract: Williams Syndrome (WS) is a rare neurodevelopmental disorder in which individuals exhibit a predisposition for social interaction, reflecting an unusual hyper-social phenotype. Past research has shown how those with WS use language in the service of their social drive. Specifically, their use of linguistic social evaluative devices e.g. intensifiers, character speech, far exceeds that of typical developing (TD) individuals (Reilly et al., 2004). Here we explore how affective prosody might also be recruited in the WS appetitive drive for sociability. In children with WS, Lando (2006) reported differences between F0 mean and an increased variation in pitch in children with WS compared to TD children. The goal of this study is to examine the use of affective prosody in adults with WS in response to emotional scenes. Narratives from the wordless picture book, Frog, where are you? (Mayer, 1969), were collected from 17 individuals with WS and 17 TD individuals. Language from four frames conveying distinct emotions was examined: Surprise, Anger, Distress, and Happy. PRAAT, a linguistic program that parses out the various aspects of prosody was used to analyze fundamental frequency (F0) mean, (F0) standard deviations, and (F0) range, along with two speech production variables; words per minute (WPS) and story frame length (SFL). Frame one was appointed as a neutral frame to establish a baseline. There were no differences in WPS and SFL between groups. There were no differences in overall prosodic measures between groups. However, A paired T-test was conducted to evaluate within group differences between emotions. The WS group had significant differences between surprise (M = 146.9) and distress (M = 168.32) p = .005, and surprise and happy (M = 171.33) p = .009 but the TD group did not show

any significant differences between emotions. This study examined whether adults with WS elicit a broader range of affective prosodic patterns, in comparison to TD's, while narrating emotional frames. These results suggest that the WS group was more emotionally engaged in the story. In contrast the TD group exhibited similar emotional responses across all frames, suggesting more cognitive distancing in their storytelling. This study can add to the social and linguistic profiles of adults with WS. Differences in affective prosody in adults with WS may reflect underlying emotional mechanisms that may contribute to their unique social qualities. Further research could examine affective prosody using a different median to elicit an emotional narrative (e.g., from a biographical interview or elicited emotional narratives from an auditory stimuli).

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Poster

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Wyncote Foundation

Title: More than Speech? Tone discrimination deficits correlate to atrophy in the logopenic variant of primary progressive aphasia

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Abstract: The logopenic variant of primary progressive aphasia (lvPPA) is characterized by progressive impairments in single word retrieval and repetition. One hypothesis attributes this to degradation of the phonological short-term memory network (Gorno-Tempini et al 2008). Consistent with this view, structural imaging in lvPPA has shown gray matter (GM) atrophy predominantly in left hemisphere posterior peri-Sylvian regions (Gorno-Tempini et al 2008), an area implicated in short-term memory. Similar regions have been previously implicated in non speech tasks involving auditory-verbal short-term memory (Hickok, 2009). Auditory discrimination tasks rely on both auditory-verbal short-term memory and auditory perceptual processes to discriminate between two sounds. This implicates several cortical areas including auditory association cortex and the more posterior peri-Sylvian regions identified in auditory-verbal short-term memory tasks. Here we examine the contribution of GM atrophy in lvPPA to performance on an auditory discrimination task using pure tones to examine whether deficits are specific to language and phonological processing. We recruited lvPPA patients (N=13) who were diagnosed using published criteria (Gorno-Tempini et al, 2011), explicitly, this included verbal short-term memory deficits. Healthy senior controls (N=12) were comparable for demographic variables. Participants heard pairs of pure tones and made same-different judgments. We performed volumetric MRI analyses in lvPPA relative to seniors, and regression analyses to relate lvPPA performance on the discrimination task to GM atrophy. Patients were significantly impaired in their judgments relative to controls. lvPPA had left lateralized GM atrophy in posterior peri-Sylvian, temporal and parietal regions. Regressions related deficits on the discrimination task to GM atrophy in a posterior portion of the superior temporal gyrus as well as regions in the inferior parietal lobe previously associated with pitch discrimination (Zarate and Zatorre, 2008). These data suggest the atrophy patterns observed in lvPPA patients impact a large-scale neural network involving broad posterior peri-Sylvian cortical regions. Impairments are observed in the auditory-verbal short-term memory system, including pure tone discrimination deficits, suggesting more than phonological processing is affected by their short-term memory deficits. These findings suggest a neuroanatomic basis for auditory-verbal short-term memory processing in patients with a focal neurodegenerative condition.

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Poster

552. Language: Neuropsychological Approaches

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Topic: F.01. Human Cognition and Behavior

Title: Language neurodevelopment in children aged 6 to 8 months old: Its relation to gender, mother's age and educational level

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Abstract: Introduction Neurodevelopment is a brain maturation process that involves genetic and epigenetic factors. Language acquisition depends on brain maturation as well as environmental stimulation. Early language development in children includes characteristically crying and babbling with vowel and consonant elements (6-8 month). The mother-child relationship at this stage plays a crucial role for language acquisition. The voice exchange establishes communication networks that increasingly become complex. Bayley scales are reliable instruments that accurately measure child cognitive and language development. Objective The aim of this work was to compare the performance of language development in children aged from 6 to 8 months old, considering the baby's gender, as well as the mother's age and schooling. Methodology Participants were 55 children from 6 to 8 months old based on Guadalajara, Jalisco, Mexico, assessed with Bayley Scales of Infant Development III. This scale evaluates receptive and expressive language components, which are important in diagnosing critical delays. The expressive communication subtest includes items that asses the young infant's ability to vocalize. Receptive communication subtest focus on the child's ability to comprehend and respond appropriately to word and requests. Results We complete a sample comprised of 55 children including 28 girls (50.9%) and 27 boys (49.1%). The mean age was 7.29 (± 0.62) months. The average mother's age was 22.57 (± 5.78) years old with a mean schooling of 4.27 (± 1.59) years. Bayleys assessment revealed that Girls (98.64) scored slightly higher than boys (94.22). Children with mothers aged 14-19 years and with 5-9 years of schooling obtained better performance (99.44 and 97.22 respectively). Intergroup and intragroup analysis revealed no significant differences for age and schooling.

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Poster

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Title: Constructing the white matter networks of semantic processing with healthy and patient populations

Authors: *Y. FANG[1,2], S. ZHONG[2], G. GONG[2], L. SONG[3], Z. HAN[2], Y. BI[2]
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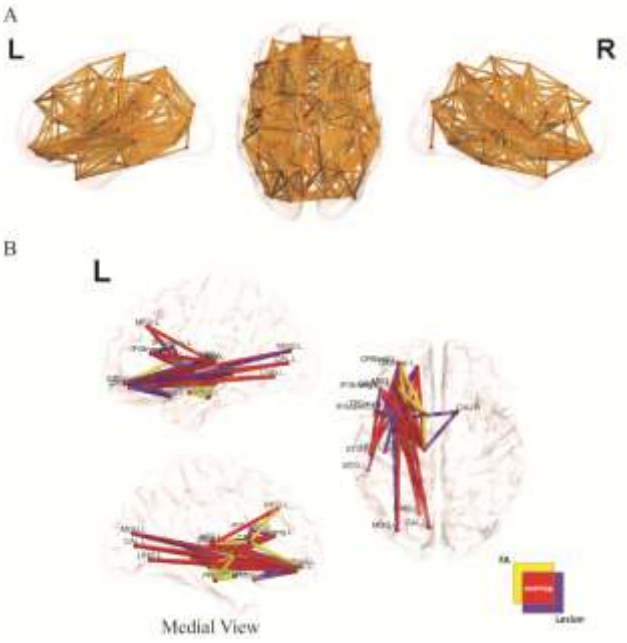
Abstract: Semantic memory stores the general knowledge of objects, people, and facts and is central to a wide range of cognitive processes. Decades of research have investigated the cortical regions (Binder et al, 2009) and major white matter (WM) tracts that support semantic memory (Han et al., 2013). An important gap between these two lines of research is that their correspondence is elusive given that the major white matter tracts identified connected numerous cortical regions. In this study, we construct a first whole-brain white matter "network" for semantic processing by directly tracking WM connections among all cortical regions (as nodes) in healthy populations and testing the effects of observed WM connections (edges) in semantic processing in patients. In a first step, we build a whole brain white matter connectivity network by conducting tractography using diffusion imaging data by 48 healthy participants, rendering a network contained 688 white matter "edges" across 90 cortical and subcortical "nodes" (Fig 1a). We then tested the relationship between the integrity of the obtained WM edges and semantic performance across 80 brain damaged patients. Semantic performance was obtained by performing principal component analyses in 8 semantic and nonsemantic tasks. Edge integrity was measured both by lesion percentage and mean fractional anisotropy. For semantic processing, we obtained 42 WM edges for lesion percentage analysis and 37 ones for mean fractional anisotropy analysis, with the cortical nodes they connect elucidated. The majority of the semantic edges landed on left inferior fronto-occipital fasciculus, left anterior thalamic radiation, and left uncinated fasciculus (Fig 1b). Using graph analyses we identified five hubs nodes with top degree (number of connections) and betweenness, including orbital part of inferior frontal gyrus, orbital part of middle frontal gyrus, insula, and thalamus and hippocampus. The results highlight the critical roles of distributed white matter connections among specific temporal and frontal regions in semantic processing.



Disclosures: **Y. Bi:** None. **S. Zhong:** None. **G. Gong:** None. **L. Song:** None. **Z. Han:** None. **Y. Fang:** None.

Poster

552. Language: Neuropsychological Approaches

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The Wyncote Foundation

Title: Impaired sentence expression in amyotrophic lateral sclerosis

Authors: *S. ASH, C. OLM, C. T. MCMILLAN, A. BOLLER, D. J. IRWIN, L. MCCLUSKY, L. ELMAN, M. GROSSMAN
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Abstract: Background: Recent studies have shown that patients with amyotrophic lateral sclerosis (ALS) may have a cognitive impairment in addition to a progressive motor disorder. There is evidence that language deficits may be prevalent in ALS, but the precise nature of such deficits has not been established. Most studies of language functioning in ALS have examined the production or comprehension of single words; there have been few studies of connected, spontaneous speech in these patients. The objective of this study was to assess sentence-level language production impairments in ALS. Methods: We examined features of spontaneous speech production in 26 patients with ALS and 19 healthy seniors. The subjects were asked to narrate the story from a wordless children's picture book, Frog, Where Are You?, by Mercer Mayer. The narrations were recorded digitally, transcribed, and analyzed for speech output, fluency, lexical access, and grammaticality. Regression analyses related grammaticality to gray matter (GM) atrophy and white matter (WM) reduced fractional anisotropy (FA). Results: Patients with ALS were impaired relative to control subjects on measures of speech production, including quantity of speech produced, speech rate, speech sound errors, and grammaticality. The measures of fluency and articulation were related to the extent of motor impairment associated with the disease. Impaired grammaticality was observed in the entire cohort of patients, in the subset of patients who did not exhibit significant executive deficits (n=22), and in the subset of patients who did not have a motor impairment causing dysarthria (n=20). Grammaticality correlated with overall cognitive status but not with the extent of a motor disorder. Regressions related grammatical expression difficulty to GM atrophy in left inferior frontal and anterior temporal regions, areas associated with grammatical expression. Regressions also related grammatical impairment to reduced FA in dorsal and ventral WM tracts that are thought to play a role in sentence processing. Conclusions: Patients with ALS exhibit deficits in sentence-level language expression. While difficulty related to the physical requirements of speech production is associated with their motor disorder, a central deficit in grammatical expression appears to a component of the cognitive disorder found in ALS.

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Poster

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Topic: F.01. Human Cognition and Behavior

Support: Doris Duke Charitable Foundation Grant 2012062

NCATS KL2TR000102

Title: Cerebellar tDCS alters articulation and verbal fluency: Location and polarity effects

Authors: *C. J. STOODLEY[1], M. K. SWEARS[1], A. DESKO[2], P. E. TURKELTAUB[2,3]
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Abstract: The cerebellum is thought to be involved in both motor and cognitive aspects of language. Cerebellar lesions can lead to dysarthria or to difficulties on naming and fluency measures, depending on the location of damage. This topography is supported by neuroimaging studies which show that articulation engages the articulatory muscle representation in anterior cerebellar lobule V extending into medial lobule VI, whereas verb generation and fluency tasks engage lateral parts of right lobule VII, which shows anatomical and functional connectivity with left frontal and parietal networks. We examined this topography for language function using anodal and cathodal transcranial direct current stimulation (tDCS) applied to two different cerebellar locations: the "motor" position (3 cm lateral to the inion) over the right anterior lobe, and the "cognitive" position (4 cm lateral to the inion and 1 cm down) over the right posterolateral cerebellum (lobule VII). The reference electrode was on the right deltoid. Healthy participants (39 females, 22 males; mean age 23.2 ± 5.6 years) completed articulation and fluency measures pre- and post- 20min of 2mA tDCS of motor (n=16 anodal, 7 cathodal), cognitive (n=15 anodal, 7 cathodal), or sham tDCS (n=16). After motor tDCS, participants in both the anodal and cathodal groups articulated fewer syllables of "ba" in a 30s period than the cognitive and sham groups. Anodal cerebellar tDCS did not selectively affect phonemic fluency, as all groups showed modest improvement/practice effects post-tDCS. However, cathodal tDCS to the cognitive position improved phonemic fluency to a greater degree than in either the motor or sham groups. During semantic fluency, anodal and cathodal tDCS to the motor position improved performance, but performance was negatively affected by both anodal and cathodal tDCS to the cognitive position. These preliminary findings indicate that, depending on electrode position and current polarity, cerebellar tDCS can differentially affect performance on articulation and fluency tasks. These findings are consistent with the concept that the cerebellum modulates both motoric and cognitive aspects of language.

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Topic: A.10. Adolescent Development

Support: R01MH085953

T32NS048004

Title: Relationship between gene expression profiles, structural neuroimaging, and clinical phenotypes in 22q11.2 microdeletion syndrome

Authors: *M. JALBRZIKOWSKI, R. JONAS, A. PATEL, L. KUSHAN, F. GAO, G. COPPOLA, C. E. BEARDEN
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Abstract: Introduction: 22q11.2 deletion syndrome (22q11DS) is a recurrent copy number variant associated with elevated rates of neuropsychiatric symptoms, particularly psychosis (≈30%), and brain structural abnormalities. Here we sought to examine how variability in gene expression profiles of 22q11DS patients may relate to psychotic symptomatology and brain morphology. Methods: Fifty-six 22q11DS patients were enrolled. Clinical symptomatology was assessed with the Structured Interview for Prodromal Symptoms (SIPS). We acquired high-resolution T1-weighted structural scans (sMRI), which were processed through a Freesurfer pipeline with manual editing. RNA was extracted from whole blood using the PAXgene extraction kit (Qiagen). Whole-genome transcriptional profiling was performed using Illumina Human HT-12 microarrays. We then conducted Weighted Gene Coexpression Network Analysis (WGCNA), a systems biology approach used to identify networks of co-expressed genes in relation to phenotypic data. To test for significance of over-representation of brain-expressed genes within identified modules, we ran hypergeometric probability tests (phyper function in R). Results: WGCNA identified 3 gene expression modules (Green, Purple, Yellow) significantly associated with psychotic symptom expression, as well as surface area measures, in 22q11DS patients. None of the genes within these modules fell within the typically 22q11.2 deleted region. Hypergeometric tests revealed significant enrichment of brain-expressed genes in all three modules. Genes co-expressed in these modules were predominantly down-regulated in 22q11DS participants with more severe psychotic symptoms (Green: p=.01, Purple: p=.001, Yellow: p=.001). Surface area in the left inferior temporal, rostral middle frontal, fusiform and right superior temporal regions was significantly associated with all 3 modules (p-value range: .05-

.006). Reduced surface area in these cortical regions was associated with down-regulated gene expression in these modules. Conclusion: Peripheral changes in gene expression are significantly related to psychotic symptomatology and underlying brain structure in 22q11DS patients. Brain regions associated with these gene expression profiles have been previously associated with idiopathic schizophrenia, providing further support for the use of peripheral tissue in the study of Mendelian forms of complex brain diseases.

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Poster

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Topic: F.01. Human Cognition and Behavior

Support: National Ataxia Foundation

Title: Prospective evaluation of the sensitivity and specificity of cognitive tasks for diagnosis of the cerebellar cognitive affective syndrome

Authors: ***F. HOCHE**[1], L. C. HORTON[1], J. A. HARDING[1], S. MANCUSO[2], R. RANGAMANNAR[2], M. G. VANGEL[3], J. C. SHERMAN[2], J. D. SCHMAHMANN[1]
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Abstract: Background: The constellation of deficits in executive function, spatial cognition, linguistic processing and affect regulation following cerebellar injury is the cerebellar cognitive affective syndrome (CCAS; Schmahmann and Sherman, 1998). Clinicians may miss the cardinal signs of CCAS on current brief tests of mental function that were not designed to detect these impairments. Tests are also needed to differentiate cerebellar from extracerebellar damage when neuropathology is not confined to the cerebellum, as in the spinocerebellar ataxias. We aim to delineate a brief set of cognitive tests to detect the CCAS in patients with cerebellar lesions, and to determine which deficits reflect the cerebellar versus noncerebellar component of cognitive impairment in patients whose underlying neuropathology includes, but is not confined to, the cerebellum. Methods: A battery of neuropsychological tests was administered to all patients with

cerebellar disease (n=71, AC); divided into n= 35 patients with isolated cerebellar pathology (IC) and n= 36 patients with complex cerebrocerebellar pathology (CC) and 59 age, gender and education matched healthy controls. Results: Significant impairments were found in the previously identified domains of the CCAS, both on standard and bed-side tests of executive function, visuospatial performance, working memory, sustained attention and language. For example, AC patients were impaired relative to controls on the Trails Test, a standard neuropsychological test of executive function ($p<0.0001$; Binomial tests with parameter 0.1), and error rate on the Go/No-Go Test, a short bedside test of mental flexibility was greater in AC patients than controls ($p=0.04$, Poisson regression). Selected bedside and neuropsychological tests differentiated IC patients from CC patients, including the Judgement of Line Orientation Test that was more impaired in CC patients than IC patients (p=0.05, Wilcoxon test). Discussion: These data provide insights into distinguishing the cognitive consequences of cerebellar versus extracerebellar pathology, and they will be used to construct a short bedside test sensitive to the CCAS.

Disclosures: F. Hoche: None. **L.C. Horton:** None. **J.A. Harding:** None. **S. Mancuso:** None. **R. Rangamannar:** None. **M.G. Vangel:** None. **J.C. Sherman:** None. **J.D. Schmahmann:** None.

Poster

553. Human Emotion: Behavioral and Neural Mechanisms

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 553.01/SS45

Topic: F.01. Human Cognition and Behavior

Support: NIMH R01 MH071589

Title: Competitive interactions between emotion and reward in the amygdala and striatum

Authors: *S. PADMALA, W. ZHAN, L. PESSOA
Univ. of Maryland, College Park, MD

Abstract: Performance improves in a diverse set of perceptual and cognitive tasks when performance-based monetary rewards are possible. At the same time, task-irrelevant negative stimuli often interfere with performance. Because investigations of how reward and negative stimuli impact brain and behavior have been largely independent of each other, our knowledge about their interactions remains rudimentary. In the current functional MRI study, we

investigated this question by manipulating both reward and emotion in a factorial design. Participants (N=32) performed a variant of the monetary incentive delay task during which a simple discrimination task was preceded by a face stimuli that served as a cue. Facial stimuli of one particular gender (e.g., male) indicated that there was a chance of winning extra monetary reward based on performance, whereas faces of the other gender (female) indicated no chance of reward. Further, half of the stimuli for each gender had neutral or fearful expressions that were irrelevant to the task. Behavioral RT data revealed a significant Motivation (reward, no-reward) x Emotion (fearful, neutral) interaction, such that behavioral improvements during reward (vs. no-reward) trials were reduced with fearful face cues compared to neutral ones. Paralleling the behavioral data, brain imaging results revealed interaction patterns in the left ventral caudate, posterior cingulate cortex, and portions of bilateral parietal cortex. In all these regions, the interaction pattern revealed that reward-related responses were decreased during fearful compared to neutral face cues. We also observed a significant interaction in the left amygdala but with a different pattern; specifically, differential fearful vs. neutral responses to faces were reduced for reward-signaling compared to no-reward-signaling gender cues. Together, our findings revealed distinct competitive interactions in key sub-cortical regions: reward-related responses in the striatum were opposed by negative emotion, whereas emotion-related responses in the amygdala were opposed by reward. Our results highlight how appetitive-aversive interactions contribute to sculpting brain responses when behavior must integrate competing signals.

Disclosures: **S. Padmala:** None. **L. Pessoa:** None. **W. Zhan:** None.

Poster

553. Human Emotion: Behavioral and Neural Mechanisms

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Topic: F.01. Human Cognition and Behavior

Support: National Natural Science Foundation of China:31000501

Shanghai Pujiang Program under Grant :11PJC044

Title: The cognitive and neural basis of myopic decisions induced by negative emotional priming

Authors: *X. LI, S. GUAN, J. LUAN, L. CHENG
East China Normal University,, Shanghai, China

Abstract: Previous studies about the influence of emotion on inter-temporal choice have been unconsistent due to the approaches to inducing emotions or the materials that induced emotions. In the present study, we first induced temporal emotional changes by presenting affective pictures in a trial-to-trial paradigm. Then we investigated the influence of emotional changes on performance of intertemporal choice task. We found that temporal negative priming resulted in much higher percentages of trials during which smaller-but-sooner rewards were chosen compared to temporal positive priming and neutral priming. In order to explore the cognitive basis of such an effect, we investigated how the emotional contexts affected time perceptions and response inhibition. When subjects conducted the time reproduction tasks, we found response times in negative priming conditions were significantly shorter than in positive and neutral conditions, which indicated that negative priming made humans overestimate the subjective experience of time. Meanwhile, temporal changes of emotion did not alter the performances of Go/NoGo task including commission errors and omissions. Therefore, we proposed that the myopic decisions induced by emotions are assosicated with altered time perceptions. Finally, we tried to explore the neural mechanism for such myopic decisions, we recorded the brain responses by employing an EEG/ERP technique when subjects performed intertemporal choices in different emotional contexts. We found that negative priming generated larger later positive components (LPCs, a time window from) around 600ms after the affective picture to the responses to alternative choices at the electrodes in posterior parietal area compared to neutral and positive priming. No differential LPCs were found in frontal areas. We proposed that the patrietal lobe might be involved in the modulation of emotion on the intertemporaldecisions.

Disclosures: X. Li: None. **S. Guan:** None. **J. Luan:** None. **L. Cheng:** None.

Poster

553. Human Emotion: Behavioral and Neural Mechanisms

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 553.03/SS47

Topic: F.01. Human Cognition and Behavior

Title: Perceptual and physiological differences between genuine and posed emotional vocalizations

Authors: *S. H. CHEN[1], S. EVANS[1], C. F. LIMA[1,2], N. DEMNITZ[1], D. BOEBINGER[1], S. SCOTT[1]
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Abstract: The ability to understand the thoughts and emotions of others is an important human behaviour. One aspect of this ability may be the skill to detect whether emotional expressions reflect a genuine emotional state or not. Indeed, a failure to distinguish genuine expressions from posed ones (i.e., non-genuine) may put one at a disadvantage in social interactions. In this study we recorded six speakers (three male) producing genuinely felt and posed positive (laughter) and negative expressions (crying), and asked a separate group of listeners to rate the recordings on a number of different scales. The emotional sounds were rated on 7-point Likert scales for their perceived authenticity, the frequency with which participants estimated they could encounter the sounds in everyday life, and the degree to which they perceived the speakers were in control of the expression. Eleven adult participants (nine female) rated 50 genuine and 50 posed laughs and 50 genuine and 50 posed cries. The ratings data were submitted to 2x2 repeated measures ANOVAs with factors: expression (positive/negative) and authenticity (genuine/posed). Authentic positive expressions were rated to be higher in authenticity than negative ones ($F(1,49)=102.305$, $MSE=0.062$, $p<0.001$), and genuine emotional vocalizations were rated as more authentic than posed ones ($F(1,49)=37.6$, $MSE=2.761$, $p<0.001$). However, laugh tokens were considered to be more genuine than crying tokens ($F(1,49)=6916.073$, $MSE=0.009$, $p<0.001$). Frequency ratings results showed that genuine emotional expressions were perceived to occur more frequently in everyday life than posed expressions ($F(1,49)=4.453$, $MSE=0.425$, $p<0.05$), and laughs were heard more frequently than cries ($F(1,49)=486.701$, $MSE=0.418$, $p<0.001$). Results of ratings of degree of control showed that the difference of control level between genuine and posed laughs were larger than that of cries ($F(1,49)=49.092$, $MSE=0.352$, $p<0.001$), also, genuine emotional tokens were perceived to be under less control than posed ones ($F(1,49)=166.464$, $MSE=0.592$, $p<0.001$). Our results suggest that listeners are indeed able to differentiate genuine from posed positive and negative emotional expressions. Cries were rated to be less genuine than laughs, which may be due to the fact that people are exposed to crying less frequently than laughter in everyday life. On-going work examining physiological responses with GSR, pupillometry and heart rate measurement to the described authenticity distinction will be discussed.

Disclosures: **S.H. Chen:** None. **S. Evans:** None. **C.F. Lima:** None. **N. Demnitz:** None. **D. Boebinger:** None. **S. Scott:** None.

Poster

553. Human Emotion: Behavioral and Neural Mechanisms

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Program#/Poster#: 553.04/SS48

Topic: F.01. Human Cognition and Behavior

Title: Development of an eeg-based neural decoding system as an affective evaluation tool

Authors: *T. FUJIMURA, R. P. HASEGAWA
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Abstract: An event-related potential (ERP) such as P300 has been traditionally detected, as a marker of cognitive function, by averaging a couple dozen trials in which targets (a stimulus to be selected in mind) are presented in a sequence of stimuli (mostly non-targets). Decoding, however, should be accomplished with speed and precision as a practical neuromarketing system, which is able to provide feedback for a user. To this end, we used linear discriminant analysis (LDA) that is one of pattern classification methods to discriminate EEG in response to targets from that to non-targets. Discriminant score for each stimulus can be calculated in real time and a stimulus with the highest discriminant score is interpreted as a target. In this study, we developed a compact wireless EEG recording system with a software to make a real-time analysis of EEG data using the LDA. We examined a possibility of our neural decoding system as an affective evaluation tool. We hypothesized that affective targets captured more attention and hence elicited greater P300 than non-affective targets. We used a set of 8 emotional faces representing a variety of affective values. We also used a set of 8 simple figures as control stimuli. EEGs were measured while participants viewed a sequence of stimuli. There were 2 sessions for emotional faces as well as simple figures. Each session was consisted of 8 games. In each game, one of the stimuli was consistently the target; each stimulus including the target was presented 8 times, resulting in 64 trials. Participants were asked to make a silent counting for the target. Each stimulus was randomly presented for 750 ms, separated by a 250 ms-blank screen in a sequence. The results showed that LDA was superior to ERP averaging in terms of discrimination between targets and non-targets from EEG. The stimuli with significant affective values were more discriminated against than the stimuli without significant affective values when they were targets. This system using LDA might be practical neural decoding system for an affective evaluation tool.

Disclosures: T. Fujimura: None. **R.P. Hasegawa:** None.

Poster

553. Human Emotion: Behavioral and Neural Mechanisms

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Topic: F.01. Human Cognition and Behavior

Title: Don't crack a smile: An fMRI study on counterproductive effect of smile

Authors: *S. PARK[1], N.-Y. SHIN[2], S.-K. LEE[2], S. HAN[1]
[1]dept. of psychology, [2]Col. of Med., Yonsei Univ., Seoul, Korea, Republic of

Abstract: Facial feedback hypothesis suggests that a smile-like facial expression can increase positive feelings. For example, people reported higher amusement ratings for a cartoon when they held pens with their teeth than on their lips. However, several behavioral studies showed that the positive facial feedback effect was dependent on given circumstances. Our study aims to find neural underpinning of the counterproductive effect of smile and individual differences using fMRI. Before scanning, 15 participants practiced to make a smile with a wooden chopstick similar to the previous cartoon study until they could make the same facial expressions regardless of the chopstick for the subsequent fMRI sessions. In emotion-inducing period, participants were shown an emotional image (negative vs. positive vs. neutral). After the image given, one of two cue words (smile vs. neutral) which were used as indicators for participants to generate facial expression was presented above the image. Four emotional and neutral words were presented in four different colors in a subsequent emotional Stroop task. Smile effect difference index (SEDI) reflecting participants' attentional biases to negative words were calculated with reaction times recorded in the emotional Stroop task. Next, we explored the counterproductive smile effect by contrasting (negative smile SEDI - negative neutral SEDI) to (positive smile SEDI - positive neutral SEDI), calling it Smile Effect Scale (SES). SES was positively correlated with participants' Social Interaction Anxiety Scale (SIAS) scores, shown that participants who had relatively high scores in SIAS were attentionally more biased toward negative words when they had to smile in negative feelings. In analysis of the fMRI data, a whole-brain general linear model (GLM) with the contrast used to calculate SES applied in the emotional Stroop task identified activations in bilateral insula, anterior cingulate cortex and right amygdala. Simple regression analysis was also performed to explore SES-related brain regions modulated by SIAS. Right inferior frontal gyrus and bilateral anterior cingulate cortex were found in the emotional Stroop task. In the facial expression generation phase given prior to the emotional Stroop task, increased activations of bilateral inferior frontal gyrus and left insula was found. Our results show that smile-like facial feedback could be ineffective and even backfires for people in negative feelings (especially for individuals with high level of social anxieties) and it is associated with an emotional conflict related brain regions such as anterior cingulated cortex, inferior frontal gyrus and insula.

Disclosures: **S. Park:** None. **N. Shin:** None. **S. Lee:** None. **S. Han:** None.

Poster

553. Human Emotion: Behavioral and Neural Mechanisms

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Topic: F.01. Human Cognition and Behavior

Title: The Multidimensional Auditory Affective Ratings Inventory (MAARI) and Computerized Emotion Recognition Toolbox (CERT): measuring facial responses to standardized, non-verbal, human vocalizations of emotion

Authors: ***M. D. MULLANE**[1], S. OKHOVAT[2], M. DIFLEY[2], J. MARTIN QUIROGA[3], S. ZIOGAS[2], M. BARTLETT[4], G. LITTLEWORT[4], A. A. CHIBA[2]
[1]Cognitive Sci. & Psychology, [2]Cognitive Sci., [3]Psychology, [4]Inst. for Neural Computation, UCSD, La Jolla, CA

Abstract: Emotional content in the world guides behavior, in part by influencing perception, memory, decision-making, and learning. There exists a voluminous literature detailing the impact of visual emotional information on various cognitive processes, making use of standard facial emotional expression coding (FACS) and emotional picture inventories (IAPS). Yet, there is a paucity of work elaborating on the influence of auditory perception of emotional information on cognitive processes. In order to examine the nature of physiological responses to human vocalizations of emotion, the Multidimensional Auditory Affective Ratings Inventory (MAARI) is being developed. To date, MAARI is an inventory of 650 spontaneous, non-verbal vocalizations of emotion, all of which hold ratings on dimensions of valence, arousal and authenticity. In order to understand whether the perception of these emotional sounds elicits emotional expressions that concur with the listener's subjective sound ratings, MAARI was presented to subjects along with video clips of facial expressions of emotion. Participants' facial expressions were recorded and analyzed using the Computerized Emotion Recognition Toolbox (CERT), which identifies facial action units (FACS), associated with prototypical facial expressions of emotion. Facial expressions of participants who exhibited a threshold level of reactivity to emotive facial expressions were then analyzed for FACS responses during their perception of emotive vocalizations. The result is a set of physiological measures associated with specific MAARI vocalizations. These measures, in conjunction with existing subjective ratings of valence, arousal and authenticity, provide a complete, standardized inventory of human

emotional vocalizations, making MAARI suitable for use as an experimental instrument to examine influences of auditory perception of non-verbal human expressions of emotion on the neural processes underlying aspects of cognition. MAARI might also be useful for gaining a further understanding of affective insufficiencies in various neurological disorders.

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Poster

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Topic: F.01. Human Cognition and Behavior

Support: NIA Grant RO1AG025340

NIA Grant K02AG032309

Title: Emotional arousal amplifies biased competition in visual processing

Authors: ***T.-H. LEE**[1], S. G. GREENING[1], N. SOSA[2], M. MATHER[3]
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Abstract: The arousal-biased competition (ABC) model predicts that arousal biases competition in favor of prioritized stimuli and against non-prioritized stimuli (Mather & Sutherland, 2011). Thus, the model predicts that arousal can lead to simultaneous enhanced processing of prioritized stimuli (by either top-down goal or bottom-up saliency) and impaired processing of non-prioritized stimuli. The goal of current fMRI study was twofold. First, it aimed to conceptually replicate the effect of competitive priority in emotional processing from recent neural evidence (Lee, Sakaki, Cheng, Velasco, & Mather, 2014). The second goal was to examine the role of the locus coeruleus (LC) in the ABC effects given it is a fundamental arousal modulator in the brain. To accomplish this goal, we used melanin-weighted MRI (e.g., Shibata et al., 2006) to localize individual's LC. Also cardiac and respiration signals were recorded to enhance the fidelity of the functional signals in the brainstem. Participants' arousal levels were manipulated on a trial-by-trial basis using fear-conditioned stimuli. In the main spatial detection task, we presented object-place image pairs as target stimuli, one of which was always given a brief luminance increase so as to render it visually salient. Participants were asked to identify the location of the salient target

location. Preliminary analyses revealed arousal-by-saliency interactions in lateral occipital complex (LOC) and parahippocampal place area (PPA); when the object image was salient, responses in LOC (i.e., responses to the prioritized object image) were enhanced on arousing compared with non-arousing trials. Simultaneously responses in PPA (i.e., responses to the non-prioritized place image) were reduced with arousal. In contrast, when the place image was salient, arousal enhanced responses in PPA whereas and reduced responses in LOC. That is, arousal biased competition in favor of prioritized information and against non-prioritized information, influencing subsequent perceptual process irrespective of stimulus type. Additional analyses will examine whether the LC plays a critical role in these arousal effects in visual processing. The current results support the ABC model by demonstrating that arousal enhanced visual processing for prioritized stimuli (i.e., increased brain response according to the prioritized stimulus categories) but simultaneously impaired processing of non-prioritized stimuli (i.e., reduced brain response to non-prioritized stimulus) compared with the non-arousing condition. The role of LC will be discussed in further detail.

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Poster

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Support: NIA R01AG025340

NIA K02AG032309

Title: Individual differences in anxiety influence the integration of early sensory signals corresponding to aversive unconditioned stimuli

Authors: *S. G. GREENING, T.-H. LEE, M. MATHER
USC, Los Angeles, CA

Abstract: In anxiety disorders, external sensory cues can trigger responses ranging from mild fear to severe panic, the strength of which can depend on attention-related biases. Research has demonstrated that accurate fear-related trace conditioning requires attention to, and awareness of, the association between the conditioned stimuli (CS+/-; i.e., a tone) and the unconditioned

stimulus (US; a shock). Intriguingly, past studies have reported increased activity in the primary sensory cortices corresponding to the US on non-reinforced CS+ relative to CS- trials. However, the functional significance of this activity has not been thoroughly examined. We tested the prediction that individual differences in trait anxiety would correlate with primary somatosensory cortex (S1) activity on non-reinforced (i.e., no shock) CS+ trials. Furthermore, given the importance of attention and awareness in trace conditioning, we predicted that activity in S1 would be correlated with activity in attention-related areas such as dorsolateral prefrontal cortex (dlPFC). Twenty healthy volunteers completed a differential-tone trace-conditioning task while undergoing fMRI. Participants heard and responded to a high and low pitch tone, one of which was paired with shock (CS+) on fifty percent of trials, the second was never paired with shock (CS-). On reinforced trials a shock occurred to the 4th and 5th fingers of the left hand after a trace interval of 1.2 seconds; these trials were excluded from all analyses. An S1 anatomical mask was used as an a priori region of interest. Skin conductance response (SCR) confirmed that participants conditioned to the CS+ compared to CS-. Consistent with our prediction, we found a positive correlation between individual differences in trait anxiety and activity in right, but not left, S1 during CS+ versus CS- conditions. A left S1 seed-based functional connectivity analysis demonstrated that trial-wise S1 activity was positively correlated with regions of dlPFC. Finally, group-wise activity in right S1 was positively correlated with activity in regions involved in both the representation of the unconditioned stimulus (i.e., A1), and the representation of fear-related emotional reactivity (i.e., amygdala). Our findings indicate that trait anxiety influences the degree of reactivity in primary sensory cortices responsible for the encoding of unconditioned aversive stimuli, and this activity is positively related to activity in dlPFC. These results suggest that individual differences in both anxiety and attention mediate the integration of emotion-related sensory signals into neural networks implicated in fear-conditioned responding.

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Poster

553. Human Emotion: Behavioral and Neural Mechanisms

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Topic: F.01. Human Cognition and Behavior

Support: U.S. Army Collaborative Agreement W911NF-10-2-0022 "The Cognition and Neuroergonomics Collaborative Technology Alliance"

Title: Competitive stimuli as an effective intrinsic motivator in the study of affect during human performance

Authors: *J. S. METCALFE[1], A. PASSARO[1], S. GORDON[2], K. OIE[3]
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Abstract: The dynamic constellation of psychological, physical, and physiological patterns that compose our emotional experiences, that is, our affective state, is an important influence on intra- and inter-individual differences seen during human performance in challenging, real-world contexts. However, because of the tight coupling between affective responses and task characteristics such as difficulty or complexity, it is difficult to distinguish independent versus mutually causal influences on affective state and performance. Here, we report preliminary findings that may support an hypothesis of an influence of motivation on affective responses that is independent of task performance. A sample of individuals who self-reported intrinsic motivation in competitive tasks were asked to perform a visual search and target classification task in a competitive video gaming context. An adaptive algorithm, denoted the "virtual competitor" (VC), simulated two degrees of challenge relative to each individual participant's real-time performance. This was accomplished by using a stochastic control system to manipulate the percentage of each 15-minute task condition that the participant was scoring higher than the VC. In the low-challenge condition, the participant outscored the VC approximately 80% of the time. The high-challenge condition, however, was chosen through pilot testing to be more closely matched at 50%. Because the VC was partly stochastic, participants were unaware of the relationship between their score and that of the VC. More importantly, task difficulty did not change with increased challenge, and this was verified by behavioral results that did not differ significantly as a function of condition. By contrast, the pattern of results across multiple subjective and psychophysiological measures indicated both physical and subjective responses were consistent with increased stress and, somewhat counterintuitively, positive affective valence as apparent challenge increased. We interpreted this in terms of an affective response driven by motivational processes. That is, participants' responses suggested a task experience that was more engaging and positive as competition increased, because the experimental manipulation structured putative rewards consistent with the subjects' intrinsic motivation by competition rather than on task-based performance measures.

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Poster

553. Human Emotion: Behavioral and Neural Mechanisms

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Topic: F.01. Human Cognition and Behavior

Title: Effects of focusing on negative and positive attributes of self-image on self-esteem and psycho-physiology

Authors: *M. C. NIEDZIELA[1], E. CARBONE[2], P. BOLLS[3]
[1]HCD Res., Lansdale, PA; [2]HCD Res., Flemington, NJ; [3]Univ. of Missouri, Columbia, MO

Abstract: Social cues have subtle non-conscious effects via implicit priming of trait associations. Priming paradigms are valuable tools for assessing implicit self evaluation. In this novel preliminary study, we tested the hypothesis that an implicit positive or negative association with self mediates physiology and behavior towards self imagery. Attitudinal, perceptual and physiological components of self image were assessed. Seventeen female participants between ages of 18-35 yrs were recruited. For pre-test, participants completed the Rosenberg Self-Esteem Scale. Participants were primed "positive" or "negative" using a facial feature description task in which they were instructed to choose three positive or negative facial features (control case, chose 3 most frequently used makeup items) after which they viewed an image of their face for 15 sec. We measured the priming effect on self assessment self-esteem scales (Rosenberg Self-Esteem Scale (post-test)), electrophysiology and eye tracking. Electrophysiological data - facial electromyography (emotional valence), heart rate (attention), skin conductance (galvanic skin response, arousal) - were collected. With this novel paradigm, we investigated how social cues modulate one's implicit self-evaluation through physiological and behavioral measures. Though not significant, positive priming (mean= 0.2) prevented a decrease in self-esteem seen in negative (mean= -0.83) and control (mean= -1.0) groups after viewing self imagery. Eye tracking results showed that when negatively primed, participants avoided features they did not like while positively primed participants were more attracted to and looked longer at features they liked. Psycho-physiological measures showed that negative priming resulted in greater changes in emotion (corrugator supercilii muscle group, negative emotion; zygomaticus major and orbicularis oculi muscle groups, positive emotion). Positive priming had a greater effect on arousal levels. Both forms of priming decreased attention compared to control. The data provide a quantitative demonstration of how implicit cues, targeting a person's self-concept, influence the way we react physiologically and behaviorally. Further means of examining cognitive priming effects on physiology are outlined.

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Poster

553. Human Emotion: Behavioral and Neural Mechanisms

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Title: Changes in autonomic arousal elicited by subcallosal cingulate DBS are associated with white matter connectivity to the mid-cingulate cortex

Authors: *C. INMAN[1], P. RIVA POSSE[2], K. CHOI[2], A. CROWELL[2], S. DANIELSON[1], S. GARLOW[2], H. MAYBERG[2], S. HAMANN[1]
[1]Psychology Dept., Emory Univ., Decatur, GA; [2]Dept. of Psychiatry, Emory Univ., Atlanta, GA

Abstract: Subcallosal cingulate (SCC) deep brain stimulation (DBS) has shown preliminary long-term efficacy and safety for treatment-resistant depression (TRD). Immediate changes in mood and behavior during intra-operative testing have also been observed. Spontaneous self-reported intra-operative effects include, "feeling lighter, calmer," and "more awake, more aware, more reactive;" indicative of changes in autonomic arousal. Autonomic changes have been observed within seconds of initiating acute stimulation and appear to relate to selection of the optimal white matter targets mediating long-term antidepressant effects. Optimal targets include locations in the white matter of the SCC whose structural connections reach the medial prefrontal cortex via the forceps minor pathway, subcortical regions via the uncinate fasciculus, and the mid-cingulate cortex via the cingulum bundle. Diffusion tensor imaging was acquired on 9 participants in an ongoing study of SCC DBS for TRD to aid in surgical planning and characterization of the white matter networks connected to the SCC. To assess the immediate effects of SCC DBS on mood, behavior, and autonomic reactivity, skin conductance response (SCR) and change in heart rate (HR) were measured during acute stimulation protocols performed during surgery, 1- and 3-months post-operatively. During surgery, increases in autonomic arousal correlated with stimulation to the most behaviorally effective contacts relative

to all other stimulations and sham. This finding is indicative of a highly location-specific effect on autonomic arousal. Correlation analyses show that these increases in autonomic arousal, particularly heart rate, positively correlate with the strength of white matter connectivity between the site of stimulation in the SCC and the mid-cingulate. In post-operative experiments, we found that as stimulation current amplitude was increased, both measures of autonomic arousal (SCR and HR) increased. In addition, post-operative stimulation of optimal versus non-optimal contacts increased autonomic arousal, also indicative of a highly location-specific autonomic response. These findings suggest that SCC DBS modulates activity through down-stream effects on brain regions involved in autonomic regulation that have strong white matter connectivity to the SCC. These findings are consistent with known anatomical connectivity of SCC to cingulate cortex and brainstem in humans and animals. Further, current- and location-dependent changes in autonomic arousal with SCC DBS provide a novel strategy for examining the interactions of affective experience and the autonomic nervous system.

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Poster

553. Human Emotion: Behavioral and Neural Mechanisms

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Topic: F.03. Motivation and Emotion

Support: KAKENHI (24730617)

NEXT program (LZ001)

KAKENHI (25245069)

Title: Neural mechanisms underlying effects of giving monetary reward and punishment on successful encoding of episodic memories in social and non-social context

Authors: *Y. SHIGEMUNE, T. TSUKIURA
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Abstract: The processing of rewards and punishments enhances episodic memories. Previous studies have reported that receiving monetary rewards and punishments enhances memory performance, and interaction between activations in the hippocampus and the reward-related region including the ventral striatum (vS) underpins this enhancement. However, there is little evidence how giving rewards and punishments modulates memory performance and memory-related neural mechanisms. Given that rewards and punishments are often given and taken in social context, we investigated this issue in both social and non-social contexts. Twenty-five college students participated in this study. During encoding with fMRI scanning, participants were presented with silhouette pictures as substitutes for students in another college (Human) and computer programs (Machine), and were required to judge whether they give monetary rewards (Reward), punishments (Punishment), or nothing (Control) to students and programs as the pictures. Participants were given a fictitious instruction that the students and programs would receive monetary rewards and punishments decided by the participants, and in return the students and programs would decide to give monetary rewards, punishments, or nothing to the participants. During retrieval, participants were presented with old and new silhouette pictures, and judged whether the pictures were old or new with two levels of confidence. Behavioral data of subjective ratings demonstrated that participants inferred other's intention and felt positive or negative emotion more strongly in Reward and Punishment than in Control, but the enhancement of inference and emotion in Reward and Punishment was larger in Human than in Machine. In addition, memories encoded in Reward and Punishment were remembered more accurately than those in Control, but there was no difference in the enhancing effects of memories between Human and Machine. In fMRI data, the lateral prefrontal cortex (lPFC) involved in the intention inference and the reward-related vS showed greater activations in Reward and Punishment than in Control, and activations in the hippocampus reflected the successful encoding of memories. In addition, correlation between activations in the hippocampus and lPFC was significantly higher in Reward and Punishment during the successful encoding of memories. These findings suggest that action of giving rewards and punishments could enhance successful encoding of memories in both social and non-social contexts, and that interactions between the lPFC and hippocampus could contribute to the memory enhancement.

Disclosures: **Y. Shigemune:** None. **T. Tsukiura:** None.

Poster

553. Human Emotion: Behavioral and Neural Mechanisms

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 553.13/SS57

Topic: F.01. Human Cognition and Behavior

Title: Relationship between brain activity and emotional state during multi task

Authors: ***T. OKAMURA**[1], U. YAMAMOTO[2], T. HIROYASU[2]
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Abstract: Objective The purpose of this study is to investigate the relationship between the brain activity and the changes in the emotional state during multi task. This paper describes that the emotional influence of multi-task differs from person to person even if they do the same task using simplified Profile of Mood States (POMS). We measure the brain activation during the multi task using functional Magnetic Resonance Imaging (fMRI), to find out a relationship between the brain activation and the emotional state. Methods Eight healthy young men and eight healthy young women (22±1 years old) participated in this study. The subjects performed online letter matching tasks, which provide a multi task and a single task. The multi task consists of a simultaneous memory of two problems at the same time. On the other hand, the single task offers that the subject memories only one problem. In both of the experiments, we investigated the relationship between the brain activation and the emotional state. In the first experiment using fMRI, we measured the brain activation during the multi tasks and the single tasks. In the second experiment using POMS, we measured the emotional changes during both of the tasks. On the basis of the emotional changes caused by each task, we classified subjects to the high-stress group or the low-stress one.The subjects with increase in emotional changes are defined as the high-stress group and those with decrease of emotional changes are defined as the low-stress group. We compared the brain activation between the high-stress group and the low-stress group. Results There were the high-stress subjects and the low-stress subjects in both tasks. The emotional influences caused by each task differed from person to person even if they do the same task. When the subjects performed the multi task, the difference of activation in the Vermis between the high-stress group and the low-stress one was observed. When the subjects performed the single task, the difference of activation in the Cerebellum between the high-stress group and the low-stress one was exhibited. Although there is not a unique region about the multi task, the results suggested that the difference in the emotional state is concerned with the brain activation in Vermis. Conclusions The emotional changes in the multi task and the single task differed from person to person. The difference in emotional changes was concerned with the brain activation even if they do the same task. This paper suggested that the emotional state during the multi task is closely related to the brain activation in Vermis.

Disclosures: **T. Okamura:** None. **U. Yamamoto:** A. Employment/Salary (full or part-time):; DOSHISHA University. **T. Hiroyasu:** A. Employment/Salary (full or part-time):; DOSHISHA University.

Poster

553. Human Emotion: Behavioral and Neural Mechanisms

Location: Halls A-C

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Program#/Poster#: 553.14/SS58

Topic: F.01. Human Cognition and Behavior

Support: Office of Naval Research (ONR) under Contract No. N00014-13-P-1078

Title: Interpreting cognitive and physiological states from biometric sensors to predict performance degradation

Authors: *B. K. BRACKEN[1], S. GUARINO[1], W. DORIN[1], P. DIZIO[2], J. LACKNER[2], V. ROMERO[1], J. PFAUTZ[1]
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Abstract: Biometric techniques for sensing peripheral nervous system indicators (e.g., heart rate, blood pressure, and galvanic skin response), when analyzed and interpreted correctly, provide an opportunity to objectively assess and forecast cognitive, physical, and emotional human states. However, the accurate interpretation of biometric data depends on the environment in which the individual is performing and on the type of task the individual is undertaking. The effort described herein focused on (1) proving the feasibility of an approach to acquire, model, and interpret cognitive, emotional, and physical state from physiological data, and (2) forecasting the effect that state will have on cognitive performance. We first identified a set of inexpensive, commercially available, off-the-shelf sensors that are simple to use, robust enough to use in real-world environments, and provide access to the raw data. We used a Zephyr Bioharness to measure heart rate, breathing rate, skin temperature, and posture; a body-mounted 9-axis accelerometer, including a 3-axis gyroscope (to measure orientation), a 3-axis accelerometer, and a 3-axis magnetometer (to measure the direction of the Earth's ferromagnetic axis); an identical ship-mounted 9-axis accelerometer for collecting environmental motion information from the boat; and a mobile device (a 10-inch Samsung Galaxy tablet) to deliver cognitive tasks. Cognitive tasks included a working memory task (dual n-back task; (Jaegi et al., 2003), a problem solving task (basic arithmetic), and an inhibition task (Stroop task; (Stroop, 1935)). Once the data were collected, we used complex event processing methods to extract and fuse the best combination of indicators of motion sickness and cognitive performance based on previous findings in the literature and subject matter expert input. Next, we modeled the fused data with a hybrid computational approach to combine different types of indicators to infer symptoms of fatigue, mood, sickness, and motivation directly from data. We then used the modeled data to

forecast cognitive degradation due to motion exposure before the individual subjectively reported her symptoms. We present the results of our pilot experiment in which we demonstrated that we could feasibly anticipate motion exposure effects on cognition for a small subject population. We believe this approach can be used across a wide range of sensing devices, assessment environments, and human states of interest.

Disclosures: B.K. Bracken: None. **S. Guarino:** None. **W. Dorin:** None. **P. DiZio:** None. **J. Lackner:** None. **V. Romero:** None. **J. Pfautz:** None.

Poster

553. Human Emotion: Behavioral and Neural Mechanisms

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Program#/Poster#: 553.15/SS59

Topic: F.01. Human Cognition and Behavior

Support: R01 AG030311

Title: Task-evoked skin conductance responses and intrinsic amygdala connectivity independently predict subjective experience of arousal

Authors: *C. XIA[1], A. TOUROUTOGLOU[1], M. ADEBAYO[1], C. CASO[1], M. STEPANOVIC[1], V. HUNTOON[1], J. ANDREANO[1], E. SIEGEL[2], K. QUIGLEY[2], B. DICKERSON[1], L. FELDMAN BARRETT[1,2]
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Abstract: BACKGROUND: Previous psychophysiologic research has shown that skin conductance response during affective reactivity tasks is related to subjective experience of arousal (Lang et al., 1998). In a recent functional magnetic resonance imaging (fMRI) study from our laboratory, we demonstrated that subjective experience of arousal is also linked to amygdala intrinsic connectivity within the salience network (Touroutoglou et al., in press). However, no study has yet examined how the physiologic responses to affective stimuli combine with salience network intrinsic connectivity to predict subjective experience of arousal. Here, we hypothesized that task-evoked skin conductance response and resting-state connectivity strength within the salience network independently predict individual differences in the experience of arousal while viewing affective pictures. METHODS: An affective reactivity task was administered to 27 young adults during which they viewed 90 items ranging in valence and arousal from the International Affective Picture System. Participants rated their subjective

experience of arousal to each image and concurrent skin conductance responses were recorded. On a separate day, resting-state fMRI data were acquired for these same participants. A multiple linear regression analysis was carried out using the average number of skin conductance responses to high arousal images (versus neutral images) and intrinsic connectivity strength of the dorsal amygdala to the rest of the salience network as independent variables, and the mean arousal ratings of high arousal images (versus neutral images) as the dependent variable. RESULTS: Participants with an increased number of skin conductance responses to high arousal images (versus neutral images) reported more intense subjective experiences of arousal. Similarly, participants with stronger resting-state connectivity within the salience network reported more intense subjective experiences of arousal for these same images. However, the task-evoked skin conductance responses and resting-state salience network connectivity strength were not correlated with each other. A linear regression analysis showed that skin conductance response and resting-state salience network connectivity strength each significantly predicted unique variance in the subjective experience of arousal. CONCLUSIONS: Our findings suggest that skin conductance response to affective stimuli and intrinsic dorsal amygdala connectivity within the salience network provide independent and complementary contributions in predicting the subjective experience of arousal.

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Poster

553. Human Emotion: Behavioral and Neural Mechanisms

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Topic: F.01. Human Cognition and Behavior

Support: BBSRC Grant BB/H011021/1

ESRC Grant ES/H016503/1

Title: Monitoring versus implementation in emotional top-down control

Authors: R. CLARKE, *T. JOHNSTONE
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Abstract: Background Successful top-down control entails monitoring and signalling the need for control and the implementation of such control. Studies in the cognitive domain suggest that these processes are distinct, with medial prefrontal cortex (PFC), particularly dorsal anterior cingulate (dACC), implicated in monitoring the need for control and lateral PFC in the implementation of control. In this study we sought to test whether the top-down control of anxiety involves similar neural circuitry. Method 22 healthy participants aged 18-21 underwent fMRI scanning while they completed a visuospatial working memory (WM) task with two load levels, in either the absence or presence of threat of electric shock to induce anxiety. We proposed that high load and threatening trials would engage top-down control in order to overcome emotional interference. Furthermore, we predicted that control mechanisms engaged on such trials would persist to subsequent trials. We thus hypothesised that control trials preceded by safe trials would primarily engage mechanisms responsible for detecting and signalling the need for greater top-down control (i.e. dACC), whilst control trials preceded by threatening trials would primarily engage mechanisms responsible for implementing control (i.e. lateral PFC) due to such mechanisms having already been established on the preceding trial. Results Contrary to our previous studies, there was no behavioural effect of emotional interference, with threat of shock improving performance at both WM loads. Previous trial threat-level had no effect on performance. Imaging results, however, showed a differential pattern of PFC activation to control trials depending on previous trial threat. Consistent with our predictions dACC activation was greater for control trials preceded by safe trials, whereas right ventrolateral PFC (vlPFC) was greater for control trials preceded by threat trials. Conclusion Our results support a model in which dACC is associated with monitoring the need for top-down control and right vlPFC activation, specifically inferior frontal gyrus (IFG), with implementing control.

Disclosures: **R. Clarke:** None. **T. Johnstone:** None.

Poster

553. Human Emotion: Behavioral and Neural Mechanisms

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Topic: F.01. Human Cognition and Behavior

Support: Arizona Alzheimer's Disease Core Center (NIA P30 AG19610-03)

State of Arizona (Arizona Alzheimer's Consortium)

The Barrow Center for Neuromodulation

Title: Mindfulness Based Stress Reduction correlates with frontal lobe changes during mood induction fMRI

Authors: *B. B. BRADEN[1], T. K. GLASPY[1], T. PIPE[2], K. STEINKE[1], C. T. ELROD[1], L. C. BAXTER[1]
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Abstract: Depression neural circuitry has been established in part through brain mapping during induction of a sad mood. Interventions such as medication and deep brain stimulation have been shown to modulate depression neural circuitry. We examined whether a behavioral intervention, Mindfulness Based Stress Reduction (MBSR), similarly modulates regional brain activity during emotional responsivity. Participants were 24 back pain patients, 13 of whom completed a 4 week MBSR class. The 11 Control participants were given MBSR reading material with no further instruction. All participants were evaluated for Beck Depression Inventory-II and Oswestry Low Back Pain and scanned on the same 3T Philips scanner a week before and after the MBSR training. During scanning, we used an fMRI mood induction paradigm that maps neural changes during transitions between sad and neutral moods. During the task, participants viewed alternating sets of sad (mourning people) or neutral (landscape) pictures for mood induction, signaling that they achieved the desired mood state (sad or neutral) with a button press. Individualized regressors linearly modeled the time periods during the attainment of sad and neutral moods. Parameters were TE/TR= 30/3000ms, FOV =24, 60x60 matrix, 40 contiguous slices 4mm thick collected in the axial plane. SPM8 was used for image processing and analyses. Paired t-tests indicated that both groups declined in depression scores (Controls; $p=0.05$, MBSR $p=0.08$ but only the MBSR group showed a significant decline in back pain symptoms ($p<0.02$; $p=0.1$ for Controls). At baseline, there were no group differences in fMRI responsivity. Post-intervention, the MBSR group showed greater BOLD signal in the middle anterior cingulate cortex (ACC; $p=0.035$, corrected), subgenual ACC ($p=0.043$, corrected), and left insula ($p=0.038$, corrected) during transitions from a sad to a neutral state, than the Control group. In summary, despite a mild improvement in depression in both groups, only the MBSR intervention improved back pain symptoms. FMRI results parallel these findings, showing greater frontal system engagement in regions important in awareness of internal states (insula) and cognitive awareness of emotional regulation (ACC). Further research is warranted to evaluate the neural correlates of MBSR as a behavioral intervention for distressed patient populations, including chronic back pain.

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Poster

553. Human Emotion: Behavioral and Neural Mechanisms

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Topic: F.03. Motivation and Emotion

Support: NSF BCS-0846892

Title: Emotion-related N250 is generated near temporo-parietal junction and is evoked both by faces and by affectively valent non-social images

Authors: J. ATHILINGAM[1], J. JONES-ROUNDS[2], D. J. POST[3], B. L. GANZEL[2,4], *M. K. BELMONTE[5,6]
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Abstract: Emotion recognition is a crucial component of social interaction that subserves the interpretation and comprehension of another actor's mental state. The current study aimed to factor out the process of emotion recognition from facial encoding by recording event-related potentials during emotion recognition of both social (face) and non-social (object) stimuli in 22 normal young adults. The N170, N250, P300, and late positive (LPP) event-related potentials were examined for the effect of task set (simple discrimination vs. emotion recognition), stimulus type (face vs. object), and valence (emotional vs. neutral). Whilst the N170 arose specifically in response to faces, P300 and LPP amplitudes increased with emotion regardless of stimulus type, suggesting that emotion processing and recognition are indeed separable from facial encoding. The LPP also seemed modulated by attention as its amplitude was greater during the emotion recognition task than during discrimination. Consistent with another recent report, the N250, the putative emotion recognition component, was greater in amplitude to neutral than to emotional faces during the emotion recognition task, possibly reflecting increased integrative processing associated with difficulty in perceiving an emotion. Independent component clustering and dipole source localisation revealed that the emotion-related N250, broadly distributed at fronto-central scalp electrode locations, is also associated with a more focal, opposite potential on occipito-parietal scalp consistent with a dipole generator near the temporo-parietal junction, a major node of information integration. These results provide evidence for a common mechanism of emotion perception in response to social and non-social stimuli, and convergent evidence for a posterior generator of the emotion-related N250.

Disclosures: **J. Athilingam:** None. **J. Jones-Rounds:** None. **D.J. Post:** None. **M.K. Belmonte:** None. **B.L. Ganzel:** None.

Poster

553. Human Emotion: Behavioral and Neural Mechanisms

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 553.19/SS63

Topic: F.01. Human Cognition and Behavior

Title: Music-induced mood improves retention in sensorimotor adaptation

Authors: K. WACLAWIK[1], L.-A. LEOW[1], *J. A. GRAHN[2]
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Abstract: During sensorimotor adaptation, individuals learn to adapt movements to distortions in sensory feedback. Rewarding feedback increases persistence of adapted movements after the distortion is removed. Positive music is also rewarding and thus might engage neural reward mechanisms during adaptation. Here, positive music increased retention of adapted movement more than negative music and silence. Rewarding feedback increased retention of adapted movements relative to no feedback. However, presence of both rewarding feedback and positive music decreased retention of the adapted movements relative to rewarding feedback alone or positive music alone. Therefore, positive music interacts with reward mechanisms during sensorimotor adaptation.

Disclosures: **K. Waclawik:** None. **L. Leow:** None. **J.A. Grahn:** None.

Poster

553. Human Emotion: Behavioral and Neural Mechanisms

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Topic: F.03. Motivation and Emotion

Support: NRF-2012R1A1A2001

NRF-2006-2005372

Title: Brain network for text based emoticons

Authors: *K.-W. KIM[1], S. LEE[1], B. JEONG[1], J. CHOI[2]
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Abstract: The text-based emoticons are a nonverbal communication tool of emotion and is a unique feature of human being. Human can extract emotional features from serial distribution of letters and/or symbols. However, little is known how the human brain recognizes emoticons and discriminate them laden with various emotions. The aim of our study was to investigate brain network for the emoticon processing using fMRI. Eighteen subjects (age=30.5±6.6 years, M: F = 10: 8) were recruited. All subjects gave written informed consent before participating in the study. They were interviewed to exclude any possible psychiatric diseases by a psychiatrist using DSM-IV criteria. The subjects didn't have any Axis-I psychiatric disorder and history of head trauma. The event-related task consisted of four emotional conditions of emoticons: happy, sad, angry/fearful, scrambled emoticons. The four different categorized emoticons were presented in pseudo-randomized order. During the fMRI scan, subjects were asked to respond by pressing a button, indicating whether the emoticon represented positive or negative emotion. The general linear model was used for linear combination of the modeled response to visual stimulation of happy, sad, angry/fearful and scrambled emoticons, excluding errors. Using FEAT of FSL software, we performed both preprocessing and statistical analysis for comparing the activation among 3 emotional and scrambled emoticons. Two distinct brain networks had important role in emoticons processing: the central executive network consisting of the dorsolateral prefrontal cortex (Z value: right=4.72, left=4.92) and the left posterior parietal cortex (Z=4.72), and the salience network consisting of the anterior insula (Z value: right=5.92, left=5.48) and the right anterior cingulate cortex (Z=5.48). The processing of emoticons was also associated with an increased activation as follows: the bilateral lateral occipital cortex (Z value: right=5.08, left=5.49), the left precentral gyrus (Z=5.28), and the left thalamus (Z=5.94) (corrected $p < 0.05$). Only sad emoticons subtracted from other conditions illustrated significantly different activity in salience network. The central executive and salience networks may play a significant role in cognitive and emotional processing of text-based emoticons.

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Poster

553. Human Emotion: Behavioral and Neural Mechanisms

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NCCAM Grant P01-AT004952

NICHD Grant P30 HD003352-449015

Title: Common amygdalar functional coupling mechanisms underlie emotional bias and explicit memory following negative-emotional processing: An evaluative conditioning paradigm

Authors: *R. C. LAPATE, T. KRAL, K. AROOR, B. SCHUYLER, R. J. DAVIDSON
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Abstract: Emotional events typically leave long-lasting fingerprints on surrounding stimuli. A prototypical laboratory example is evaluative conditioning, whereby a novel neutral stimulus paired with a negative stimulus is subsequently rated as more negative. Evaluative conditioning is a context in which implicit emotional regulation is operative, but its neural mechanisms remain unclear. Here, we combined resting state and task-based indices of amygdalar functional connectivity to test whether the magnitude of evaluative conditioning across individuals is associated with amygdalar-prefrontal cortical (PFC) coupling mechanisms implicated in favorable emotion-regulatory outcomes. While in the scanner, healthy participants (N=72, 26 – 65 y old, 47 female) watched 72 negative, neutral and positive pictures presented for 4000 ms. Following the presentation of 2/3 of the pictures, neutral faces were flashed on the screen for 500 ms. Each neutral face was presented twice, and consistently paired with one picture valence. Resting state data were also acquired. Three days later, individuals were presented with the previously shown neutral faces, as well as foils, and asked to rate each face for likeability (indexing evaluative conditioning) and recognition (indexing stimulus memory). Although individuals reported on average not remembering the previously shown neutral faces, $t(70)= -7.9$, $p < .001$, faces following negative images were significantly less liked, $F(1,71) = 16.8$, $p < .001$, and better remembered, $F(1,71) = 9.7$, $p < .01$, than faces following neutral images. Task-based functional connectivity analysis (psychophysiological interaction) with the amygdala as a seed indicated that the negative coupling between the amygdala and common regions of the inferior frontal gyrus (IFG), insula (INS) and basal ganglia (BG; including putamen and pallidum) following negative (relative to neutral) pictures predicted less evaluative conditioning as well as greater recollection of neutral faces following negative (relative to neutral) pictures, $z > 2.3$, $p < .05$, whole-brain cluster-corrected for multiple comparisons. Further, positive coupling between the amygdala and the BG at rest predicted less evaluative conditioning and greater negative

coupling between the amygdala and the BG, INS, and IFG during the task, ps < .03. Thus, these results extend the functional relevance of amygdalar-PFC coupling to improved memory for and reduction of negative bias toward neutral stimuli following evaluative conditioning, while highlighting a role for amygdalar-BG coupling at rest in predicting amygdalar-PFC dynamics during a negative emotional challenge.

Disclosures: **R.C. Lapate:** None. **T. Kral:** None. **K. Aroor:** None. **B. Schuyler:** None. **R.J. Davidson:** None.

Poster

553. Human Emotion: Behavioral and Neural Mechanisms

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Topic: F.01. Human Cognition and Behavior

Support: NIH MH097320

Title: The role of the human amygdaloid complex in fear conditioning: A functional connectivity analysis

Authors: ***S. YIN**[1], Y. LIU[3], A. KEIL[2], M. DING[1]
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Abstract: The Role of the Human Amygdaloid Complex in Fear Conditioning: A Functional Connectivity Analysis Siyang Yin, Yuelu Liu, Andreas Keil, Mingzhou Ding Work in the rodent models of fear conditioning has demonstrated that the amygdaloid complex plays a central role in mediating defensive responses to conditioned threat cues. In human imaging studies of aversive conditioning, however, activation of the amygdala by the conditioned stimulus is not always observed. We examined this problem from a functional connectivity perspective. Functional magnetic resonance imaging (fMRI) data were recorded from 18 subjects during a classical differential fear conditioning paradigm. Two Gabor patches (45° and 135°) were used as conditioned stimuli (CSs). One Gabor patch, denoted as CS+, was occasionally paired with an aversive human scream used as the unconditioned stimulus (US; 25% reinforcement rate), whereas the other Gabor patch (CS-) was never paired with the US. Comparing BOLD activity evoked by the CS+ (in unpaired trials) and by the CS-, we found that ACC and insula, but not the amygdala, were selectively activated by CS+. Furthermore, applying the beta series method, the

time courses of single trial beta values from CS+ unpaired trials and from CS- trials were statistically indistinguishable in the amygdala. A ROI-based connectivity analysis showed that relative to CS-, a significant increase of functional connectivity between the amygdaloid complex and cortical structures including ventral anterior cingulate cortex (vACC), insula, and medial prefrontal cortex (mPFC) was observed for CS+. The connectivity change between amygdala and vACC/mPFC was negatively correlated with the subjects' heart rate change, whereas the connectivity change between amygdala and insula/putamen was positively correlated with the subjects' heart rate change. These results suggest that in the present paradigm the amygdala contributes to fear acquisition and autonomic response regulation via its functional interaction with other cortical and subcortical structures.

Disclosures: S. Yin: None. **Y. Liu:** None. **A. Keil:** None. **M. Ding:** None.

Poster

553. Human Emotion: Behavioral and Neural Mechanisms

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Program#/Poster#: 553.23/SS67

Topic: F.01. Human Cognition and Behavior

Support: NIH RO1 MH098348 (Mrug & Knight)

UAB OVPED - CMFSDP (Harnett)

Title: Affective state is associated with changes in the neural response to threat

Authors: *N. G. HARNETT, M. D. WHEELOCK, K. H. WOOD, S. MRUG, D. C. KNIGHT
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Abstract: During Pavlovian fear conditioning, the unconditioned response (UCR) elicited by an aversive threat (i.e. the unconditioned stimulus; UCS) is diminished when preceded by a warning signal (i.e. the conditioned stimulus; CS). This phenomenon is called conditioned UCR diminution. Relatively limited research has studied the neural mechanisms of conditioned UCR diminution, and even less research has investigated whether UCR diminution is influenced by individual differences in emotion processes. A better understanding of the impact that individual differences in cognitive and emotional function have on the neural response to threat would provide valuable insights into this type of emotional learning. Therefore, the present study investigated neural activity, using functional magnetic resonance imaging, to identify brain

regions that mediate learning-related changes in the emotional response to a threat (i.e. conditioned UCR diminution). In addition, we assessed the effect that individual differences in locus of control and positive/negative affect have on conditioned UCR diminution in these brain regions. Whole brain analyses revealed conditioned UCR diminution within the insula, dorsomedial prefrontal cortex (PFC), dorsolateral PFC, and inferior parietal lobule. Whole brain regression analysis revealed that brain activity varied with positive/negative affect. Further, the relationship between brain activity and positive/negative affect appeared to vary depending on whether the UCS was predictable (e.g., UCS alone or UCS preceded by the CS+). These findings suggest that individual differences in affective state influence the neural response to threat, and are dependent on stimulus predictability.

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Poster

553. Human Emotion: Behavioral and Neural Mechanisms

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Topic: F.01. Human Cognition and Behavior

Support: Capes

Faperj

CNPq

IBN Net

Title: Threat imminence influences emotional and defensive brain resposnses

Authors: L. L. PORTUGAL[1], O. F. JUNIOR[1], R. S. ALVES[1], F. S. ERTHAL[2], E. VOLCHAN[2], T. A. SANCHEZ[2], S. PADMALA[3], L. OLIVEIRA[1], L. PESSOA[3], *M. G. PEREIRA[1,1,2,3]
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Abstract: Privileged processing of cues that signal danger in the environment and initiation of appropriate defensive behaviors is essential for survival. Thus, it is reasonable to expect a strong

interaction between emotional processing and prompting of action tendencies. The aim of this study was to investigate how different threatening stimuli drive the activity of brain networks, especially in motor related areas. The direction of threat stimuli was the key factor manipulated to attempt to activate different defensive responses. We used functional MRI to investigate brain responses of 31 volunteers (14 women) during a simple motor task while they viewed 42 threat and 42 matched neutral pictures. Threat stimuli were divided into two sets - one set composed of photographs with a person directing a firearm toward the observer (directed towards threat), and another composed of photographs in which a person was pointing a fire arm away from the viewer (directed away threat). Matched neutral stimuli were also divided into two sets in a similar fashion. Each picture was presented for 5 sec and an overlapped central circle appeared between 3.7 to 4.2 sec after picture onset. Participants were instructed to press a button as quickly as possible whenever a circle appeared. Imaging data analysis was performed using AFNI, with directed threat, directed away threat, directed neutral and directed away neutral as regressors in the general linear model. We conducted a repeated-measures ANOVA at the second level analysis with the factors Valence (threat or neutral) and Direction (directed towards or directed away). We found a main effect of Valence (i.e., threat > neutral) in several regions including amygdala, visual cortex, mid cingulate cortex, precentral gyrus (PG), supplementary motor area (SMA) and insula. More focused ROI analysis also revealed an interaction between Valence and Direction in subset of these regions such that threat (vs. neutral) responses were stronger in directed towards compared to directed away condition. These results suggest that viewing of threatening pictures increase motor preparation, especially when threat is directed towards the observer, and probably reflects that different defensive reactions are implemented depending on the perceived threat level.

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Poster

553. Human Emotion: Behavioral and Neural Mechanisms

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 553.25/TT1

Topic: F.01. Human Cognition and Behavior

Support: NIMH R01 MH071589

Title: Motivation counteracts aversive processing in the amygdala and visual cortex

Authors: *M. SIRBU, S. J. E. LANGESLAG, S. PADMALA, L. PESSOA
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Abstract: Positive motivational processes such as those manipulated via monetary rewards typically improve cognitive task performance, whereas task-irrelevant aversive stimuli often interfere with performance. We have recently reported that performance-based rewards reduced the interference effect of task-irrelevant aversive stimuli in a behavioral setting (Padmala & Pessoa, Emotion, in press). In the current fMRI study, we sought to determine the neural correlates of this behavioral effect. In line with the behavioral findings, we hypothesized that rewards would lead to decreased aversive processing in regions such as the amygdala. Additionally, we investigated how aversive processing was influenced by distinct motivational manipulations: when participants sought monetary gains or when they avoided monetary losses. Participants (N=27) performed a visual discrimination task where on each trial they judged whether two circular grating stimuli (above and below fixation) had the same or different line orientation. Along with the grating stimuli, two faces of the same identity and expression (fearful or neutral presented to the left and right of fixation) served as task-irrelevant distractors. Positive motivation (i.e., seeking monetary gains), negative motivation (i.e., monetary loss avoidance), and control (i.e., no gains/losses) conditions were presented in a blocked fashion and signaled by a cue at the start of each block. At the end of each block, participants received feedback about their earnings in that block and their cumulative earnings. As expected, we observed distractor effects in the amygdala and fusiform gyrus such that fearful faces elicited stronger responses compared to neutral ones. We also observed evidence for an interaction between Positive motivation (reward, control) and Distractor type (fearful, neutral) in the amygdala and fusiform gyrus, such that increased fearful (vs. neutral) responses in the control condition were reduced during the reward condition. In contrast, we did not detect an interaction between Negative motivation (punishment, control) and Distractor type (fearful, neutral) in the amygdala. Our preliminary analyses suggest that potential reward reduces aversive distractor processing in the amygdala and visual cortex, thus potentially reducing their detrimental effect on behavior. Furthermore, these results support the notion that processing of aversive information is not obligatory (i.e., automatic) but can be modulated by motivational processes.

Disclosures: **M. Sirbu:** None. **S. Padmala:** None. **L. Pessoa:** None. **S.J.E. Langeslag:** None.

Poster

553. Human Emotion: Behavioral and Neural Mechanisms

Location: Halls A-C

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Program#/Poster#: 553.26/TT2

Topic: F.01. Human Cognition and Behavior

Support: NIH Grant R21-MH098149

NIH Grant R01-DA033369

Title: Decoding spontaneous emotional states from neural activation during rest

Authors: *P. A. KRAGEL, A. R. HARIRI, K. S. LABAR
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Abstract: As emotional states are conventionally elicited through stimulus presentation or internally-guided imagery, studying the nature of neural systems underlying emotion in the absence of experimental tasks remains a challenge. Identifying neural activity that corresponds to spontaneous fluctuations in emotional states may be important for characterizing individual differences in susceptibility to psychiatric illness, assessing the outcome of intervention strategies, and quantifying emotion elicitation in individuals who have little insight into their emotional experience. In the present study, we use multivariate classification models to predict the occurrence of basic emotional states (i.e., contentment, amusement, surprise, fear, anger, sadness, and neutral states) from patterns of neural activation during resting state fMRI. Utilizing classifiers trained on fMRI data from task-based emotion induction in an independent sample, we examined the frequency and temporal dynamics of emotional states spontaneously elicited at rest from a large sample of over 400 individuals. Additionally, we assessed whether individual differences in personality traits (i.e., neuroticism and extraversion) influence the occurrence of individual emotions at rest. We found that emotional states exhibited a non-uniform distribution during rest, with contentment being predicted the least and neutral states occurring most often. Analysis of temporal dynamics revealed significant changes in classification scores over time for all seven emotions. Notably, scores for classifying fear were the highest at the onset of resting-state scans - when participants were initially placed into the MRI scanner - and decreased over time. Regression analyses revealed that the frequencies of emotional states were associated with personality differences: the number of fear classifications increased with neuroticism, whereas the frequency of surprise increased and the frequency of sadness decreased with increasing extraversion. These findings demonstrate the capacity of multivariate biomarkers to detect emotional states at rest and illustrate their sensitivity to individual differences in personality traits that impact spontaneous emotional experience. Further, these results have practical implications regarding the nature of emotions at rest. As they are not uniformly distributed or temporally invariant, using a resting state baseline for task-based emotion induction may obscure or bias results, particularly depending on the personality traits of the sample.

Disclosures: **P.A. Kragel:** None. **A.R. Hariri:** None. **K.S. LaBar:** None.

Poster

553. Human Emotion: Behavioral and Neural Mechanisms

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Topic: F.01. Human Cognition and Behavior

Support: NIH Grant MH098348

UAB Faculty Development Grant

Title: The neural response in humans is diminished to predictable and controllable threats

Authors: ***K. H. WOOD**[1], M. D. WHEELOCK[1], J. R. SHUMEN[1], L. W. VER HOEF[2,3], D. C. KNIGHT[1]
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Abstract: Susceptibility and resilience to stress is mediated, in part, by our ability to predict and control threats in our surroundings. Prior animal research has demonstrated a diminished emotional response in anticipation of predictable and controllable stressors, whereas unpredictable and uncontrollable stressors result in an enhanced emotional response. The present study was designed to better understand the effect of predictability and controllability on threat-related brain activation. Two groups of healthy volunteers participated in a novel Pavlovian fear conditioning study during functional magnetic resonance imaging (fMRI). Similar to prior animal research, the groups consisted of yoked pairs in which one group (Controllable Condition; CC) was able to terminate the unconditioned stimulus (UCS), and the other group (Uncontrollable Condition; UC) was not able to terminate the UCS. The threat-related fMRI signal response was diminished on predictable compared to unpredictable trials within the dorsolateral prefrontal cortex (PFC), dorsomedial PFC, ventromedial PFC (vmPFC), ventrolateral PFC, and posterior cingulate for both CC and UC groups. A predictability x controllability interaction was observed within the vmPFC and hippocampus. Specifically, the threat-related response within these brain regions was diminished on predictable vs. unpredictable trials for the CC. Further, the threat-related response was enhanced on predictable trials for the UC compared to the CC within the vmPFC and hippocampus. The current findings suggest the vmPFC and hippocampus play a key role in regulating the emotional response to a

threat. To our knowledge this is the first human neuroimaging study to demonstrate that controllability and predictability impact the emotional response elicited by the threat itself using a yoked fear conditioning paradigm. These findings provide a better understanding of the neural circuitry that supports the modulatory effects of predictability and controllability on the emotional response to a threat.

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Poster

554. Working Memory II

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 554.01/TT4

Topic: F.01. Human Cognition and Behavior

Title: Mobile brain/body imaging: Combined EEG and motion capture during a dart throwing visuospatial working memory task

Authors: *R. J. GOUGELET[1,2], M. MIYAKOSHI[2], A. BIRGER[1], J. LE[1], A. WOOTEN[1], S. MAKEIG[2]
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Abstract: With an embodied cognition perspective at the forefront of recent cognitive theory, the link between brain activity and full body motion will become increasingly important. Few studies combine full body motion and electroencephalography (EEG), especially in visuospatial working memory tasks. This study involves humans performing a novel visuospatial delayed response working memory task. For each trial, a projector projects a target stimulus onto a 1.8 x 1.2 m white cork board, onto one of 17 fixed positions in the visual field of subjects. The target stimulus is similar to a dart board, with a bull's-eye surrounded by concentric circles corresponding to decreasing distance and point values. The subjects fixate on a center crosshair for a 3 s preparatory period, the target is displayed for 250 ms before the target either disappears for the target absent condition, or remains for the target present condition. After a randomly variable 3 to 9 s integer delay, the subjects throw a dart to the displayed or remembered position of the target, the dart's distance from the target is measured and they receive motivating points based on their performance. Continuous EEG data were collected using 128 active electrodes with a 512 Hz sample rate. Electrooculography (EOG) and electromyography (EMG) from the right throwing arm were also collected. EEG, EOG, and EMG data were analyzed using

EEGLAB. EEG data were epoched into 3 s periods at the start of the delay conditions. Epochs with excessive eye and muscle artifacts were removed. The continuous EEG data were also decomposed into maximally independent components using independent component analysis (ICA). Full-body kinematic motion data were collected from 32 LED locations on the limbs, torso, and head of each subject. A Shapiro-Wilk's test ($p > 0.05$) showed that both target absent and target present distance scores were not approximately normally distributed. A non-parametric two-sample Kolmogorov-Smirnov test between the target absent and target present conditions demonstrated that the distributions were significantly different between conditions ($p < 0.05$), with greater mean and median distance, greater variability, and greater kurtosis in the target absent condition, suggesting decreased precision and accuracy when throwing to remembered targets. These performance differences should be reflected in kinematic differences between conditions, but analyses are forthcoming. Preliminary EEG results show strong theta (4-7 Hz) activity in both target absent and target present conditions during the initial 3 s of the delay period, with distinct theta ICA components spatially localized to the frontomedial cortex.

Disclosures: R.J. Gougelet: None. **M. Miyakoshi:** None. **A. Birger:** None. **J. Le:** None. **A. Wooten:** None. **S. Makeig:** None.

Poster

554. Working Memory II

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Program#/Poster#: 554.02/TT5

Topic: F.01. Human Cognition and Behavior

Title: 2D:4D, mental rotations, SQ and EQ in a typical population

Authors: *P. T. ORR, C. E. LOWE, B. E. ZIMMERMAN, M. R. BUSCH
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Abstract: Autism Spectrum Disorders (ASD's) are characterized by 3 major symptom domains: social withdrawal, impaired language development, and repetitive behaviors or restricted interests. One etiologic explanation suggests that this developmental pattern could result from atypical patterns of steroid hormone exposure. The Extreme Male Brain theory of Autism suggests that the pattern of behavior seen in children with ASD is similar to a stereotypically male pattern of behavior (low empathy and high systemizing) and that this may result from androgenic overexposure (Baron-Cohen, 2002). However, although the ratio of the length of the second digit of the hand to the fourth digit of the hand (2D:4D), a stable marker of the ratio of

androgens to estrogens to which a fetus is exposed in the uterine environment (Zheng & Cohen, 2011), is altered in individuals with an ASD, 2D:4D is not strongly associated with typical measures of the ASD behavioral phenotype, the Empathizing Quotient (EQ) and the Systemizing Quotient (SQ; Teatero & Netley, 2013; Honekopp, 2012) Mental rotation and other spatial working memory tasks typically show sex differences in performance (Voyer, Voyer & Bryden, 1995), and performance on these tasks are related to 2D:4D in both males and females (Peters, et al., 2007). Though not diagnostic, memory deficits have been identified in individuals with ASD's. Although verbal working memory appears to be intact, spatial working memory is disrupted (Williams, et al., 2005; Ozonoff & Strayer, 2001). As such, spatial working memory deficits may represent an independent behavioral dimension of ASD's, but there is little work investigating the relationship between spatial working memory, systemizing, and empathizing. 36 undergraduates (61.1% female), aged 18-24, were assessed using SQ, EQ, and a battery of cognitive tasks, including tests for word, shape, and face memory, and mental rotations. 2D:4D was also measured in these participants. Overall, there was no correlation between 2D:4D and EQ or SQ. There was a strong negative correlation between 2D:4D and mental rotations score in females for both left ($r(9) = -.736$, $p = .006$) and right ($r(9) = -.665$, $p = .025$) hands. There was also a strong correlation in females between right hand 2D:4D and errors in immediate recall of faces ($r(9) = .789$, $p = .004$) and shapes ($r(9) = .761$, $p = .007$), but not words. Mental rotations and recall were not significantly correlated with SQ or EQ. Overall, these results indicate that spatial memory is relatively independent of empathizing and systemizing and suggest that the ASD-related differences in 2D:4D should be examined in relation to spatial memory deficits, rather than SQ and EQ.

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Poster

554. Working Memory II

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Program#/Poster#: 554.03/TT6

Topic: F.01. Human Cognition and Behavior

Support: University of Guanajuato

Title: Effects of estrogen receptor alpha gene on emotional processing in middle-age women

Authors: *M. SOLIS-ORTIZ, M. E. FAJARDO-ARAUJO, M. L. GUTIÉRREZ-MUÑOZ
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Abstract: There is evidence that recognition of emotional facial expressions change depending on the levels of estrogen in young women during the menstrual cycle. Estrogen receptor alpha gene (ESR1) polymorphisms have been associated with susceptibility to develop some cognitive deficits in middle-age women. The effect of these polymorphisms on emotional processing in women with low estrogen levels is not well known. The aim of this study was to examine the influence of the PvuII polymorphism of gene ESR1 on working memory for recognition of emotional facial expressions in healthy female. We genotyped 70 middle-age healthy women volunteers between 49 and 60 years old for the ESR1 polymorphisms, using DNA from peripheral blood leukocytes. We analyzed the effects of PP, Pp and pp genotypes on a working memory task for the recognition of emotional facial expressions, which included fear, sadness, anger, surprise and happiness. Number of correct responses, errors, omissions, reaction time and accuracy for each expression were computed. Women carriers of the pp genotype, with more estrogens receptor expression, did not show differences in task performance. In contrast, female carriers of the PP genotype, con lower estrogens receptor expression, showed more correct responses ($p=0.01$), fewer errors, reduced reaction time ($p=0.02$) and less omission. The accuracy in recognizing emotional expressions of sadness was significantly higher ($p=0.001$). These findings indicate that middle-aged women carriers of the PP genotype of ESR1gene influences on recognition of negative emotional expressions, probably due to the lower expression of receptors inducing emotional susceptibility.

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Poster

554. Working Memory II

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Program#/Poster#: 554.04/TT7

Topic: F.01. Human Cognition and Behavior

Title: Cholinergic enhancement improves visual short-term memory performance

Authors: *S. M. YOUSEF[1,2], S. L. SHEREMATA[4], R. K. KANETA[3], A. N. HAREWOOD[2], M. A. SILVER[2,3]

[2]Vision Sci., [3]Sch. of Optometry, [1]Univ. of California, Berkeley, Berkeley, CA; [4]Psychology, George Washington Univ., Washington, DC

Abstract: Visual short-term memory (VSTM) refers to the retention of visual information from the immediate environment over brief intervals. In patients with mild cognitive impairment, pharmacologically increasing synaptic levels of acetylcholine (ACh) facilitates VSTM by boosting the rate of information processing (Bublak et al., 2011). We, therefore, hypothesized that cholinergic enhancement would improve VSTM performance in healthy subjects when stimulus duration limited memory performance. Synaptic ACh levels were elevated by administration of the cholinesterase inhibitor donepezil in a placebo-controlled, double blind crossover design. Subjects performed a color change detection task in which a set of colored squares was presented for either 100 or 200ms. Consolidation of the set was disrupted by subsequent presentation of a visual mask. In order to control task difficulty across subjects, we assessed the effects of donepezil for set sizes that were based on each subject's VSTM capacity (k), measured prior to the pharmacological manipulation. We found that for 100ms, but not 200ms, stimulus presentation, cholinergic enhancement improved VSTM performance. Consistent with the hypothesized role of ACh on information processing in VSTM, our results demonstrate that cholinergic enhancement improves VSTM performance when it is limited by stimulus presentation time.

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Poster

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Topic: F.01. Human Cognition and Behavior

Support: GCOE Project: Human-friednly Robotics Based on Cognitive Neuroscience

JSPS Grand to MO 23240036

Title: Temporal desynchronisation may underlie audio-spatial binding in working memory: An EEG study under anesthesia in humans

Authors: *T. MINAMOTO[1], T. IKEDA[2], K. ENDO[4], A. NAKAE[3], S. HAGIHIRA[3], Y. FUJINO[3], T. MASHIMO[5], M. OSAKA[1]
[1]Human Sci., [2]Engin., [3]Med., Osaka Univ., Suita, Japan; [4]Psychology, Kyoto Univ., Kyoto, Japan; [5]Toyonaka Municipal Hosp., Toyonaka, Japan

Abstract: Binding of stimulus features is thought to depend on neural synchronization across brain regions. Using the anesthesia-induced sedation, the present study examined its effect on audio-spatial binding. Given that the anesthesia disturbs neural synchronization, the feature binding should be impaired with the neural disturbance. Participants received either propofol or midazolam, and their performance of an audio-spatial working memory task was measured. In the task, two tones were given serially from either right or left speakers, and they remembered the tones and their location for two seconds delay. A probe tone was presented and they judged whether it was matched to one of the original tones from the original location. The task was given before anesthesia (baseline), and three different sedative phases (deep, moderate, and light). EEGs over the Cz and Fz were also measured during the task. Due to large amount of trial omissions under the deep anesthesia, the phase was removed from the analysis. As for the behavioral performance, participants showed impaired performance in the binding under the moderate phase in comparison to the baseline and the light phases. ERPs over the Cz and Fz did not differ among conditions, where positive component was obtained 400ms after the stimulus onset. However, oscillation between the two regions differed in the moderate phase in comparison to the baseline and light phases. Specifically, as opposed to our prediction, the oscillation in the beta-band remained strong across time in the moderate phase, while tentative decrease was observed in the other conditions 400-500ms after stimulus onset. The tentative decrement of the oscillation across regions may reflect functional dissociation of distinctive neural populations, and such a neural characteristic may play a critical role in feature binding.

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Poster

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Topic: F.01. Human Cognition and Behavior

Support: DARPA N66001-14-C-4016

WFBMC Dept. of Neurosurgery

Title: Differential encoding of behavioral tasks by stereotaxically recorded CA1 and CA3 neurons in human hippocampus

Authors: M. R. WITCHER[1], D. E. COUTURE[1], A. M. LAXTON[1], G. POPLI[2], M. J. SOLLMAN[3], C. A. SEXTON[4], S. A. DEADWYLER[4], *R. E. HAMPSON[5]
[1]Neurosurg., [2]Neurol., [3]Neurology/Neuropsychology, Wake Forest Baptist Med. Ctr., Winston-Salem, NC; [4]Physiol. & Pharmacol., [5]Wake Forest Sch. of Med., Winston-Salem, NC

Abstract: Recent animal research has illustrated multineuronal ensemble firing patterns of CA1 based on the recorded discharge patterns of CA3 (Berger et al., 2011; Hampson et al., 2013), have specific discharge timing contributing to task-specific performance. Here we extend this work to study human memory formation and retrieval in patients suffering from pharmacologically refractory epilepsy who undergo intracranial monitoring with implanted electrodes for seizure localization.. Studies have shown this population to provide an excellent model to understand discharge patterns of single neuronal units within the mesial temporal lobe during learning and memory tasks (Suthana and Fried 2012), since patients do retain the ability to form new memories even when hippocampally-mediated memory formation is altered by epilepsy, (Suthana, Haneef et al. 2012). These recordings thus provide valuable insight into the mechanisms underlying human memory encoding and formation. Adult subjects underwent surgical implantation of FDA-approved hippocampal electrodes with recording site capable of field potential (macro-) and single-unit recording (micro-) recording (Ad-Tech Medical Instrumentation Corporation, Racine, WI) for localization of seizures. Inclusion in this study was voluntary and consented separately from the surgical procedure and all study participants underwent appropriate clinical epilepsy screening evaluations. A frameless Brainlab Cranial Navigation System (BrainLab North America, Westchester, IL) was used to plan and guide electrode entry points, stereotaxic electrode trajectories and targets within the CA3 and CA1 subfields of each hippocampus. Electrode localization was confirmed using postoperative MRI. Single unit neural activity was isolated and recorded using Plexon MAP or Blackrock Cervello electrophysiological recording systems. Neurocognitive experiments consisted of visual object-oriented Delayed-Match-to-Sample and spatial-oriented Paired Associate Learning tasks. Results yielded similar task-related firing correlates for CA1 and CA3 on both DMS and PAL tasks, but differential firing between anterior/posterior hippocampal locations as well as differences in event-related encoded in the object vs spatial tasks. Hippocampal neurons exhibited the same differential encoding and correlation to successful vs. failed behavioral outcome as shown in prior rodent and nonhuman primate studies, suggesting the utility of this model for eventual testing of a neural prosthetic for human memory.

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Poster

554. Working Memory II

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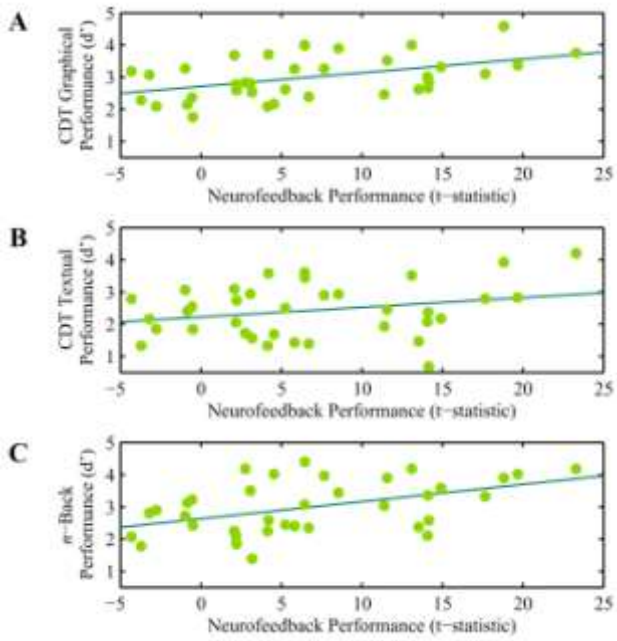
Topic: F.01. Human Cognition and Behavior

Support: AFRL Contract FA8650-11-C-6157

Title: Conscious control over the BOLD effect in the DLPFC is linked with task performance

Authors: *M. S. SHERWOOD[1,2], J. H. KANE[1], M. P. WEISEND[1], J. G. PARKER[1,2]
[1]Wright State Res. Inst., Dayton, OH; [2]Dept. of Biomedical, Industrial and Human Factors Engin., Wright State Univ., Dayton, OH

Abstract: Neurofeedback training (NFT) using real-time functional magnetic resonance imaging (rt-fMRI) can be used to train localized, conscious regulation of neuronal activity in humans. As a therapeutic technique, rt-fMRI NFT has been shown to successfully reduce the symptoms of a variety of neurologic disorders. To date, few studies have examined the use of rt-fMRI NFT to enhance cognitive performance. This work investigates the utility of rt-fMRI NFT as a tool to enhance human cognition by training healthy individuals to consciously control activity in the left dorsolateral prefrontal cortex (DLPFC). A cohort of 18 healthy participants underwent rt-fMRI NFT of the left DLPFC in five training sessions completed across two weeks. Working memory (WM) performance was evaluated before and after the two-week training regimen using two computerized tests, one of which was a dual-task. Standard statistical tests were used to investigate brain control performance of the group across training sessions, the group change in WM after the 2-week training regimen, and the relationship between individual brain control and performance on the WM tasks. The group demonstrated a significant increase in the ability to self-regulate the left DLPFC across sessions and a significant improvement in task performance after the 2-week training regimen. Additionally, individual brain control was found to correlate positively with performance on two of the three tasks performed. These results provide the evidence that humans can increase conscious control of the left DLPFC using NFT from rt-fMRI. Furthermore, these results indicate that an increased ability to self-regulate the left DLPFC is associated with superior WM performance. This study provides the foundation for future controlled studies to further investigate the effect of NFT from rt-fMRI on cognition.



Disclosures: **J.G. Parker:** None. **J.H. Kane:** None. **M.P. Weisend:** None. **M.S. Sherwood:** None.

Poster

554. Working Memory II

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Topic: F.01. Human Cognition and Behavior

Support: This research was funded by Bayer.

Title: Investigating the effects of 4-weeks multivitamin/mineral supplementation on functional brain activity during working memory

Authors: *D. J. WHITE[1], M. HUGHES[2], A. PIPINGAS[1], A. B. SCHOLEY[1]
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Abstract: There is growing evidence supporting positive effects of multivitamin/mineral (MV) supplementation on both mood and cognitive function. A double-blind, placebo-controlled study

examined, the impact of 4-weeks MV supplementation on functional brain activity using Steady State Topography (SST) and functional magnetic resonance imaging (fMRI) during completion of working memory tasks. A total of 40 healthy young adults (mean age 26.6 ± 5.27 years) consumed a daily dose of MV or placebo for 4-weeks using a balanced, parallel groups design. Assessment of neurocognitive function took place at pre-treatment baseline and after 28-days of supplementation. Brain electrical activity was assessed using SST during spatial working memory task completion, which explores changes in the Steady State Visually Evoked Potential (SSVEP) using a task-irrelevant visual flicker during periods of cognitive engagement. A subset of participants also underwent fMRI while completing a Rapid Visual Information Processing (RVIP) task. Changes in SSVEP response during the spatial working memory task showed significant latency reductions in centro-parietal regions after MV treatment, while no differences were observed in the placebo group. Analysis of fMRI activation during RVIP task performance showed significant treatment effects across task-related parietal lobe regions, whereby greater activation was seen following 4-week MV supplementation with respect to placebo. These findings suggest functional brain activity can be influenced by chronic MV supplementation, with patterns of activation following MV administration consistent with benefits to working memory systems.

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Poster

554. Working Memory II

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Topic: F.01. Human Cognition and Behavior

Support: Georgetown and Howard Universities Center for Clinical and Translational Research

Title: Cholinergic activity in human medial temporal lobe during active maintenance of configural information

Authors: *K. SHATTUCK[1], J. W. VANMETER[2]
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Abstract: Though short-term memory has traditionally been associated with neural processing in fronto-parietal networks, recent work emphasizes the importance of medial temporal lobe structures (MTL) for short-term memory of configural information. Increases in MTL neuronal activity during the active maintenance (AM) period between stimulus exposure and recognition test correlate with subsequent memory performance, indicating a role for MTL in transitioning current activity to long-term encoding. Importantly, cholinergic neurotransmission mediates AM processes as shown by systemic drug administration to human subjects: both MTL activity during AM and subsequent recall are increased by cholinesterase inhibitors and decreased by muscarinic acetylcholine receptor antagonists. Our current work addresses whether cholinergic activity during AM occurs locally in MTL. Human subjects complete a configural visual object recognition task that can be solved only by remembering feature arrangements rather than individual features themselves. BOLD-fMRI results confirm that neuronal activity increases in right MTL during AM between stimulus exposure and recognition test. Preliminary functional 1H-MR spectroscopy results show rises in signal peaks from unbound choline-containing metabolites in a 2x2x3cm voxel including right parahippocampal gyrus and hippocampus, suggesting short-timescale (60 second) increases in MTL cholinergic metabolism during AM of task information. No changes during task blocks are observed for other major MR spectroscopy peaks, in particular those associated with N-acetylaspartate or creatine.

Disclosures: **K. Shattuck:** None. **J.W. VanMeter:** None.

Poster

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Title: Direct recordings of sustained theta-band electrical activity in the human auditory cortex during working memory for tones

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Abstract: Working memory is the capacity to hold and manipulate behaviourally relevant information in mind in the absence of ongoing sensory input. Here we tested the hypothesis that auditory working memory for tones requires ongoing activity in auditory cortex, and examined the form of such activity in neuronal ensembles. We recorded local field potentials from two human subjects undergoing invasive monitoring for pre-surgical localization of epileptic foci. The subjects were implanted with depth electrodes along the axis of Heschl's gyrus (HG) containing primary cortex in the medial part, and subdural grids over temporal and frontal cortex. Following a visual alert subjects were presented with a pair of tones (0.5 s duration, 1 s ISI) belonging to two different categories ('Low': 300-570 Hz; 'High': 2000 -2800 Hz). A visual cue (1.5 s) then informed the subjects which tone (first or second) to keep in mind. A 3 s retention period was followed by a tone which could be the same or different (frequency difference ±20%) from the tone held in mind. The subjects made a same/different decision by pressing a button. A total of 96 trials (48 each of 'Low' and 'High' tone retention) were presented. We measured average ERPs and carried out single-trial time-frequency analysis using a wavelet transform. Both the magnitude of ERPs (~100 ms after stimulus onset) and gamma-band (60-120 Hz) power in electrodes located in HG and lateral superior temporal gyrus (STG) showed category-specific responses during the perception of tones. High tones elicited stronger responses in medial HG and low tones in lateral HG. During the retention period, a sustained theta-band (2-6 Hz) activity was observed in all contacts that showed gamma-band responses during perception. Power in this band also showed a recency effect: a greater response in HG electrodes was observed for retention of the most recently presented (second) tone. On the STG, however, the opposite effect was observed: a greater 2-6 Hz power for retention of the first compared to the second tone. The data demonstrate: 1) theta-band correlates of tone retention in auditory cortex in the same neural ensembles that are active in the gamma band during perception 2) neural bases in the auditory cortex for interference effects within tonal working memory.

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Poster

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Topic: F.01. Human Cognition and Behavior

Title: Are default mode and task network activity anti-correlated?

Authors: *J. M. JANSMA[1], T. R. VAN RAALTEN[2], J. H. CALLICOTT[3], N. F. RAMSEY[1]
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Abstract: Background. The default mode network (DMN) is defined as a set of brain regions that decrease their activity during goal oriented behavior. It has been hypothesized that the level of decrease in DMN is anti-correlated with the level of increase in the network of task specific regions that increase their activity during goal oriented behavior. However, there is yet little actual evidence to support this hypothesis. In this study we tested this hypothesis by measuring the change in activity in DMN and task specific activated regions before and after practice of a working memory task. As a control measurement, we measured and correlated the within network change in activity between regions in left and right hemisphere. Method. 46 healthy volunteers participated in the fMRI experiment, and performed a Sternberg task with novel and a shortly practiced target-set. Scan parameters: 3T GE Scanner; TE/TR = 30/2000, FA = 90, FOV = 24, voxel size = 3.75 x 3.75 x 5, matrix = 64 x 64 x 24, 192 reps. Scan preprocessing included movement correction, normalization into MNI space, spatially smoothing (FWHM: 8mm), voxel-wise high-pass filtering (0.0005 Hz), voxel-wise signal normalization. Individual subject analysis was performed according to a blocked design, with regressors for the novel and practiced condition. We identified regions of interest (ROIS) by clustering voxels that showed a significant effect of practice ($|t| > 5.1$) and divided these regions in two groups: regions that were activated in the novel condition (task network ('TN')) and regions that were deactivated in the novel condition (DMN). We calculated the correlation between the average activity change in DMN and TN, as well as in left and right ROIs is within DMN and within TN. Results. The within network correlation between left and right hemisphere appeared to be significant and very high for both the TN ($r = 0.78$; $p < 0.001$) and DMN ($r = 0.65$; $p < 0.001$). The correlation between the change in activity in TN and DMN appeared not to be not significant ($p = -0.24$; $p = 0.11$) Discussion. Our results indicate that on a network level, the change in activity in TN is not correlated with the change in activity in DMN, while our study was powerful enough to detect network correlations, as demonstrated by the observed strong correlation between left and right hemisphere in both TN and DMN. This result suggest that there is no strong functional connectivity between the DMN and TN. This result however does not imply that there are no subnetworks or single regions within the DMN and TN that do show anticorrelated activity.

Disclosures: **J.M. Jansma:** None. **T.R. van Raalten:** None. **J.H. Callicott:** None. **N.F. Ramsey:** None.

Poster

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Support: DFG grant Mu1364/4-1

Title: Attentional filtering and working memory storage in mild cognitive impairment and Parkinson's disease

Authors: ***N. G. MUELLER**, J. BLATT, A. VELLAGE
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Abstract: Working memory (WM) involves the two subprocesses attentional selection (i.e. filtering out of irrelevant information) and information storage. These two processes have been proposed to be sustained by different neurotransmitters, namely acetylcholine for attentional selection and dopamine for memory storage. This hypothesis is challenged by the assumption that a dopaminergic deficit leads to filtering rather than storage deficits. We tested the these hypotheses in two patient cohorts which either served as models for a cholinergic or a dopaminergic deficit. The first group comprised 18 patients with amnestic mild cognitive impairment (aMCI), the second 22 patients with Parkinson's disease (PD). The two groups did not differ regarding their overall cognitive abilities. Both patient groups as well as a control group without neurological deficits (n=25) performed a visuo-spatial working memory task in which both the necessity to filter out irrelevant information and memory load, i.e. the number of items to be held in memory, were manipulated. In accordance with the primary hypothesis, aMCI patients displayed problems with filtering, i.e. were especially impaired when the task required ignoring distracting stimuli. PD patients on the other hand showed difficulties when memory load was increased suggesting that they mainly suffered from a storage deficit.

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Poster

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Topic: F.01. Human Cognition and Behavior

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Title: rTMS/EEG of MT+ during STM for transparent motion

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Abstract: Although the role for area MT/V5 in the perceptual processing of visual motion is well established, its role in the short-term retention of motion information, during tests of short-term memory (STM) and working memory, remains uncertain. Here, we applied repetitive transcranial magnetic stimulation (rTMS) to MT+ while simultaneously recording the electroencephalogram (EEG) in healthy human subjects performing STM for visual motion. Subjects fixated centrally while the sample stimuli ─ random-dots moving in two directions, giving rise to the percept of two superimposed transparent surfaces moving in different directions - were presented for 1000 msec in an aperture to the left of fixation. One sec after sample offset, a cue indicated which of the two remembered directions was to be recalled by aligning a radial vector with the cued direction. Precision of recall was estimated from the error between subjects' response and the cued direction. In a fully crossed within-subject design, a 10 Hz, 1 sec-long, train of rTMS was delivered (or not) concurrent with either sample presentation or the delay period, and with the stimulating coil positioned over right MT+ or right post-central gyrus (somatosensory cortex as the control). rTMS delivered during sample presentation had no effect on mnemonic precision. In contrast, delay-period rTMS produced large individual differences. In some, delay-period rTMS of MT+ produced a drop in recall precision, and in others it produced an improvement. (This pattern is consistent with previous findings using other types of stimuli and targeting other areas.) Inspection of the delay period EEG data for the electrode nearest MT+ (P8) revealed that each TMS pulse within the 10 Hz train evoked roughly 3 cycles in the ERP waveform. Consistent with this observation, the time-frequency spectrogram showed that 10 Hz rTMS produced elevated power at 30 Hz and higher harmonic frequencies.

However, the behavioral effects of rTMS on MT+ did not relate to these higher frequencies. Rather, they corresponded to rTMS-related changes in delay-period spectral power in the 10-15 Hz band. Further, the direction of these changes were predicted by individual differences in this frequency band that were observed during rTMS-absent trials, and that have been shown previously to reflect trait-like differences in the spectral pattern of delay-period activity (Kundu et al., 2014).

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Poster

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Topic: F.01. Human Cognition and Behavior

Support: JSPS Garnt to NO 22220003

Title: Rinzai Zen meditators are superior to resolve semantic interference: An fMRI study based on a Stroop paradigm

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Abstract: Rinzai Zen is one of the traditional Japanese meditations, and the professional Zen monks are thought to accept the environmental world as it is, shedding their own bias that is often mediated by language. A color Stroop task is a suitable experimental paradigm to investigate such characteristic of the Zen monks, as the task requires to focus on the sensory information while ignoring higher order meaning. Given that the Zen monks are superior to avoid higher order semantic processing, the Stroop effect should disappear and neural regions related to the effect should be less activated. Nineteen Zen monks (M = 39.26, SD = 5.91) and eighteen age- and education-matched healthy male volunteers (M = 38.83, SD = 6.35) were participated in the present study. All the monks finished Zen training, which had lasted for at least four years, and mean training year was 7.53 (SD = 2.50). Participants in the matched group had not experienced meditation training. A standard color Stroop task was given in the MRI scanner, and brain regions related to the Stroop interference were measured. The Stroop task consisted of three types of block: congruent, incongruent, and neutral block. The behavioral

results of reaction time and accuracy showed a significant Stroop effect in the control group, while such interference was not observed in the meditators. As for the fMRI results, significant interactions were found in the anterior cingulate cortex, the dorsolateral prefrontal cortex, and the posterior parietal cortex, which are known to be involved in conflict resolution. Specifically, control subjects showed greater activation of the regions in the incongruent condition than the congruent condition, while meditators showed equivalent activation across conditions. Those results provide empirical evidence that Rinzai Zen monks, using cognitive control, focus on sensory processing with avoiding higher semantic processing.

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Title: Changes in global brain connectivity predict behavioural performance across task difficulty

Authors: ***D. VATANSEVER**, D. K. MENON, A. MANKTELOW, E. A. STAMATAKIS
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Abstract: Introduction: Resting state functional connectivity analysis has revealed a number of large-scale brain networks (LSNs) including default mode, fronto-parietal control, dorsal attention, somatomotor, vision, and audition. Despite the visual similarity of these LSNs to those obtained from task-based cognitive paradigms, their behavioural significance remains to be

elucidated. The aim of this study was to investigate the changes in whole-brain functional connectivity in a working memory paradigm and to relate these alterations to LSN connectivity and performance outcomes. **Methods:** A group of 22 healthy adults (19-57 years old, mean = 35.0, SD = 11.2) were scanned (Siemens Trio 3T MRI scanner) during a block designed N-Back (1,2,3-Back) paradigm. Behavioural analysis indicated a decrease in correct responses with increasing task difficulty. Subject-level, condition-specific intrinsic connectivity contrast (ICC) maps were calculated to measure global brain connectivity. Second level imaging analyses involved a one-way ANOVA to assess group level changes in ICC across the 1,2 and 3-Back blocks. The clusters with the highest ICC change were subsequently used as regions of interest (ROI) for seed-based functional connectivity analyses. Functional connectivity maps from these two ROIs were related to reaction times for correct responses. The reported clusters were corrected for multiple comparisons at the FWE 0.05 level of significance. **Results:** The greatest changes in ICC-based global connectivity across increasing task difficulty were centred on the left and right angular gyri. Seed-based functional connectivity from these two regions revealed connections to the default mode, fronto-parietal control, dorsal attention and visual networks. Increased connectivity between the left angular gyrus and posterior cingulate cortex (default mode network) predicted faster reaction times to correct responses. Increased connectivity between the right angular gyrus and the left calcarine sulcus (visual network) also predicted improved reaction times. **Conclusions:** Changes in ICC with increasing task difficulty reflected task-induced alterations in global brain connectivity. The bilateral angular gyri demonstrated the most significant ICC changes, suggesting a key role for these regions in facilitating communication between LSNs. Furthermore, functional connectivity between these two regions and both the default mode and visual networks predicted behavioural outcome, which indicates some relevance in task performance. Overall, the results suggest that LSN connectivity may contribute to working memory task execution.

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Poster

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Topic: D.05. Visual Sensory-motor Processing

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Title: Ghost in the machine: Neural mechanisms of spatial working memory

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Abstract: In a previous study we found that spatial memory can be biased toward the memoranda of the previous trial. (Ferdoash, Papadimitriou, & Snyder, SFN 2011 Poster). In the current study we look for neural correlates of this behavior in frontal memory circuits. We find clear effects of the previous memoranda on neuronal activity, but these effects are not congruent with the behavior we observe in the animal. We recorded from frontal eye fields (FEF) while three macaques performed memory guided saccades. We found markedly different neural effects in trials when the previous trial and current trial targets were far apart, compared to trials when the previous and current targets were close to one another. When the previous and current targets were far apart, the neurons activated in the previous trial remained slightly active during the current trial. A vector sum readout of activity in these trials predicts an attractive bias towards the previous trial's target location. We observed an opposite effect in trials for which the previous and current targets were close to one another. On these trials, the neurons activated in the previous trial showed reduced activation in the current trial. A vector sum readout on these trials predicts that behavioral responses will be repulsed away from the previous trial's target location. Thus the previous target location had opposite effects on the frontal memory neurons depending on whether the current target activated the same or different neurons. We conclude that frontal memory circuits show two separate carryover effects from the target location on the previous trial, depending on the relative locations of the two targets. These results suggest that either other memory circuits are involved in producing the observed behavior, or that a different, more nuanced readout of these circuits is used by the brain to produce behavior.

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Poster

555. Human Decision-Making: Cognition and Computation

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Topic: F.01. Human Cognition and Behavior

Support: European Research Council Grant (ERC-2009-AdG#250106 to E. K.)

Title: Adaptation to action-related changes in a volatile environment

Authors: *H. THÉRO, V. CHAMBON, E. KOECHLIN
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Abstract: Sense of agency refers to the feeling of controlling one's own actions and, through these actions, events in the outside world. A subjective experience of control implies the ability to discriminate the external events that are caused by our own actions from the events that are not. Ultimately, this processing of action-related changes is crucial to adapt to a volatile environment, structured with multiple informational levels. We hypothesized that such ability would depend on the active monitoring of three different types of statistics: 1) the mutual dependency between the action performed and the perceived event (i.e., action-outcome contingencies), 2) the rewarding value of the perceived event, and 3) its variability over time. In a modified reversal-learning task, human subjects played two slot machines simultaneously, and had to find out which machine was influenced by their choices. Critically, unpredictable reversals could occur so that subject's influence over one machine abruptly transferred to the other one. We found that subjects dynamically adjusted their response to these reversals, and quickly retrieved control over the relevant machine through active monitoring of 1) action-outcome contingencies, 2) the rewarding value of the machine's outcome, or 3) variability of this outcome across trials. These results showed that human subjects were influenced by these three classes of information to construct action outcomes from perceptual events. In order to further assess the respective contribution of these three factors to the feeling of control, we tested two contrasted families of models. Reinforcement-learning models make choices and guide behavior based on reward prediction errors, while Bayesian models dynamically compute optimal actions through performing inferences about states of the world, irrespective of the rewarding value associated with each alternative. The fitting of these two types of models suggests that the subjects' behavior was best explained by a reward-based heuristics of Bayesian inference. An fMRI experiment will be conducted to better understand the neural correlates of this sense of agency.

Disclosures: H. Théro: None. **V. Chambon:** None. **E. Koechlin:** None.

Poster

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Topic: F.01. Human Cognition and Behavior

Title: The role of working memory in the Iowa Gambling Task

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Abstract: The Iowa Gambling Task (IGT) was designed to study decision-making deficits in patients with ventromedial prefrontal cortex (vmPFC) damage (Bechara et al., 1994). Based on their impaired IGT performance, the Somatic Marker Hypothesis (SMH) was developed (Bechara et al., 1997; Damasio, 1996). The SMH emphasized the contribution of emotion (as opposed to cognition) to decision-making. Subsequent studies found that IGT performance was also impaired after damage to the dorsolateral prefrontal cortex (dlPFC) (Fellows & Farrah, 2005). This raises the possibility that non-emotional, i.e., cognitive processes, contribute to decision-making in the IGT. One cognitive process that is implicated in dlPFC function is working memory (WM). Presently, there is a debate regarding the relative contribution of WM and emotion to IGT performance (Dunn et al., 2006). The present study investigated the role of WM on IGT performance in two experiments. Experiment 1 investigated the effects of increasing WM load during the IGT by having participants intermittently read and remember either a social or analytical scenario. Respectively, these scenarios putatively engage the Default Mode Network (DMN which involves vmPFC) and the Task Positive Network (TPN which involves dlPFC) (Jack et al., 2013). Results revealed that males' IGT performance did not differ with either scenario condition. However, females' IGT performance, as well as their conscious knowledge of the IGT rule increased with either scenario relative to a traditional IGT (no scenarios). Experiment 2 measured participants' working memory capacity (independent of the IGT) to determine if this was correlated with IGT performance or conscious knowledge of the rule of the IGT. Subjects performed the IGT and then their WM capacity was assessed using the Automated Operation Span (AOSPAN; Unsworth et al., 2005). Results showed that there was no significant correlation between WM span and IGT performance; however, there was a significant, positive correlation between WM span and the frequency of correctly stating which two decks were the good decks (i.e., conscious knowledge of the rule). In conclusion, this study found: (1) paradoxically, in females IGT performance and knowledge of the IGT rule improved when WM was loaded with social or analytical scenarios suggesting that the type task used to load working memory can differentially affect IGT performance. (2) WM capacity was correlated with stating the correct rule on the IGT, but not on performance on the IGT, indicating that participants may be aware of the correct decision, but that they do not always perform accordingly.

Disclosures: A. Pierce: None. **M. Sauls:** None. **W.H. Overman:** None. **R. Tank:** None.

Poster

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Topic: F.01. Human Cognition and Behavior

Title: EEG correlates of activity within salience, central executive and default-mode networks after errors

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Abstract: fMRI research has revealed that activity in the 'salience network' (SN), involving medial prefrontal (MPFC) and anterior insular cortices, is thought to reflect a monitoring process engaged after salient events such as errors. This network has also been suggested to control switching between the default-mode network (DMN, more active during internally oriented mental activity), which includes posterior cingulate (PCC) and ventral medial prefrontal cortices (VMPFC), and the central executive network (CEN, more active during externally oriented activity), which includes dorsolateral prefrontal (DLPFC) and parietal cortices. Due to fMRI's limited time resolution and indirect measurement of brain activity, however, the interactions between these regions during task performance have yet to be investigated on a rapid timescale. We used EEG to analyze connectivity using seeds in three electrode sites that have previously been used to study error monitoring (FCz, MPFC), internally directed attention (Pz, PCC) and goal-directed mental activity (F5-F6, DLPFC). We sought to analyze whether time-frequency signals in these electrodes can be seen as an EEG index of the SN, DMN and CEN. We hypothesized that FCz, Pz and F5-F6 would show a stronger phase coherence with other areas attributed to the SN, DMN and CEN respectively, and that areas of the SN would correlate positively with the CEN and negatively with the DMN. In agreement with these hypotheses, our results indicated that phase coherence between the MPFC seed and all the other electrodes across the whole brain showed prominent connectivity with task-specific areas in the occipital region after errors. On the other hand, the PCC seed was significantly more connected with anterior prefrontal areas (VMPFC) while the DLPFC was more connected with parietal areas. Moreover, the time courses of these three electrodes revealed a positive correlation between the MPFC and the DLPFC ($p < 0.005$) and a negative correlation between these two and the PCC area ($p < 0.005$). These results agree with previous fMRI data that show a negative correlation of the DMN and a positive correlation of the CEN with the SN, respectively. Lastly, when compared to correct responses, our data show a stronger connectivity between the areas after errors,

supporting the idea that the SN may signal the need for enhanced cognitive control (CEN) and inhibition of non task-related activity (DMN) during situations of increased conflict. Ongoing work will further analyze the direction of the interactions between these areas and the correlations between this EEG connectivity and fMRI-derived networks.

Disclosures: A. Navarro-Cebrian: None. **A.S. Kayser:** None. **T.A. Vega:** None.

Poster

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Title: Dynamics of human anterior cingulate cortex responses during cognitive control

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Abstract: How the Anterior Cingulate Cortex (ACC) and Prefrontal Cortex (PFC) interact to orchestrate cognitive control is not known. Existing theories based on neuroimaging and single unit recordings in human dorsal ACC posit that the ACC is the source of conflict monitoring, which then triggers control processes in the PFC. Here we test this theory by recording intracranial field potentials from 1,728 electrodes implanted in 18 epilepsy patients for clinical purposes while patients performed a Stroop task. In both ACC and PFC, we find that power in the gamma band (70-100 Hz) is increased when incongruent stimuli are presented compared to congruent stimulus trials. This conflict-modulating activity arises with latencies of ~500ms after trial onset. By virtue of the high spatiotemporal resolution of the intracranial recordings, we present a characterization of the relative latencies between ACC and PFC during the Stroop task. We further describe the interactions between these two areas during cognitive control.

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Poster

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Title: Cortical activity reflecting a tactical thinking to follow a rule: An fNIRS study

Authors: ***N. MIURA**[1], N. SHIRASAWA[1], S. KANOH[2]
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Abstract: Introduction A rule prescribes human's behavior in social relationships, and it is important to follow a rule when avoiding a risk of existing in society. In order to follow the rule, a tactical thinking is required to consider the appropriate behavior within the limitation of the rule, and that appropriate behavior may be different from optimal behavior in case the rule does not exist. And, it is expected that the tactical thinking is affected by the cognitive processing to determine the appropriate behavior independently from the difficulty of behavior. In this study, we investigated a cortical activity when performing an experimental task that is operated both existence of a rule which has a risk to violation and level of task difficulty is measured by using functional near-infrared spectroscopy (fNIRS). Methods Twenty healthy Japanese volunteers participated in this study. All participants provided written informed consent to an experimental protocol approved by the Ethical Committee of Tohoku Institute of Technology. The participants performed four conditions of experimental task like a video game to catch the balls falling down from the upper part of a screen. They were instructed to catch the ball by the object which is controlled by a computer mouse, and to acquire the high score. Four conditions were prepared by changing the task difficulty and existence of rule. The task difficulty was defined by the width of the playable space where the ball falls. And, on the conditions with the rule, a score of experimental task will be reduced greatly when the ball of a specific color cannot be caught. The fNIRS data were measured from a forehead region covering the frontal to temporal area. Two regions of interest (ROI) located on the bilateral prefrontal area were determined for this analysis. A three-way repeated-measures factorial ANOVA was performed using a condition-related signal change on each ROI. Each factor was determined by the difference of task

difficulty, existence of rule, and location of the ROI. Result and discussion The result showed that significant interaction effect between the factors of the existence of rule and the location of the ROI, that is, the left lateral prefrontal area showed significant signal increase when the rule existed in the task. When the rule exists, the subject has to catch the ball with the specific color to avoid a decline in his/her score. Then, it is necessary to take the tactics for catching more preferentially than the balls with different color in order to get higher score. Thus, increase of the left prefrontal activation reflects the cognitive processing underpinning that tactical thinking in consideration of the rule.

Disclosures: **N. Miura:** None. **S. Kanoh:** None. **N. Shirasawa:** None.

Poster

555. Human Decision-Making: Cognition and Computation

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Support: Lundbeck Foundation, Grant of Excellence "ContAct" R59 A5399

Copenhagen University SUND-FAK PhD Award

Title: Transcranial magnetic stimulation in human inferior frontal cortex alters voluntary action decisions

Authors: ***S. ANGSTMANN**[1], J. ZHANG[2], B. HALLSSON[1], K. H. MADSEN[1], J. B. ROWE[2], H. R. SIEBNER[1]
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Abstract: Voluntary actions involve a special form of decision-making process that select between action alternatives. Our previous study proposed that during voluntary action decisions, the intentions of selecting different actions are accumulated over time and competed against each other until a response threshold is reached, and the right inferior frontal gyrus (rIFG) plays a crucial role in mediating people's tendency to inhibit the repetition of a previous action. Here, we set out to test whether voluntary actions showed altered characteristics when transiently perturbing rIFG by combining transcranial magnetic stimulation (TMS) and functional MRI. 15 healthy subjects performed a finger-tapping task in two fMRI sessions (active and inactive sham

TMS stimulation session, 80% and 30% resting motor threshold) on separate days. In each session, the subjects were instructed to voluntarily choose any one of three permitted buttons (480 trials) or to press with a specified button (240 trials). Before each session, the rIFG was localized on the basis of our previous published coordinates (x = 56, y = 14, z = -2) and stimulated using continuous theta-burst TMS (cTBS, 600 pulses). We found longer response times for choice than for specified trials. An interaction effect between trial type (choice vs. specified) and repetition characteristics indicated that response in the previous trial had different influences on the current trial. Active TMS shortened the response times both in the choice and in the specified condition, but irrespectively of response history ($p < .05$). This effect was observable in the first 30 to 35 minutes after stimulation which is consistent with previous reports of the duration of cTBS effects. The shortened response time after TMS suggests an unspecific pre-charging of all valid/specified neural accumulators via a rIFG pathway. This explains why the relative response pattern remains unchanged but results in a global response time decrease.

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Poster

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Support: Canadian Institutes of Health Research (CIHR)

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Title: Identification of context enables use of model-based learning strategies in value-based decision making

Authors: *M. BALCARRAS[1], O. ABID[1], T. WOMELSDORF[2]
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Abstract: Decision-making strategies, such as using an internal model versus habitual model-free responses, provide advantages when used correctly, but deployment of an appropriate decision-making strategy requires correctly identifying the current context. Models of value-based decision-making often assume that the subject identifies the task context correctly (Wilson and Niv, 2011, Daw et al., 2005), however it is not clear that even well-informed subjects understand the experimental framework as assumed by the investigator (Shteingart and Loewenstein, 2014). We designed a task to test how behavioural adaptation to changing task conditions reflects latent identification of a decision-making context and utilization of a decision-making strategy. In our task subjects are required to learn stimulus values through trial and error, with discrete trials grouped into blocks by stimulus sets. In half the blocks, different reward probability is associated with a particular stimulus feature (i.e. just shape or colour), whereas in the other half of blocks, reward probability is associated with combinations of features that form an object. Importantly, blocks are organized into repeating pairs such that an object block precedes a feature block followed by a second pair using a new stimulus feature set but with the same relevant feature type as in the first feature block. Subjects can either use feedback to learn stimulus values and receive rewarding outcomes, a model-free strategy, or subjects can improve performance by learning the structure of block pairs, a model-based strategy. We analyzed subject choices across blocks and sessions to determine markers of model-free and model-based strategies. We observed that subjects (n=12, 2 sessions) reached a performance criterion of 80% average correct choices in 87.5% of all blocks (70% of subjects reached criterion in all blocks) indicating that they were able to use locally learned values to guide future choices. Subjects (86%) also took longer to reach criterion in feature blocks, reflecting the greater complexity of feature blocks. Importantly though, we observed 68% of subjects increased their learning rate and overall performance in the second feature block compared to the first session, and all of these subjects outperformed those subjects that don't show this increase. These results indicate that context identification and structure learning enable use of model-based strategies that improve performance in complex task environments. Developing markers of decision-making strategies is key for proper model development and improves the impact of fitting model components with neural activity.

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Poster

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Topic: F.01. Human Cognition and Behavior

Support: University of Minnesota McKnight Presidential Chair in Cogntive Neuroscience

Title: Cognitive mechanisms underlying instructed choice exploration of small city maps

Authors: *S. SAKELLARIDI[1], P. CHRISTOVA[2], J. PEPONIS[3], A. P. GEORGOPOULOS[1,2]
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Abstract: The ability to explore novel environments and make spatial decisions, such as selecting a place to live or choosing a landmark to visit, is a fundamental and highly evolved behavior that requires the coordination of cognitive functions. But how people explore novel environments to make spatial decisions is still poorly understood. To address this question, we conducted a psychophysical experiment in which subjects explored a set of real maps of various U.S. metropolitan cities, exemplifying different street network layouts, in order to place a hypothetical post office at one of two possible locations. Results suggest that people developed highly stereotyped strategies for evaluating and comparing the two potential options. Particularly, monitoring subjects' eye positions revealed restricted map exploration determined by the position of the two alternative options and the centers of the maps. We also found that subjects were continuously exploring the areas around the two options and the center of the map by repeatedly looking back and forth between them before deciding which option to choose, presumably implementing a comparison process. Another key finding is that the selection of an option was highly associated with the time spending exploring the area around that option, with subjects spending more time exploring the area around the option they finally selected than the option that was not selected at the end of the trial. Finally, we found that first and last fixation sets have an important role in influencing the value of the alternative options, and thus, biasing the decision. Overall, the initial fixations favored the location ultimately chosen. The first fixation bias occurred as early as 230 ms after the stimulus onset, whereas the final fixation bias appeared at about 345 ms prior to make a decision. All these findings suggest that human strategies are highly stereotyped in exploring novel environments for making spatial decisions, and these strategies share many common characteristics with the way that humans make economic choices.

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Topic: F.01. Human Cognition and Behavior

Title: Identifying the relative influence of multiple prior events on predictions of a probabilistic future: An artificial neural network analysis

Authors: J. FREEDMAN[1], A. AMLIE-WOLF[2], R. WITTENBERG[1], O. SHOHAM[1], S. R. ARONSON[3], *M. D. LOOSE[1]
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Abstract: When predicting future events most individuals tend to match their rate of predicting an outcome to the probability of that outcome occurring. We used an artificial neural network trained via back-propagation (ANN) to test the hypothesis that multiple types of information influence such predictions. The model used events of recent previous trials as inputs (14 input nodes) with two or five hidden nodes and two output nodes. The sign of the difference between the two output nodes determined the network's guess. Training sets were constructed (N=22) from behavioral data collected for an event-related potential study in which the probability ratio was 63:37 (Society for Neuroscience 2012, 101.22). Each training set contained a 50:50 ratio of high and low probability predictions while the test set retained the original choice ratio (range of high predictions: 54% - 82%). Ten networks, each initialized with random weight values, were trained for each of the 22 data sets. After training, the ANNs were more accurate compared to before training at predicting subjects' choices with the training set (mean improvement: 14.9 %, SD = 5.0%, $p < 0.001$), as well as with the test set (mean improvement: 9.5%, SD = 11.2%, $p < 0.001$). We compared 3 metrics for rank ordering the contributions of the various inputs to accuracy of the networks. We tested a new metric that used the magnitude of output node difference values (as a measure of network confidence) and two established metrics, a lesion method and a connection weight strength algorithm. We examined four types of input patterns that could have influenced each decision: Patterns that defied the expected probability, behavioral streaks, accuracy of the previous guess, and speed of the previous guess. The strongest correlations between input pattern metrics and actual behavior were achieved with the synaptic weight algorithm (mean r = .83) and the confidence metric (mean r = .81). Both metrics were more highly correlated ($p < 0.05$) than the lesion metric (mean r = .49). Each input pattern was found to be important for a subset of individuals. The interactions of two or more patterns

often were important to successful predictions by the ANN, and no one strategy was found to be strongly predictive for all participants. Our results suggest that for simple probabilistic predictions people vary their strategies depending upon recent events and their decisions are influenced simultaneously by multiple types of information. We infer that attempting to fit probabilistic decisions with a single strategy like the win-stay lose-shift or the expectation matching strategy will fail to capture significant components of the decision making process.

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Poster

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Support: European Research Council Grant (ERC-2009-AdG#250106 to E. K.)

Title: Cue and outcome-based task retrieval in human

Authors: *M. E. EKOVICH, E. KOECHLIN
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Abstract: Humans need to adapt to uncertain, changing, and open-ended environments. In such situations, decision-making involves exploring, adjusting and exploiting multiple task sets - defined as flexible sensorimotor mappings associating stimuli, actions, and expected outcomes. Collins and Kœchlin proposed a computational model that controls the creation, learning, storage, retrieval, and selection of such task sets for driving action (Collins & Kœchlin, 2012, PloS biology). The model monitors the reliability of a collection of concurrent task sets - i.e., the ability of alternative task sets to correctly predict action outcomes. Task set reliability is updated in a Bayesian manner according to outcomes and contextual information and arbitrates between exploiting the most reliable task set or exploring new ones to drive action. It has recently been shown that the reliability of alternative learned task sets is monitored in frontopolar cortex (Donoso, Collins & Kœchlin, submitted). However it remains unclear how task sets are actually retrieved for subsequent use. The goal of this study is to investigate the neural mechanisms that subserve the retrieval of stored task sets according to contextual cues or action outcomes. We designed an fMRI experiment requiring healthy human subjects to learn by trials and errors and

to switch between multiple task sets associated with various contextual cues. Experimental conditions varied unpredictably such that (i) previously learned task sets re-occurred with either the same or new contextual cues, (ii) new task sets that needed to be learned occurred with new cues or previously encountered ones. Behavioral results and model fits show that subjects learned, monitored and switched across an expanding repertoire of task sets as predicted by the computational model. More specifically: (i)Known contextual cues were used proactively to select the corresponding task set (ii)When previously learned task sets re-occurred with unknown contextual cues, subjects selected the stored task set based on action outcomes. Preliminary fMRI results revealed that lateral prefrontal cortex is engaged in the selection process in both cases. However distinct networks are involved depending on whether the retrieval is cue or outcome-based. On the one hand, cue-based retrieval relies on a ventral pathway including ventromedial prefrontal cortex, striatum and bilateral hippocampus. On the other hand, outcome-based retrieval relies on a frontal network including frontopolar cortex and dorsolateral frontal cortex. Overall these results suggest a possible dissociation between two types of task sets retrievals in humans.

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Poster

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Support: NSERC Discovery Grant

Title: Electroencephalographic correlates of system two decision making

Authors: *O. E. KRIGOLSON, C. HASSALL
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Abstract: Evidence suggests that human decision-making is the product of two distinct systems - a fast system that supplies well known or reflexive answers (System I) and a slow system that supplies more deliberative answers (System II)(Kahneman, 2011). Extending from this, decision making theory posits that early in learning System II is engaged as one works to link decision options to outcomes. However, with learning, and as the linking of decision options to outcomes becomes automated, there is a shift to rely on System I to provide intuitive responses. Here, we

hypothesized that early in learning decisions made by System II would be characterized by enhanced alpha activity in the human electroencephalogram over pre-frontal and medial-frontal cortex, and that with learning, a decrease in alpha activity would reflect a shift in reliance to System I. To test this, we had participants perform a perceptual learning task that required them to categorize "blobs" based on a subordinate level naming structure. Initially, participants had to classify the blobs into one of four families. In a key manipulation, at the midpoint of the experiment two new families of blobs were added while two were removed - thus, at this juncture in the experiment, there were "new" and "familiar" blobs. Our analysis of the EEG data revealed a decrease in alpha activity from the start of the experiment to the midpoint at the time of the blob classification decision. After the midpoint, enhanced alpha activity was observed over pre-frontal and medial-frontal cortex when classifying new blobs but the alpha activity associated for the classification of familiar blobs was still reduced. We suggest that the decrease in alpha activity with learning reflects a diminishment in the activation of the System II decision network and thus is reflective of a shift to the System I decision network. Importantly, our results provide novel electroencephalographic evidence for the two system model of decision making proposed by Kahneman and others.

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Poster

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Support: NIH Grant R01-DA029149

Title: Is there overlapping neural activation from different sources of decision difficulty?

Authors: *B. E. KIM-VIECHNICKI, J. W. KABLE
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Abstract: Objective: The subjective experience of difficulty can feel similar across many different decision situations, but it is unknown whether this similarity in experience is accompanied by a neural signal that is common to different forms of decision difficulty. Regions in a fronto-parietal network have been implicated in detecting subjective difficulty and responding to cognitive demand of many different forms. Previous studies have typically used a

single manipulation of cognitive demand, however. This study aims to address these limitations with a cognitive conjunctive design across multiple kinds of decision difficulty. Methods: We measured BOLD signal during a well-studied delay discounting task, with four different manipulations to induce increased difficulty: 1) time pressure, 2) more options, 3) disfluency, and 4) options close in subjective value. Easy control trials did not induce time pressure and involved only two options presented numerically that were far apart in subjective value. Trials were blocked and after each block subjects rated how difficult they thought the last set of decisions were. Results and Conclusions: All four of the manipulation blocks were rated as significantly more difficult than the easy control blocks. Subjects also spent significantly more time on hard trials than on control trials for the disfluency, 4-options, and close in value manipulations and spent significantly less time on hard trials than on control trials for the time pressure manipulation. Not surprisingly, unique activity was found in left inferior frontal regions for disfluency and early visual regions for increased number of options. We also found widespread activation in fronto-parietal regions for all manipulations. Surprisingly, however, we did not find a common frontal region of increased activation that was reliable across all four manipulations, and only found a small overlapping area in parietal cortex. These results suggest that, while all forms of decision difficulty are associated with greater activation in fronto-parietal regions generally, these activations in response to cognitive demand may not overlap at a more fine-grained level.

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Poster

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Dana Foundation

Title: Frequency-dependent feedback-related information exchange in the human medial and lateral prefrontal cortex

Authors: *S. A. SHETH[1], G. BANKS[1], M. K. MIAN[2], S. R. PATEL[2], E. N. ESKANDAR[2], E. H. SMITH[1]

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Abstract: Rapid adaptation to a changing environment is integral for survival. Feedback, both internally and externally generated, plays a pivotal role in our ability to learn to survive in complex environments. Several salient feedback signals have been characterized in the human brain using scalp electroencephalography. One such signal, the feedback-related negativity (FRN), is a difference in the e¬¬voked potential between trials in which the subject receives positive and negative feedback. FRN is usually observed 100 to 200 milliseconds after a subject has registered a behavioral response, and is greatest on central EEG contacts. Source localization studies have implicated the dorsal anterior cingulate cortex as generating this potential. Recent studies in primates have provided evidence for an anatomical substrate for interactions among medial and lateral prefrontal regions, and physiological evidence for how medial and lateral prefrontal cortex may be interacting during environmental feedback. Here we show a frequency-dependent mechanism for information exchange between the human medial and lateral prefrontal cortices during feedback. This study examined intracranial electrocorticographic (ECoG) signals in 6 patients undergoing monitoring for medically refractory epilepsy. We examined local field potentials (LFP) recorded from the medial and lateral contacts on eight-contact depth electrodes implanted through the mediolateral extent of prefrontal cortex (12 left hemisphere and 8 right hemisphere). Recordings were acquired while patients performed the Multi-Source Interference Task, a Stroop-like cognitive task, with alternating feedback and non-feedback blocks. FRN was observed in the low frequency LFP on all contacts, though was significantly greater, relative to event-related potential size, on the medial contacts. FRN observed in high frequency LFP was also significantly greater on medial contacts, and in the left hemisphere. LFP coherence in theta and high gamma bands increased for feedback trials during the feedback period, compared with a baseline period. Conditional mutual information and Granger causality support medial prefrontal regions leading lateral prefrontal regions in processing feedback. These human ECoG recordings describe an oscillatory mechanism for propagation of feedback related information between medial and lateral prefrontal cortex. These results have implications for understanding cognitive control and reinforcement learning.

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Poster

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Title: Measuring and manipulating satisficing decision strategies in models, humans, and monkeys

Authors: H. OH, P. ZHU, K. RAFIE, S. FERRARI, J. BECK, T. EGNER, *M. A. SOMMER
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Abstract: The study of rational decision-making assumes that decision-makers have perfect knowledge of possible choices and outcome probabilities, as well as ample time and computational resources to optimally integrate this information. In real life, however, some decision-relevant information is typically unknown or uncertain. Thus natural decision-making may be bounded by limited time and mental resources. Humans are thought to overcome such limitations through satisficing: fast but "good-enough" heuristic decision-making that prioritizes some sources of information while ignoring others. To start to elucidate the principles that govern satisficing, we developed a new protocol for measuring and manipulating decision strategies in a probabilistic classification task. The paradigm is suitable for testing computational models, humans, and non-human primates. Here we focus on human subjects. On each trial, participants were presented with compound cue stimuli consisting of multiple features, each of which could take multiple values, which can be treated as random variables associated with some implicit conditional distributions (reward probabilities) that must be learned via probabilistic feedback. We manipulated the amount of decision time available, and employed Bayesian inference to quantify subjects' decision-making strategies. We found that under low time pressure, participants performed comparable to an ideal observer, correctly weighting and integrating all available cues to arrive at near-optimal decisions using a Bayesian Network. With increasing time pressure, however, subjects gradually shifted their decision strategies by taking only a subset of the most predictive cues into account to arrive at fast, yet good-enough, decisions. Our data document adaptive re-weighting of cue values to compensate for limited decision time and thus support "bounded rationality" models of human decision-making.

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Poster

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Coghlan Fellowship at Stanford

Stanford Neuroventures

Title: Exploration and exploitation in action selection in humans depends on striatal GABA

Authors: *A. S. ROKEM[1], G. S. TANG[1], T. LUCAS[2], A. THAMRONGRATTANARIT[3], L. BALTUSIS[1], R. F. DOUGHERTY[1], R. MATA[4], L. L. CARSTENSEN[1], G. R. SAMANEZ-LARKIN[5], S. M. MCCLURE[1]
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Abstract: Balancing exploration of the environment with exploitation of known sources of reward is a ubiquitous behavioral challenge. Optimal performance requires exploring sufficiently to discover the best behaviors for each situation. Simultaneously, exploring too long runs the risk of deviating from known sources of reward only to sample novel, but inferior alternatives. Various strategies may be used to resolve exploration-exploitation trade-offs. One strategy that has received particular attention is to maintain estimates of uncertainty about behaviors and adaptively sample from unknown, but potentially valuable actions (Frank et al., 2009). An alternative strategy is simply to incorporate noise into the decision-making process so that choices do not always follow the option with the highest expected reward. In simple decision environments, such as multi-armed bandit tasks, this noise-based exploratory strategy best describes behavior (Daw et al., 2006). Our project had two goals. First, we wished to develop a multi-armed bandit task for which (1) the optimal exploration-exploitation trade-off varies in an adaptive manner, and for which (2) a noise-based exploration strategy accounts for behavior. To accomplish this, we took advantage of the fact that for longer time horizons, greater exploration increases total expected reward. We used a probabilistic learning task (multi-armed bandit) with two different task lengths. As predicted, behavior was well matched by a noise-based exploration model with greater exploration in the longer task condition. Second, we wished to test the hypothesis that noise-based exploration is related to the viability of the GABAergic inhibitory

network in the striatum. The basal ganglia are known to mediate action selection, and activity of striatal medium spiny neurons (MSNs) is believed to be critical to the selection process. Specifically, lateral inhibition among MSNs as well as from GABAergic interneurons is believed to underlie the competition among possible actions. We constructed a neural network model that accounts for action selection in terms of the inhibition applied by striatal MSNs. The model predicted that greater density of MSN processes (and hence greater total striatal GABA) would give rise to greater exploration. To test this prediction, we measured 1-H Magnetic Resonance Spectroscopy (MRS) and assessed *in vivo* concentrations of GABA in the striatum. In agreement with our model, we found that participants with larger striatal GABA concentrations had higher estimated exploration, suggesting that striatal GABA levels play an important role in mediating exploration.

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Poster

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Title: Cortical-subcortical circuits underlying parallel behavioural control systems

Authors: ***V. VOON**[1], L. MORRIS[1], P. KUNDU[1], M. IRVINE[1], T. ROBBINS[1], E. BULLMORE[1], N. DAW[2]
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Abstract: Background: The capacity to flexibly adapt behavior is crucial to negotiating the vicissitudes of daily life. Behavioural flexibility or compulsivity can be divided into subtypes orchestrated by overlapping yet distinct regions of fronto-striatal circuitry. These processes include habit formation, reversal learning (RL), and extradimensional set shifting (ED) and more basic processes of perseveration and shifting. We focus on the intrinsic functional connectivity of neural networks underlying habit formation (model-free learning) or the tendency to select a previously reinforced action, which co-exists in parallel with goal-directed behaviours (model-

based learning) involving forward planning of the task structure (Daw, Neuron, 2011; Voon, Mol Psychiatry, 2014). We compare these with RL and its counterpart, acquisition learning and ED. We further examine the influence of outcome valence on acquisition and RL. Methods: Resting-state fMRI was collected from 66 healthy volunteers. We used a novel multi-echo planar sequence allowing a more precise distinction between neuronal (BOLD-like) and non-neuronal (non-BOLD-like) components allowing for enhanced signal-to-noise ratio (Kundu, PNAS, 2013). Seed-based functional connectivity of distinct fronto-striatal circuits were mapped. Behavioural function was mapped onto fronto-striatal circuitry based on the compulsivity measures of habit formation using a two-step task, RL using a one-step task and a task testing ED shift. Acquisition learning and RL with both positive and negative outcomes were assessed. Results: We show a fine grained parcellation of motor, cognitive and limbic fronto-striatal connectivity maps. Goal-directed model-based learning using a two-step task was associated with enhanced mOFC-VS connectivity, and habitual model-free learning was associated with enhanced SMA-posterior putamen connectivity. Reward-related acquisition learning errors using a one-step task was associated with enhanced vmPFC-VS and reward reversal-learning errors were associated with enhanced mOFC-VS connectivity with loss errors associated with the opposite direction. ED shift errors were associated with lower dlPFC-VS connectivity. Conclusions: We map the functional substrate of parallel fronto-striatal circuits highlighting the distinct yet overlapping neural networks of subtypes of compulsivity.

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Poster

555. Human Decision-Making: Cognition and Computation

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 555.17/TT36

Topic: F.01. Human Cognition and Behavior

Support: NSERC Graduate Scholarship

NSERC Discovery Grant

Title: Electroencephalographic evidence for the sparsity heuristic

Authors: ***C. D. HASSALL**, P. C. CONNOR, T. P. TRAPPENBERG, O. E. KRIGOLSON
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Abstract: Decision making in a complex world is aided by heuristics or “mental shortcuts”. One such heuristic, sparsity, guides our choices by focusing learning on only a small subset of available features. Alternative strategies, such as valuing specific combinations of features, have been shown to result in poorer performance when sparsity information is available (that is, when only certain dimensions are relevant). Here, we extended existing work suggesting that humans use sparsity when making complex choices, and found novel behavioral and electroencephalographic (EEG) evidence implicating selective attention in this process. In particular, we had participants choose between two complex stimuli that varied randomly along three dimensions: shape, color, and texture. Only one of these dimensions was relevant in each block, and reward probability was determined by a value map within the target dimension. Target dimensions and value maps were chosen randomly for each block, and unknown to participants. Thus, on each trial there was always a higher valued stimulus and a lower valued stimulus, as determined by the value map within the target dimension. We observed that participants were faster to respond to attentional probes that appeared unexpectedly within higher valued stimuli compared to probes within lower valued stimuli. Our EEG result supported our behavioural result: we observed a value-dependent change in the N2pc, a component of the human event-related potential (ERP) related to attention. Specifically, we observed an increase in the N2pc in response to higher valued stimuli compared to lower valued stimuli. Thus, our behavioral and ERP results suggest that selective attention may be one of the mechanisms behind the use of sparsity in decision making.

Disclosures: C.D. Hassall: None. **P.C. Connor:** None. **T.P. Trappenberg:** None. **O.E. Krigolson:** None.

Poster

555. Human Decision-Making: Cognition and Computation

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Support: Defense Science and Technology Agency Singapore (POD0713897)

National Medical Research Council Singapore (StaR/0004/2008)

Title: Calibration of persistence based on delay-timing distribution is preserved in sleep deprivation

Authors: *S. A. MASSAR, M. W. L. CHEE
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Abstract: Persistence in waiting for a reward depends on the expectations of the statistical distribution of the possible delay durations (McGuire & Kable 2013). Under some distributions it can be appropriate to be completely persistent, whereas in other situations more optimal returns can be obtained if waiting is abandoned after a short delay. In a recent study it has been shown that human decision makers can accurately calibrate their persistence based on the statistical distribution of delays that they are exposed to (McGuire & Kable 2012). In the present study we sought to examine whether this ability is affected by sleep deprivation. Studies on the effects of insufficient sleep on intertemporal choice have yielded mixed findings. Traditional methods of delay discounting using delays ranging from days to months have shown no changes under sleep deprivation. However, some studies in which the delays, in the range of seconds, were actually experienced during the decision-making task show indications of decreased tolerance to delays during sleep deprivation. Whether this would affect the ability to discriminate different delay distributions and adjust behavior accordingly is not known. Here, participants performed a willingness-to-wait task once after a night of normal sleep, and once after a night of sleep deprivation. The task required subjects to make continuous decisions about whether to wait for a larger reward (15 ¢) or to abandon waiting in favor of a smaller reward (1¢). Crucially, the delay durations were drawn from either a uniform distribution (0-16 seconds) where being persistent is the optimal strategy, or alternatively from a heavy-tailed distribution where total persistence would lead to a suboptimal outcome since occasionally very long delays were presented (up to 90 seconds). In line with earlier findings participants showed stronger persistence under the uniform distribution compared to the heavy-tailed distribution. Interestingly, this ability to distinguish different delay distributions and adjust behavior accordingly was mostly preserved after sleep deprivation. Despite marked increases in subjective sleepiness and impairments of attentional performance in a psychomotor vigilance task, participants maintained sensitivity to delay distributions in the willingness-to-wait task.

Disclosures: S.A. Massar: None. **M.W.L. Chee:** None.

Poster

555. Human Decision-Making: Cognition and Computation

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Topic: F.01. Human Cognition and Behavior

Support: NSF CRCNS IIS1207833

John Templeton Foundation

Title: A general form for state-space representation in frontal and temporal cortex

Authors: *M. M. BOTVINICK[1], C. DIUK[2], D. YEE[3], J. CHEONG[1], A. WEINSTEIN[1], Y. NIV[1], A. BARTO[4]
[1]Princeton Univ., Princeton, NJ; [2]Facebook, Inc., Menlo Park, CA; [3]Washington Univ., St. Louis, MO; [4]Univ. of Massachusetts, Amherst, MA

Abstract: Data from frontal and temporal cortex convincingly show that the neural representation of particular stimuli can be strongly shaped by the tasks into which they fit. Although this has been demonstrated mainly in the context of simple classification tasks, it suggests something general, namely that the neural representations of state space — the set of stimuli or situations arising within a particular task — assume a form that facilitates the computations necessary for task performance. The present work takes this broad idea and translates it into a formally explicit account of how task structure influences state-space representation, culminating in an experimental test. Our computational theory starts from the assumption that any task can be understood as comprising a set of action-outcome contingencies and an objective function favoring reward-maximizing behaviors. Optimal control theory identifies a fundamental consistency constraint in this context, expressed in the famous Bellman equation (the foundation for well known reinforcement learning algorithms). Building on previous work in machine learning, we note that the Bellman equation implicitly embeds a particular kind of state-space representation, one that encodes individual states or stimuli in terms of what they predict about the future. On a theoretical level, this result is intriguing for its deep connections with a variety of other formal constructs, including slow feature analysis, spectral embedding, and principal components analysis. However, our focus in the present work is on implications for neuroscience. We hypothesize that the brain represents state space in precisely the format recommended by the Bellman equation. This gives rise to a specific, testable prediction, which is that task stimuli that are associated with overlapping predictions should give rise to similar neural representations. We tested for this effect in human subjects, using the Tower of Hanoi, a problem-solving task that has been used extensively in research on executive function and planning. Using fMRI and multi-voxel pattern analysis, looking within areas of inferior frontal and anterior and medial temporal cortex previously implicated in state-space encoding, we confirmed that the representation of state-space in these regions displays a similarity structure consistent with the predictions of the theoretical account. The results reported have direct implications not only for understanding state-space encoding and the function of the cortical regions involved, but also for understanding how the brain decomposes tasks into coherent subtasks, thereby facilitating planning in complex domains.

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Poster

555. Human Decision-Making: Cognition and Computation

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Topic: F.01. Human Cognition and Behavior

Support: NIH Grant EY019889

Title: Source of choice behavior variability in probabilistic events: Noise vs suboptimal inference

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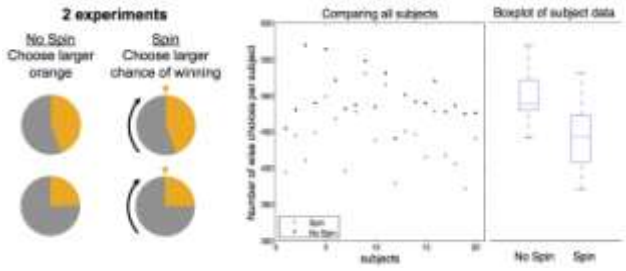
[1]Dept. of Psychology, [2]Inst. for the Interdisciplinary Study of Decision Making, [3]Ctr. for Neural Sci., New York Univ., New York, NY

Abstract: INTRODUCTION Choice behavior can vary from trial to trial, even with near-identical stimuli. This variability is often attributed to internal noise (i.e. the brain's sensory limitations). A recent work proposed the alternative idea that, in theory, variability could also arise from suboptimal inference [1]. It is, however, difficult to attribute the exact source of variability due to confoundment. We designed two complementary tasks in an attempt to isolate the sources. METHODS During each trial in Task 1 (i.e. No Spin), a subject was presented with two roulette wheels where a fraction of each wheel is colored orange. The subject was asked to choose the wheel that has the larger proportion of orange. Making the correct choice won a monetary prize. In Task 2 (i.e. Spin), a subject was presented with the same pair of wheels and was asked to choose the wheel that has the larger chance of winning. After choosing, the wheels were spun and the subject won a monetary prize if the chosen wheel stopped in the orange. A staircase procedure was used to vary one wheel while the other acted as the test condition. There were 20 test conditions, uniformly distributed between [0.025,0.975] with 30 trials/condition. 20 naïve subjects performed both tasks. RESULTS We compared the total number of wise (i.e. correct) choices made by each subject for both tasks. While the total number of wise choices differs across subjects, all subjects made more wise choices in the No Spin task than the Spin task. The former has a median of 489/600 while the latter's median is markedly lower at 444/600.



DISCUSSION Both tasks have the same optimal solution (i.e. the wheel with more orange). The No Spin task established a baseline for internal noise error while the Spin task measured the incremental error arising from suboptimal inference. The substantial difference in the results of both tasks supports the theory that suboptimal inference is an alternative source of behavioral variability. [1] Beck JM, Ma WJ, Pitkow X, Latham PE, Pouget A. 2012. Not noisy, just wrong: the role of suboptimal inference in behavioral variability, Neuron 74(1):30-39.

Disclosures: J. Tee: None. **L.T. Maloney:** None.

Poster

555. Human Decision-Making: Cognition and Computation

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Topic: F.01. Human Cognition and Behavior

Support: NSERC Grant

CIHR Grant

Title: A core brain network for mixed-strategy decision-making

Authors: *A. C. PARR[1], B. C. COE[1], M. C. DORRIS[2], D. P. MUNOZ[1]
[1]Ctr. for Neurosci. Studies, Queen's Univ., Kingston, ON, Canada; [2]Inst. of Neurosci., Shanghai Inst. for Biol. 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 employ the use of mixed-strategies; that is, choosing among available actions unpredictably and stochastically, to avoid exploitation from opponents. The neural mechanisms that calculate abstract value representations and transform these into specific actions during human decision

making are currently not well understood. We have previously demonstrated that strategic decision-making is associated with the activation of a distributed corticostriatal network in humans; however, the contribution of motor structures to strategic action selection remains largely unknown. The current study used functional magnetic resonance imaging (fMRI) to dissociate the core elements of a strategic brain network from those brain structures involved in controlling specific motor effectors, in this case, the eye and the hand. A colour-based version of the strategic game, Matching Pennies, was played against a dynamic computer opponent that exploited biases in player's response patterns. Participants selected one of two different coloured visual targets, and were rewarded if their selection matched that of the opponent. We employed a block design, wherein participants indicated their choices with either the eye (saccade) or the hand (button-press). Brain activation during the saccade condition was contrasted with that observed during the button-press condition; any difference in response patterns should highlight processes related to controlling individual motor effectors. Strategic decision-making, *regardless of the particular effector used*, was associated with the activation of a highly distributed network including the head of the caudate nucleus, dorsolateral prefrontal, anterior cingulate, parietal, insular and orbitofrontal cortices. We propose that this network represents the key elements of a functional network underlying strategic forms of decisions. Conversely, we observed effector-specific activation of cortical motor structures, *depending on the particular effector used* to make choices. Activation of the supplementary motor area (SMA) and the pre-SMA was associated with choices made using a button press, while activation of the frontal eye fields was associated with choices made using a saccadic eye movement. These results suggest that strategic forms of decisions activate a common brain network, distinct from brain regions involved in controlling specific motor effectors.

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Poster

555. Human Decision-Making: Cognition and Computation

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Topic: F.01. Human Cognition and Behavior

Title: Dynamic normalization in cascaded decision circuits

Authors: *T. LOFARO[1], K. LOUIE[2], P. W. GLIMCHER[2]
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Abstract: Decision-making is a complex cognitive function involving multiple processes including sensation, memory, reward processing, and option selection. Although the decision process likely involves multiple brain regions, neurophysiological studies primarily focus on the activity of neurons in a single brain area and little is known about how the decision process is coordinated across different neural circuits. Here, we use a dynamic network model of value coding to characterize neural activity in interconnected decision-related brain areas. In Louie et al (2011) a static normalization model was shown to explain neural activity in monkey lateral intraparietal area (LIP) under varying value conditions. Recently, we have shown that a differential equation model of normalization qualitatively predicts both the time-independent features of the original model as well as the temporal evolution of LIP firing rates. However, these previous models did not specify how value information reaches parietal circuits and did not incorporate other brain regions such as the orbitofrontal cortex (OFC), which is thought to store information about option values. We build on our previous work to model a cascaded network from OFC to LIP again using circuit-motivated normalization models. A key feature in the development of these models is the distinct time constants associated with OFC versus LIP neural activity, a feature that allows the model to capture the long timescale range-normalization empirically observed in OFC. Two distinct models are presented and analyzed for similarities and differences; in particular, we explore dynamical properties that might be used to experimentally distinguish between these two models.

Disclosures: T. Lofaro: None. **K. Louie:** None. **P.W. Glimcher:** None.

Poster

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LU2 grant ANR-11-BSV4-0006

Title: Investigation of the effect of different dopaminergic levels on the basal ganglia action selection properties using a biologically constrained computational model

Authors: J. BELLOT[1], J. LIÉNARD[3], M. KHAMASSI[1], *B. GIRARD[2]
[1]ISIR, [2]CNRS / UPMC, Paris Cedex, France; [3]Washington State Univ., Vancouver, WA

Abstract: The basal ganglia have been widely studied for their action selection properties [1] and their link with numerous pathologies involving dopamine dis-regulations such as Parkinson's disease. Numerous computational models of the basal ganglia have been developed for these purposes. However, many of the current models of the whole basal ganglia circuitry only reproduce the global network connectivity without much constraints over the strength of the connection weights, producing an output not comparable to the actual activity of the basal ganglia in living animals. [2] developed a biologically constrained mean field model of the primates basal ganglia that produces plausible activity for the different nuclei, while having action selection properties. We developed a reduced version of this model, based on the leaky integrator formalism, by replacing the dynamics of the post synaptic potentials with the corresponding integrated activity over time. We show that this simplification does not alter the ability of the model to (1) perform action selection, (2) produce plausible firing rates and (3) display beta oscillations with low levels of dopamine. This model has the specificity of not assuming the existence of the direct and indirect pathways associated with D1 and D2 dopamine receptors. If this dissociation seems to exist in mice, anatomical studies in primates revealed that these two pathways are, at best, marginally dissociated [3]. Consequently, changes in DA level have a different impact on the model than in the previous ones. We thus investigated these changes in two contexts: first, does the level of DA in the system regulate the exploration exploitation trade-off, as suggested by the work of [4]? Second, to what extent is the segregation in two different pathways necessary to reproduce the differences in reward and punishment sensitivity observed in Parkinsonian patients with or without medication [5]? [1] Mink, J. W. (1996). The basal ganglia: focused selection and inhibition of competing motor programs. Prog neurobiol. [2] Liénard, J., & Girard, B. (2013). A biologically constrained model of the whole basal ganglia addressing the paradoxes of connections and selection. J comput neurosci. [3] Lévesque, M., & Parent, A. (2005). The striatofugal fiber system in primates: a reevaluation of its organization based on single-axon tracing studies. PNAS. [4] Humphries, M. D., Khamassi, M., & Gurney, K. (2012). Dopaminergic control of the exploration-exploitation trade-off via the basal ganglia. Front neurosci. [5] Frank, M. J., Seeberger, L. C., & O'reilly, R. C. (2004). By carrot or by stick: cognitive reinforcement learning in parkinsonism. Science.

Disclosures: J. Bellot: None. **M. Khamassi:** None. **B. Girard:** None. **J. Liénard:** None.

Poster

555. Human Decision-Making: Cognition and Computation

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Topic: F.01. Human Cognition and Behavior

Support: Army Research Office (W911ND-11-1-0482)

Title: Divisive normalization and neurobiological constraints on decision-making

Authors: *R. WEBB[1], P. W. GLIMCHER[2], K. LOUIE[2]
[1]Rotman Sch. of Mgmt., Univ. of Toronto, Toronto, ON, Canada; [2]Ctr. for Neurosci., New York Univ., New York, NY

Abstract: Biology places constraints on the form of neural computations that ultimately characterize choice behaviour. Consistent with the result of a constrained optimization over evolutionary timescales, certain canonical computations occur widely and repeatedly in neural circuits. Recently, it was shown that value coding in decision-related circuits is implemented via divisive normalization, a computation extensively seen in sensory processing. However, the relationship between normalization, inherent constraints of biological information processing, and choice behavior are unknown. Here, we show that divisive normalization produces an efficient representation of value information under simple assumptions about neurobiological constraints. We further show that this optimization predicts specific context-dependent choice effects driven by the composition and size of the choice set. Evidence for such context-dependent choice behaviour is provided from two behavioural experiments, and these behavioral patterns are more accurately captured by a choice model incorporating normalization compared to common econometric specifications. These results emphasize both the positive advances offered by developing choice models grounded in neuroscience, and the normative role neurobiological constraints can play in the study of decision-making.

Disclosures: R. Webb: None. **P.W. Glimcher:** None. **K. Louie:** None.

Poster

555. Human Decision-Making: Cognition and Computation

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Topic: F.01. Human Cognition and Behavior

Title: Analogical mapping within and across modalities: Modular abilities or analo-g?

Authors: *G. ENGLISH[1], N. PARROTT[2], E. FEARON[2], N. LIU[2], N. GALLAGHER[2], A. GREEN[3]
[2]Psychology, [3]Psychology Department, Interdisciplinary Dept. in Neurosci., [1]Georgetown Univ., Washington, DC

Abstract: The ability to connect seemingly unlike things via analogical reasoning (mapping) underlies social, practical, and scholastic learning. To date, research on analogical mapping has focused on connecting items within a single information modality, especially verbal and visual analogies. The capacity to perform analogical mapping across (rather than within) a modality has remained unexplored. This project examined cross-modal analogical ability in young adults. 52 participants (56% male; M age=21.98, SD=2.91) underwent cognitive testing including WASI-II full-scale IQ and four blocked conditions of analogical reasoning. Analogical reasoning stimuli were 4-term proportional true or false analogies, interpreted as "A is to B as C is to D". In three conditions, the first two terms were line arrangements. The third and fourth terms varied by condition: line arrangements, words, or sounds. Each initial pair appeared in each of the three modalities. In addition to these Line-Line, Line-Word, and Line-Sound sets, 51 participants performed a fourth condition: 4-term true-false word-word analogy task. Participants exhibited good accuracy in all three conditions (Line-Line M Acc = 0.84, SD = 0.08; Line-Word M Acc = 0.82, SD = 0.06; Line-Sound M Acc = 0.92, SD = 0.07; Word-Word M Acc = 0.87, SD = 0.08). Word-Word accuracy had a significant positive correlation with Line-Word ($r=0.321$, $n=51$, $p=0.021$), but not Line-Line or Line-Sound. Line-Line and Line-Word accuracy were significantly positively correlated with Line-Sound accuracy ($r=0.348$, $n=52$, $p=0.012$; $r=0.464$, $n=52$, $p=0.001$). Line-Line and Line-Word accuracy correlated at a trend level ($r=0.264$, $n=51$, $p=0.058$). Verbal Comprehension, Perceptual Reasoning, and Full-Scale IQ all significantly positively correlated with Word-Word accuracy ($r=0.378$, $n=51$, $p = 0.006$; $r=0.329$, $n=51$, $p=0.018$; $r=0.438$, $n=51$, $p=0.001$), but none of the other modalities. High participant accuracy on the three nonstandard analogy conditions (Line-Line, Line-Word, and Line-Sound), as well as the Word-Word analogies, strongly supports the ability of individuals to reason both within and across modalities, expanding on current research. The positive correlations between participants' accuracy on the three nonstandard modalities indicate that these abilities access some of the same underlying mechanisms despite sensory input differences. However, the failure of Line-Line, Line-Word, and Line-Sound analogies to correlate significantly with IQ measures, as well as the significant correlation of Word-Word analogies only with Line-Word analogies, may imply some degree of verbal modularity in analogical reasoning ability.

Disclosures: G. English: None. **N. Parrott:** None. **E. Fearon:** None. **N. Liu:** None. **N. Gallagher:** None. **A. Green:** None.

Poster

556. Human Cognition: Timing and Temporal Processing

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Program#/Poster#: 556.01/TT45

Topic: F.01. Human Cognition and Behavior

Support: NSF Grant 1231515

Title: Sleep inspires temporal insight but not categorical insight

Authors: *L. CANNELLA, I. LERNER, D. OHIOMA, M. A. GLUCK
Rutgers Univ. Newark, Newark, NJ

Abstract: It is well documented that sleep has beneficial effects on memory and learning in humans. In particular, sleep has been shown to facilitate insight in associative-learning tasks that are known to depend on the hippocampus. However, tasks used to demonstrate sleep effects on hippocampal-dependent insight in humans were predominantly focused on the need to discover a hidden rule with a temporal structure. Whether such results can generalize to hippocampal tasks requiring non-temporal insight remains an open question. To investigate this issue, we developed two novel tasks, one requiring temporal insight and the other requiring insight that does not have temporal aspects. In each trial of the first task, subjects had to guide an avatar through five rooms. Each room had three different colored doors in different spatial locations. Perfect performance could be achieved by memorizing the correct sequence of doors or colors by trial and error; however, realizing that the sequences across all trials have a repetitive temporal structure, namely, that the location of some doors could be predicted by the location of other doors, could considerably improve performance. In the second task, subjects were instructed to sort visual objects that differed in shape, size, color, and pattern into category A or B based on trial and error. Optimal categorization could be achieved by either memorizing the features of individual objects, or by gaining the insight that a non-trivial systematic combination of features reliably determines the correct classification. We assessed if subjects discovered this hidden rule by testing them on new objects that complied with the same rule (‘generalization’). Subjects were assigned to either a ‘sleep’ group, in which they were tested in the evening and in the following morning with a 12-hour period of sleep in between; or to a ‘no-sleep’ group, in which they were tested twice on the same day with no intermediate sleep. In the temporal task, the sleep group showed a significantly reduced number of errors for predictable doors than the no-sleep group, and was also more able to explicitly point out the temporal rule, signifying that sleep

promoted insight. In the non-temporal task, whereas the sleep group showed increased performance in memorizing individual object categorization compared to the no-sleep group, generalization performance did not differ between the groups and no difference in explicit knowledge of the categorization rule emerged, indicating no improved insight. These results suggest that the facilitation of insight by sleep in hippocampal-dependent associative learning tasks is specific to those with a temporal structure.

Disclosures: **L. Cannella:** None. **I. Lerner:** None. **D. Ohioma:** None. **M.A. Gluck:** None.

Poster

556. Human Cognition: Timing and Temporal Processing

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Topic: F.01. Human Cognition and Behavior

Support: NSF Grant 1231515

Title: A neurocomputational model of how memory replay during slow-wave sleep inspires insight

Authors: *I. LERNER, M. GLUCK
Rutgers Univ., Newark, NJ

Abstract: In the current study, we present a neural network model with temporal-based learning mechanisms that accounts for how insight in humans is inspired by sleep. During the last decade, sleep has been shown to facilitate the sudden identification of hidden temporal structures within sequences of stimuli, a finding demonstrated using cognitive tasks such as the Number reduction Task (NRT) and the Serial Reaction Time Task (SRTT). Evidence suggests that this 'temporal insight' is related to hippocampal activation during Slow-Wave-Sleep (SWS). SWS, in turn, has been shown in rodent studies to express 'memory replay': hippocampal reactivations of previously encoded wake experiences in a time-compressed manner. Here, we suggest that compressed replay may in fact be responsible for the sleep-inspired insight by providing 'temporal scaffolding' to the process of memory consolidation. Specifically, we posit that extraction of unattended temporal structure contained within wake experiences is often beyond the reach of regular timescales of synaptic plasticity mechanisms; however, time-compressed reactivation of these experiences during SWS allows neurons to become sensitive to such structure. We demonstrate this mechanism using an integrate-and-fire neural network simulation.

Inputs to the network included sequences of stimuli that corresponded to those used by human experiments in the NRT and SRTT. These sequences could be presented to the network either in their original 'wake-time' pace, or in a compressed time-scale representing memory replay. The network attempted to learn economical representations of these sequences using unsupervised Hebbian-learning mechanisms based on spike-timing-dependent-plasticity; and to predict future desired responses by converting these representations to output signals using supervised temporal-learning mechanisms inspired by the Tempotron model. When stimuli were presented in regular 'wake' pace, no neural representations of the temporal structure were formed, preventing the network from successful prediction of future outputs. However, when accelerating the time-scale of stimuli presentation, the network became sensitive to the temporal structure of the tasks and yielded correct predictions. Thus, our model suggests a simple, biologically-driven mechanism, which accounts for the way sleep inspires temporal insight. In addition, it suggests a novel functional role for the time-compression characterizing memory replay in the hippocampus. We point to the entorhinal and prefrontal cortex as candidate brain regions in which these mechanisms may take place.

Disclosures: I. Lerner: None. **M. Gluck:** None.

Poster

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Title: Dissecting human cortical oscillations with surgical transections: White and grey matter contributions to independent oscillatory units

Authors: *A. H. HAWASLI[1,3], D. KIM[3], N. M. LEDBETTER[3], S. DAHIYA[2], D. L. BARBOUR[3], E. C. LEUTHARDT[1,3]
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Abstract: Oscillating brain activity reflects changes in cortical potentials caused by activity in neuronal populations. Despite routine use of human cortical oscillations in neuroscience and medicine, the circuits and physiology underlying them remain largely unknown. The modulation of low-frequency oscillations tends to correlate across relatively large areas of cortex while the modulation of high-frequencies tends to be local. From this observation, we hypothesized that (1) low- and high-frequency oscillations preferentially reflect long- and short-range communications, respectively; and (2) synchronization between low-frequency phase with high-frequency amplitude (i.e. phase/amplitude coupling) represents a mechanism that enables large-scale distributed brain-networks to coordinate with local cortical processing. We tested these hypotheses by selectively disrupting white or grey matter connections (i.e., long- or short-range) to cortical sites in humans undergoing neurosurgical resection while recording surface field potentials. We found that selective disruption of white matter connections reduced oscillation power in low-frequencies more than high-frequencies, as expected. Contrary to our hypotheses, however, white matter transection increased functional connectivity with adjacent cortical sites (predominantly at low-frequencies) and did not alter phase/amplitude coupling. Disruption of surrounding grey matter connections did not significantly alter oscillatory power but increased functional connectivity in higher-frequencies and substantially increased cross-frequency phase/amplitude coupling at a synchronized phase. When the lesions were combined at the same site, endogenous oscillations and complex oscillatory relationships were maintained or enhanced. Oscillations persisted even after cortical tissue was completely removed from the brain. These findings suggest that cortex consists of independent oscillatory-units (IOUs) that maintain their own complex endogenous rhythm structure. IOUs are differentially modulated by their white and grey matter connections, where long-range connections maintain topographical heterogeneity (i.e. separable processing along the cortical surface) and short-range connections segregate cortical synchronization patterns (i.e. separable processing in time). Modulation of these distinct oscillatory modules allows for functional diversity necessary for complex signal processing in the human brain.

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Poster

556. Human Cognition: Timing and Temporal Processing

Location: Halls A-C

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Topic: F.01. Human Cognition and Behavior

Support: Grant-in-Aid for Scientific Research on Innovative Areas "The study on the neural dynamics for understanding communication in terms of complex hetero systems (No.4103)" (21120005) of MEXT, Japan

Title: Temporal coordination in human communications - Phase-oscillator analysis of a two-person alternate tapping task

Authors: Y. CHENG[1], M. KAWASAKI[2], K. KITAJO[3,4], *Y. YAMAGUCHI[5,1]
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Abstract: Our conversation can be considered as a temporal evolution of alternate events between two (or more) speakers. In a two-person alternate speech task, common rhythms are known to emerge spontaneously between the speakers (Kawasaki et al. 2013). In this paper we aim to elucidate the principle of dynamical system in alternate communication events. We employed a two-person alternate finger-tapping task. Each pair of subjects participated in several alternate tapping tasks, in which they were required to make their inter-tapping-interval (ITI) as similar as possible to the ITI made by the partner's latest tapping. A visual signal was shown to both the subjects after each tap, where the color of the signal depended on who made the tap. We designed three types of alternate tapping tasks: human-human (HH), in which the two subjects played with each other; human/computer-follow (HC-F), in which each subject played with a computer program implemented to reply its ITI same as the preceding one plus the Gaussian noise; human/computer-step (HC-S), in which each subject played with a computer program changing its ITI stepwise after a certain number of taps. The change of partner from human to computer program was not notified in both HC-F and HC-S tasks. 19 pairs (38 subjects) participated in the experiment. We analyzed the time series data of ITIs based on phase-oscillator model coupled with phase response curves (PRC). Although the performance of HH task varied largely through pairs, the alternate tapping sequence of every pair was found to be the outcome of an attractor formed by phase locking. PRCs of all the subjects, obtained in HC-F task, were either the types 0 or 1. In HC-S task, on the other hand, three types of adaptation to the sudden step change of ITI were observed. In addition, after the behavioral tasks, the questionnaire for autism-spectrum quotient (AQ) known to be available for evaluation of communication ability of healthy subjects, was applied to the subjects. The performance in HC-F task was found to correlate with the score of AQ. Thus, our findings imply the importance of dynamical system theory in social communications.

Disclosures: **Y. Cheng:** None. **M. Kawasaki:** None. **K. Kitajo:** None. **Y. Yamaguchi:** None.

Poster

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Topic: F.01. Human Cognition and Behavior

Support: Wellcome Trust

NIHR

Title: Temporal orienting of attention can be preserved in normal ageing

Authors: *J. CHAUVIN, C. GILLEBERT, G. ROHENKOHL, G. HUMPHREYS, A. NOBRE
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Abstract: Studies using temporal orienting cues have discovered that it is possible to orient attention to moments in time, optimizing behavioural performance in young adults (Davranche et al., 2011; Nobre & Rohenkohl, 2013). It is generally accepted that older adults show deficiencies or impairments on timing tasks (Balci et al., 2009), though there have been few studies examining the effects of temporal orienting in elderly participants. Zanto and colleagues (2011) demonstrated that elderly participants exhibited what they called an 'expectation deficiency' on a temporal orienting task. To investigate the effects of temporal expectation in ageing further, two experiments in healthy young and old adults were conducted. For the first experiment, 18 younger (<30 years) and 18 older (>60 years) participants took part in blocked versions of two temporal orienting tasks: a speeded motor reaction timing task and a rapid search visual presentation (RSVP) task. In both tasks, two temporal cues in the form of a beep (high and low pitch) indicated the likelihood of a target item occurring after a short or longer period of time. In the motor reaction time task, participants were instructed to use the cues to help them respond as quickly as they could to seeing a green patch appear at the centre of the computer screen. In the RSVP task, participants used temporal cues to orient their attention in time in order to help them perceive a target letter in a stream of rapidly presented letters. In a second experiment, 18 younger (<30 years) and 19 older (>60 years) participants took part in a trial-by-trial version of these tasks. In both experiments, it was found that elderly participants were able to use temporal orienting cues to enhance RT performance and sensitivity to the target stimuli when validly cued for short foreperiod relative to invalid cues. Contrary to previous findings, our results suggest that the ability to allocate attention to moments in time can be preserved in normal ageing. Further research is needed to ascertain whether similar networks are used or whether compensatory mechanisms are at work.

Disclosures: **J. Chauvin:** None. **C. Gillebert:** None. **G. Rohenkohl:** None. **G. Humphreys:** None. **A. Nobre:** None.

Poster

556. Human Cognition: Timing and Temporal Processing

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Topic: F.01. Human Cognition and Behavior

Support: NIH Grant NS078127

Fund from McGovern Institute for Brain Research at MIT

Title: Integration of short-term and long-term temporal information in interval timing

Authors: ***S. W. EGGER**[1], M. JAZAYERI[1,2]
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Abstract: Humans are sensitive to the passage of time and use temporal cues to coordinate their actions. However, tracking time is inherently noisy and is thus associated with significant behavioral variability. Humans employ various computational strategies to combat this variability. For example, the ability to measure or produce intervals is improved if the interval is presented within the context of a rhythm. This indicates that subjects utilize the short-term information available within a rhythm to mitigate interval timing variability. It has also been shown that subjects rely on the temporal statistics of the environment learned over longer time periods to improve estimates of time intervals (Jazayeri and Shadlen; 2010). It is not known, however, whether and how the information inferred from a rhythmic structure and the prior knowledge about temporal statistics are integrated to further improve timing behavior. Therefore, we developed a task in which subjects could rely on both prior expectations (based on previous trials) and the rhythmic structure (within a trial) to improve timing behavior. Subjects fixated a central spot and observed 2 to 5 visual spots in the periphery flashed successively. The number and position of flashes were known at the beginning of the trial. The interval between successive flashes (sample interval) was fixed within a trial creating a visual rhythm. Across trials, the sample interval was drawn from a uniform distribution (prior distribution) that ranged between 500 and 900 ms. Subjects had to measure the sample interval and reproduce it immediately after the last flash by a key press, continuing the rhythm for one beat beyond the last flash. We found that subjects' relied on both the long-term prior distribution and short-term rhythmic structure of

the stimulus to improve performance. With 1 sample interval, production intervals exhibited a trade off between bias and variance predicted by a Bayesian model. As the number of sample intervals increased, performance improved but the bias persisted to a greater degree than predicted by the model. The persistence of the bias despite additional sensory evidence suggests that the integration of multiple intervals within a rhythm is imperfect and that the internal estimate is subject to a diffusion process that over time relaxes to the mean of the prior. This observation indicates that subjects use their prior knowledge to compensate for the additional uncertainty due to an imperfect integration of short-term information.

Disclosures: S.W. Egger: None. **M. Jazayeri:** None.

Poster

556. Human Cognition: Timing and Temporal Processing

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Support: ACC Creativity and Innovation Fellowship

Diversity Research Grant

Title: Mindfulness meditation alters subjective experience of time

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Abstract: Evidence shows that mindfulness meditation reduces stimulus-independent thought or "mind-wandering," by focusing attention and awareness on the present moment through mediation of the default mode network. Despite the relatively limited knowledge of the cognitive mechanisms underlying the default mode network, recent literature suggests that perception of time and mental time-keeping strategies may play a key role in the attentional lapses that lead to mind-wandering. Given the increased focus of attention on moment-to-moment awareness, meditation may modify the way we attend to our subjective experience of time. However, few studies have examined the interaction between the meditative state and temporality. In the present experiment, we measured time perception on a verbal time estimation task before and after meditation or relaxation training. We found that meditation training improved performance on a verbal interval estimation task, whereas the relaxation training control group showed no

enhancement in duration estimates. Since experienced meditators report a sense of time slowing down both during meditative practice and everyday life, this experiment has important implications for how meditation alters perception of time and the resulting behavioral consequences of this contemplative practice.

Disclosures: D. Smith: None. **A. Cate:** None. **M. Koffarnus:** None.

Poster

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Topic: F.01. Human Cognition and Behavior

Support: NSF RX4235406

Title: Neural substrate of omitted stimulus response: A simultaneous EEG-fMRI study

Authors: *I. B. SAMUEL, H. HUANG, A. RAJAN, M. DING
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Abstract: An expected but missing stimulus is often accompanied by an endogenously generated brain response called the omitted stimulus response (OSR). Neuronal mechanisms of OSR in humans have been mainly studied with EEG and MEG techniques. Given these techniques' limited spatial resolution the anatomical substrate of OSR remains not well understood. We addressed this problem by conducting a simultaneous EEG-fMRI experiment. Participants were presented with a rhythmic stimulus train of random duration in the visual domain (inter-stimulus interval=2s) and instructed to anticipate the onset of the next stimulus upon each visual stimulation. Since the number of stimuli in the train was random it was expected that the omitted stimulus response should occur 2s after the termination of the stimulus train. This prediction was confirmed by an ERP analysis. A GLM analysis of the fMRI data revealed the activation of the anterior cingulate cortex and the dorsal lateral prefrontal cortex. To further examine the relation between the ERP response and fMRI response to the omitted stimulus, omitted stimulus ERP was estimated on a trial by trial basis using the ASEO method (analysis of single-trial event-related potentials and ongoing-activity) and used as a regressor in the fMRI analysis.

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Poster

556. Human Cognition: Timing and Temporal Processing

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Program#/Poster#: 556.09/TT53

Topic: F.01. Human Cognition and Behavior

Title: Processing of sub- and supra-second intervals in primate brain results from the calibration of multiple neuronal oscillators via sensory/motor and feedback processes

Authors: *D. S. GUPTA
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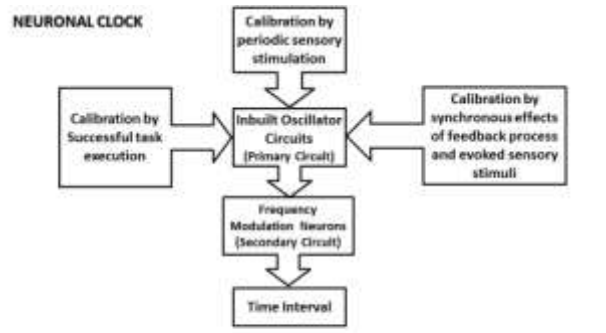
Abstract: Processing of time intervals by brain is critical for our interaction with the surroundings. Conscious processing of time intervals predicts movements in a vehicular traffic or the temporal delay in finger movements while playing a musical instrument. Processing of time interval also occurs at a subconscious level, which helps to determine the speed of individual muscle contractions, coordinating our movements. To understand how brain processes various sub- and supra-second intervals, I have proposed inbuilt neuronal oscillators within various circuits in brain, which helps to form neuronal clock mechanisms. Proposed oscillators include pacemaker neurons, tonic inputs and, synchronized excitation and inhibition of inter-connected neurons. Time is represented within proposed neuronal clock mechanism by intervals, called 'sensory moment', between two spikes or spike bursts produced by oscillators. Outputs from oscillators are processed to generate changes in states of next level of circuits, represented by rates of changes in frequency and/or frequency of neuronal activity, which encode time intervals. Inbuilt oscillators are proposed to be calibrated by (a) feedback processes (b) input of time intervals resulting from rhythmic external sensory stimulation and (c) synchronous effects of feedback processes and evoked sensory activity. Proposed mechanism of calibration also imposes fluctuations on the regular activity of oscillators. Differences with the pacemaker/accumulator model are discussed, which proposes a separate oscillator with a stable frequency. Multiple calibration mechanisms account for redundancies in the timing mechanisms. Furthermore, abnormal feedback processes associated with motor symptoms could be responsible for the impairment of temporal processing, as seen in schizophrenics.



Above schematic shows the circuitry of the proposed neuronal clock. A neuronal clock is formed by an inbuilt oscillator circuit (one per circuit), which is calibrated by different mechanisms involved in the homeostasis of the primary or the main circuit. A calibrated oscillator circuit activates a second level of circuits, which contains frequency modulator (FM) neurons, exhibiting short term plasticity. Different states of second level of circuits, produced with the help of the modulation in the activity of FM neurons, encode various types of circuit or task specific sub- and supra-second intervals.

Disclosures: D.S. Gupta: None.

Poster

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Support: KAKENHI (21670004)

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KAKENHI (23650335)

KAKENHI (25242058)

Title: Transcranial magnetic stimulation over the right dorsal premotor cortex increases dependence on prior information during tactile temporal order judgment

Authors: *M. MIYAZAKI[1], S. TAKEUCHI[2], H. SEKIGUCHI[2]
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Abstract: We can improve perceptual and behavioral accuracy using prior information. In the Bayesian estimation model (Körding & Wolpert, 2004), an optimal estimate for a sensorimotor task can be computed by combining prior information on the task statistics with the likelihood

obtained from sensory input. Psychophysical studies (Miyazaki et al., 2006; Yamamoto et al., 2012; Nagai et al., 2012) indicate that the brain implements Bayesian estimation during temporal order judgment (TOJ). Based on our preliminary study using electroencephalography (partially reported in Takeuchi et al., 2013), we hypothesized that the dorsal premotor cortex (PMd) is associated with Bayesian estimation during tactile TOJ. Here, we tested this hypothesis using disruptive transcranial magnetic stimulation (TMS). Twelve subjects performed TOJ with two tactile stimuli, one in each hand (detailed in Miyazaki et al., 2006). The stimulus onset asynchronies (SOAs) were sampled from the Gaussian distribution, biased to the right-hand side first (mean = 80 ms, SD = 80 ms; R1st-bias condition) or to the left-hand side first (mean = -80 ms, SD = 80 ms; L1st-bias condition). Each participant completed two sessions (1600 trials/session). TMS was applied over the right PMd in one session and over the left PMd in another session (3 cm anterior to hot spots for the motor evoked potentials of the abductor pollicis brevis; van der Berg et al., 2010). For each session, the TMS and no-TMS conditions were interleaved every 200 trials. Under the TMS condition, TMS was applied in 50% of the trials, with the SOA within -40 to 40 ms. We used a paired pulse (ISI = 10 ms) for TMS. The TMS intensity was 110% of the resting motor threshold. The TMS timing was 80 ms after the first tactile stimulus in each trial. Using the proportion of "right-hand side first" judgments as a function of the SOAs, we calculated the difference in the point of subjective simultaneity between the R1st-bias and L1st-bias conditions (ΔPSS). Positive ΔPSS values imply that the brain implements Bayesian estimation using the biased distributions as prior information. The results supported our hypothesis. TMS of the left PMd did not affect ΔPSS (mean ± SEM: no-TMS, 67.8 ± 17.2 ms; TMS, 82.3 ± 20.7 ms; $P = 0.51$, paired t-test with Holm correction), whereas TMS of the right PMd increased ΔPSS (no-TMS, 68.2 ± 17.8 ms; TMS, 91.3 ± 16.2 ms; $P = 0.02$). A greater ΔPSS implies increased dependence on prior information. That is, applying disruptive TMS to the right PMd led to overuse of prior information, suggesting that the right PMd is involved in processing of the likelihood.

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Poster

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Topic: F.01. Human Cognition and Behavior

Support: ERC-YSt-263584

ANR- JCJC-1904

Title: Electromagnetic signatures of mental travel in time and space

Authors: *B. GAUTHIER, K. PESTKE, V. VAN WASSENHOVE
Cea/dsv/i2bm/Neurospin Center/Cognitive Neuroimaging Unit, Gif-sur-Yvette, France

Abstract: The Human ability to navigate through space and time mentally has been widely explored with both psychophysics and fMRI and produced two major frameworks (ATOM: Walsh, 2009 and MTL theory: Bonato, 2012) that diverged on the functional relationship between time and space but critically converged on the involvement of parietal cortex in manipulation of these two dimensions. Linking serial stages of cognition and brain imaging demands to access to the finest temporal resolution of brain activity but surprisingly few M/EEG characterizations have been reported that would allow establishing the time course of these high level cognitive functions. In this context, we explored the M/EEG responses related to the temporal and spatial distance effects with a combined mental time and space travel paradigm. First, subjects memorized a set of events with their historical context, date and location. One day after, subjects performed a temporal or a spatial judgment task (TJ and SJ, respectively) while being recorded with MEEG. TJ and SJ conditions were randomly intermixed in a block-design. In a given trial, participants were asked to mentally place themselves in a given temporal and spatial position and asked a Before/After or East/West question with regards to an event presentation - e.g. Ouragan Katrina. Subjects responded in a 2-AFC by button press. The references that were tested were ego- and allo-centric, namely: Present, 9 years ago or after for TJ, and Paris, Cayenne or Dubaï for SJ. A significant decrease was found in both reaction times and accuracy as a function of temporal and spatial distance for both TJ and SJ respectively, replicating previous results interpreted as a psychological correlate of a mental mapping of time and space metrics. MEEG analyses were performed using event-related potentials (ERPs) and fields (ERFs). Contrasting TJ vs. SJ revealed a long-lasting frontal positivity 300 ms post-stimulus onset in both MEG and EEG. Psychological distance effects were indexed as 300 ms post-stimulus parietal differences between close and far conditions, followed by a late parietal negativity at ~700ms. These patterns were notably observed prior to the comparison stage differentially implicating frontal cortices. The neural latencies and topographies are globally congruent with previous results (Arzy et al, 2008). As predicted, we observed a shared parietal lobe response for time and space mapping but interestingly, with a specifically early implication of frontal region for TJ.

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Poster

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Topic: F.01. Human Cognition and Behavior

Title: The role of the supplementary motor area and the cerebellum in beat-based and non-beat-based timing

Authors: *L.-A. LEOW, J. A. GRAHN
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Abstract: Time intervals in a rhythm can be represented as absolute durations (non-beat-based timing) or relative to a recurrent beat (beat-based timing). Neuropsychological and neuroimaging work show that the supplementary motor area (SMA) and the cerebellum are involved in beat-based and non-beat-based timing (Grahn and Brett, 2007, Grube et al., 2010), however, the specific role of these structures remains unclear. Here, we assess their roles in beat- and non-beat-based timing by modulating excitability in each area using anodal and cathodal 2mA transcranial direct current stimulation. During stimulation, subjects performed a discrimination task with beat and non-beat rhythms to test beat-based and non-beat-based timing, respectively. Discrimination of beat rhythms was better when SMA excitability was increased and worse when SMA excitability was decreased. Discrimination of non-beat rhythms was worsened when SMA excitability was decreased. Conversely, discrimination of beat (but not non-beat) rhythms was worse when cerebellar excitability was increased and better when cerebellar excitability was decreased. Discrimination of non-beat rhythms was not significantly affected by altering cerebellar excitability. Overall, the data suggest both areas play a critical role in the timing of rhythmic sequences.

Disclosures: L. Leow: None. **J.A. Grahn:** None.

Poster

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Topic: F.01. Human Cognition and Behavior

Support: National Science Foundation (II-1114833)

NIH: MH60163

Title: Novel regimes in dynamic attractor networks account for timing and temporal warping

Authors: *N. HARDY, D. V. BUONOMANO
Neurobio., UCLA, Los Angeles, CA

Abstract: The ability to encode time and generate timed responses is essential to motor control. A general feature of motor control is the ability to produce motor patterns, such as reaching, typing, and speaking, at a range of different speeds ("temporal warping"). Though a number of models address how the brain produces complex spatiotemporal motor patterns, few have addressed how such patterns could undergo temporal warping. One general class of models ("population clocks") proposes that the timing necessary for motor activity arises from the temporally dynamic patterns of neural activity, or neural trajectories, of recurrent neural networks (RNNs). In order to reliably encode time and produce complex spatiotemporal motor patterns such networks must exhibit reproducible dynamics. However, the dynamics of most RNNs studied to date are highly-sensitive to noise and often formally chaotic. We have recently established that firing-rate based RNN models can exhibit highly stable and computationally powerful dynamic regimes by appropriately tuning the recurrent connections (Laje & Buonomano, 2013). In these dynamic attractor network models, triggered activity patterns follow a trained neural trajectory and will return to the ongoing neural trajectory if perturbed by an external stimulus or internal noise. Here, we show that these dynamic attractor networks can also account for temporal warping. Specifically, the network can be trained to complete the same arbitrary "innate" trajectory at different speeds (i.e., trajectory durations). This is accomplished by delivering a constant tonic input which signals the network to temporally scale the speed of its current trajectory. Once trained, the network can generalize its speed based on interpolated untrained velocity signals. While the RNN is trained to produce the same warped trajectory at different speeds, the network converges to states in which very similar "parallel" trajectories in neural space can be generated at different speeds. Because of the large dimensionality reduction of the RRN patterns at the level of the output units, the output patterns are virtually indistinguishable other than the fact that they are temporally warped. Our results describe a general computational framework that accounts not only for the generation of complex spatiotemporal patterns, but also the ability to rescale such patterns_thus capturing a heretofore unaddressed but fundamental feature of motor control. These findings have implications for the problem of temporal warping of motor patterns as well as the neural mechanisms underlying the generation of complex spatiotemporal motor patterns.

Disclosures: N. Hardy: None. **D.V. Buonomano:** None.

Poster

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Research in Autism, Intellectual and Neurodevelopmental Disabilities (RAIND, PI: McAuley)

Michigan State University Radiology Pilot Scan Program

Michigan State University Cognitive Science Program

Michigan State University College of Communication Arts & Sciences

Michigan State University Department of Communicative Sciences & Disorders

Title: Brain activity differences during rhythm discrimination in adults who stutter

Authors: *E. A. WIELAND[1], J. D. MCAULEY[2], D. ZHU[3], L. C. DILLEY[1], S.-E. CHANG[4]
[1]Communication Sci. & Disorders, [2]Psychology & Neurosci. Program, [3]Radiology, Michigan State Univ., East Lansing, MI; [4]Psychiatry, Univ. of Michigan, Ann Arbor, MI

Abstract: Stuttering affects the timing and rhythmic flow of speech production. Interestingly, when speech is synchronized with an external pacing signal (e.g., a metronome), stuttering can be markedly alleviated. This suggests that people who stutter may have difficulty generating an internal rhythm to pace their speech. We hypothesized that adults who stutter (AWS) would have different brain activity patterns relative to controls during a rhythm discrimination task, specifically in a network of regions that have been shown to support rhythm processing (i.e., putamen, supplementary motor area (SMA), auditory, and premotor regions; Grahn & Rowe, 2009). Eleven AWS (6 M) and 11 controls (5 M) (aged 18-46 years) participated in an fMRI experiment where on each trial, participants heard two successive presentations of a standard rhythm and then judged whether a third (comparison) rhythm was the same or different from the standard. Rhythms were either simple (accents at regular periodic intervals) or complex (accents at irregular intervals). Trials were presented using an event-related design divided into six

functional runs. Whole-brain EPI volumes were acquired (44 contiguous 3 mm axial interleaved slices, TR = 2.5s, TE = 27.7 ms, FOV = 22 cm) on a 3T GE Signa HDx scanner with an eight-channel head coil. Statistical inference was based on a whole-brain corrected $p < 0.05$. Consistent with previous research, simple rhythms were discriminated significantly better than complex rhythms. There was no overall significant effect of group, but there was a tendency for AWS to have more difficulty discriminating complex rhythms than controls. Although the behavioral results were comparable in both groups, fMRI results revealed that AWS had significantly greater brain activity than controls in right-hemisphere regions previously implicated in rhythm processing, regardless of rhythm type. AWS showed bilateral activity in the SMA and putamen, whereas controls showed lateralized left-sided activity in these areas. AWS also showed dispersed hyperactivity in the premotor and auditory regions in both hemispheres relative to controls. AWS seem to exhibit less efficient neural network activity patterns to support rhythm processing. This may underlie deficiencies in internal rhythm generation, in turn affecting timing of speech movements.

Disclosures: **E.A. Wieland:** None. **J.D. McAuley:** None. **D. Zhu:** None. **S. Chang:** None. **L.C. Dilley:** None.

Poster

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Program#/Poster#: 556.15/TT59

Topic: F.01. Human Cognition and Behavior

Support: ANR-10-ORAR-006-03

Title: Motor activity improves interval timing judgments

Authors: *E. THOMAS[1], L. FAUTRELLE[2], D. MARESCHAL[3], R. FRENCH[4]
[1]INSERMU1093, Univ. De Bourgogne, Dijon, France; [2]Univ. Paris Ouest, Paris, France; [3]Univ. of Birkbeck, London, United Kingdom; [4]CNRS, Dijon, France

Abstract: Interval timing involves the judgement of durations ranging from 0.5 seconds to some minutes. Some of the brain areas known to be involved in interval timing are also important in motor activity. These include the basal ganglia and the presupplementary and supplementary motor areas [1]. This raises the possibility that motor acts accompanying interval timing judgment might influence the latter process and especially its learning. In order to test this

hypothesis, we carried out interval timing tests following different types of training, some of which included motor acts. The pre and post training tasks consisted of a button press upon rhythmic presentation of a visual stimulus. Improvements in reaction times were taken as indicating improvements in interval judgment [2]. Training also consisted of asking the subjects to respond to visual presentations of the stimulus, but with a different response mode. Depending on the group to which the subject belonged, the individual was asked to either 1) point with a whole body movement 2) point with the arm from a sitting position 3) imagine a whole body pointing 4) simply watch the stimulus presentation. Subjects undergoing training in interval estimation using motor activity showed significant improvements on these judgments compared to individuals who had not. While the improvements were slightly less for the motor imagery group, this group also showed significantly lower response times than those without any motor training. Control tests were carried out in order to ensure that the improvements obtained with motor training could not simply be attributed to an improved attention or facilitation in sensory motor coordination for this group. In the latter case, this consisted of training with a whole body pointing movement in response to irregular stimuli. No significant improvements in response times were obtained with this group. Similar performances in a secondary task for all groups also indicated that there were no major differences in attention levels between the different training groups. Our results indicate that motor activity can improve interval timing judgments. [1] Coull JT, Cheng RK, Meck WH. (2011) Neuroanatomical and neurochemical substrates of timing. Neuropsychopharmacology. 36(1):3-25. Review. [2] Correa A, Lupianez J and Tudela P (2005) Attentional preparation based on temporal expectancy modulates processing at the perceptual level. Psychonomic Bulletin & Reviews 12: 328-334.

Disclosures: **E. Thomas:** None. **L. Fautrelle:** None. **D. Mareschal:** None. **R. French:** None.

Poster

556. Human Cognition: Timing and Temporal Processing

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 556.16/TT60

Topic: F.01. Human Cognition and Behavior

Title: Representation of elapsed time in the human posterior parietal cortex

Authors: *M. I. LEON, N. B. FLYNN, J. E. MEDINA
Cal State Univ, Bakersfield, BAKERSFIELD, CA

Abstract: We measured broadband (1-60 Hz) EEG power from scalp electrodes positioned over the posterior parietal cortex of human participants performing a time-discrimination task. Subjects were required to judge whether the duration of a tone (test cue) was longer or shorter than the duration of a remembered standard cue. Subjects indicated their decision after a delay period by making a left-arm or right-arm reach movement toward one of two choice buttons of a response box. Across all subjects, the probability of choosing "long" increased sigmoidally with the duration of the test cue. Using a short (1.5 sec) and long (3 sec) standard was found to affect participants' bisection point, the test cue duration at which short and long choices are equally likely. Throughout the presentation of the test cue, the signal power appeared to encode the participants' perception of elapsed time relative to the standard duration. During the test cue period, the signal power initially decreased, and then later increased with the passage of time. The test cue duration at which the signal power reversed its trajectory depended on the standard duration and correlated with the bisection point. This experiment lends novel insight into the neural representation of human time sense.

Disclosures: **M.I. Leon:** None. **N.B. Flynn:** None. **J.E. Medina:** None.

Poster

556. Human Cognition: Timing and Temporal Processing

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 556.17/TT61

Topic: F.01. Human Cognition and Behavior

Title: The effects of energy drinks on cognitive performance on the Stroop test

Authors: *B. C. NOLAN, G. L. HUBER
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Abstract: The present study examines the cognitive effects of energy drinks. A computerized Stroop Test, which measures cognitive processing of color-word congruent and color-word incongruent conditions, was used to evaluate 4 conditions: Energy Drink, Decaffeinated Energy Drink, caffeine pills, and acetaminophen pills (placebo). Participants were college age volunteers randomly placed into 1 of the 4 conditions, and then administered the appropriated drug/drink. Participants filled out a screening questionnaire to assess recent caffeine (previous 4 hours) and typical caffeine consumption on an average day. Participant took the Stroop Test twice. A pre-test was given, and then again 40 minutes after consuming their drug. A 2x2x2 ANOVA and t-test were used for statistical analysis. The data suggest that caffeine leads to faster reaction

times regardless of condition, but showed the lowest accuracy in discrimination between congruent and non-congruent conditions. The Energy Drink conditions showed better discrimination between the congruent and non-congruent conditions. Taken together, this suggests that while caffeine speeds up processing, other ingredient of the energy drinks may help increase the accuracy of cognitive performance.

Disclosures: B.C. Nolan: None. **G.L. Huber:** None.

Poster

556. Human Cognition: Timing and Temporal Processing

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 556.18/TT62

Topic: F.01. Human Cognition and Behavior

Support: CONACYT Grant 151223

PAPPIT Grant IN201214-25

Title: Analysis of the trajectories of the neuronal population activity in the premotor cortex of Rhesus monkeys during a Synchronization-Continuation Task

Authors: J. GAMEZ, R. BARTOLO, *H. MERCHANT
Inst. de Neurobiologia UNAM, Queretaro, Mexico

Abstract: Time in the range of hundreds of milliseconds is the context in which different behavioral responses, such as target interception and speech recognition-production, are organized. The neural basis of temporal information processing is not well understood; although it has been proposed that time could be an emergent property of neural network dynamics. In the present work, we used Principal Component Analysis on the neuronal activity of extracellular recordings of the medial premotor cortex of monkeys performing a Synchronization-Continuation Task (SCT). In each trial of the task, the animals first synchronized their tapping with a metronome to produce 3 intervals, and then continued tapping for other 3 internally timed intervals, without the metronome. Five target intervals (450-1000 ms) were used. We found highly stereotyped trajectories that varied according to the target interval performed by the monkey. Moreover, the neural trajectories followed the scalar property increasing their variability for longer intervals. Using a neural-network based classifier; we found that

combinations of Principal Components contained information about the target interval, sequential order, and phase (synchronization or continuation) of the SCT task.

Disclosures: J. Gamez: None. **R. Bartolo:** None. **H. Merchant:** None.

Poster

556. Human Cognition: Timing and Temporal Processing

Location: Halls A-C

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Program#/Poster#: 556.19/TT63

Topic: F.01. Human Cognition and Behavior

Support: Ellison Medical Foundation

Santa Fe Institute Consortium

Elizabeth H. Solomon Center for Neurodevelopmental Research

Title: Theta oscillations during processing of rapid frequency change differ between infants with a family history of Specific Language Impairment and controls

Authors: *N. A. CHOUDHURY[1,2], S. ORTIZ-MANTILLA[2], J. PARASCANDO[2], A. A. BENASICH[2]
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Abstract: The ability to perform fine-grained acoustic analysis in the tens of millisecond range during infancy is a robust predictor of language development and subsequent language disorders. However the basic neural mechanisms underlying infant processing of pre-linguistic auditory information remain unclear. In this study we examine the temporal dynamics of brain activity that may support rapid auditory processing abilities in a group of 6-month old infants at higher risk for language impairment as a function of family history of Specific Language Impairment (FH+, n=17) and control infants without such a history (FH-, n=20). An oddball paradigm to complex tone-pairs (standard:100-100 Hz, deviant:100-300 Hz [15%]) with 70ms between pair ISI was employed. Dense array EEG/ERP's were collected using a 62-channel EGI geodesic net. Co-registered, age-appropriate MRIs were used to model a two-dipole source solution for each individual at the largest positive peak representing sensory discrimination. Temporal-spectral analysis was conducted in source-space using a 2-50 Hz frequency band from -300 to 915 ms, with 1Hz wide frequency bins and time resolution of 50 ms. Changes in frequency band

amplitude as a function of time relative to stimulus presentation was evaluated using TSE (temporal spectral evolution). Permutation testing, in combination with data clustering analyses, confirmed that theta oscillations underlie evoked responses in the auditory cortices for both groups of infants and that there is a relative increase in power during processing of the deviant signal for both groups. However, there were significant group difference in theta power (5-8 Hz) between 300 and 550ms; infants in the FH+ group had attenuated theta oscillatory activity as compared to FH- infants, and showed atypical lateralization. While control infants showed enhanced theta activity in left cortical regions when processing rapid rate stimuli, FH+ infants showed attenuated activity in left as compared to right regions. Previous research has established the significance of left hemisphere activation to frequency change when processing rapid rate auditory stimuli, as well as highlighting the crucial role of processing rapid spectrotemporal change for efficient language acquisition. Thus, our findings confirm and extend differences reported in the neural substrates that underlie basic auditory detection and discrimination in infants at familial risk for language learning disorders when compared to infants without such risk.

Disclosures: N.A. Choudhury: None. **S. Ortiz-Mantilla:** None. **J. Parascando:** None. **A.A. Benasich:** None.

Poster

556. Human Cognition: Timing and Temporal Processing

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 556.20/TT64

Topic: F.01. Human Cognition and Behavior

Support: CONACYT-129337

Title: Saccadic eye movements during a bisection timing task

Authors: *A. TOSCANO ZAPIEN[1], D. DANIEL VELAZQUEZ-LOPEZ[2], D. VELAZQUEZ-MARTINEZ[1]
[1]Dept. de Psicofisiología, Facultad de Psicología., Univ. Nacional Autónoma De México, Mexico, D.F., Mexico; [2]Dept. de Matematicas, Facultad de Ciencias, Univ. Nacional Autónoma de México, Mexico, Mexico

Abstract: Timing refers to a wide range of behaviors that reveals the sensitivity of organisms to the duration of events on the range of seconds to minutes. Many species produced similar

behavioral patterns on a variety of timing procedures, revealing its adaptive significance. On a bisection task, first, subjects are trained to emit differential responses to a short or to a long stimulus; thereafter, subjects are confronted with intermediate durations. Several cognitive models assume that organisms uses a pacemaker to estimate durations and that the rate of the pacemaker is related to the bisection point (subjective equality between short and long stimuli), while the Weber Fraction and Limen is related to the sensitivity to the stimulus dimension and the attention paid to that dimension. In this study with human subjects, gaze variables (duration of fixations, pupil diameter, sequence of fixations, latency to fixation, latency of response, etc) were recorded while the subjects were trained in a bisection task (200 vs 800 msec) and then confronted intermediate durations of visual stimuli presented randomly at 5 locations on a screen. Data analysis included fixation to a stimulus by at least 100 msec and with a latency larger than 100 msec; with this criteria, two different populations emerged: subjects with a gaze that attempted to follow the location of the stimulus and a different population whose gaze remained in the center of the screen. Despite these differences, similar bisections point were obtained in the two populations but differences in Weber Fraction emerged; these differences may be related to the duration of fixations and to se sequence of fixations, revealing differences in the attentional mechanism used.

Disclosures: A. Toscano Zapien: None. **D. Daniel Velazquez-Lopez:** None. **D. Velazquez-Martinez:** None.

Poster

556. Human Cognition: Timing and Temporal Processing

Location: Halls A-C

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Program#/Poster#: 556.21/TT65

Topic: F.01. Human Cognition and Behavior

Support: ERC-YSt-263584

ANR- JCJC-1904

Title: The role of multisensory signals in interval timing: An MEG study

Authors: *T. W. KONONOWICZ, L. LECOUTRE, V. VAN WASSENHOVE
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Abstract: Duration perception has been shown to be influenced by the sensory modality; for instance, for the same physical durations, auditory stimuli have often been reported to be perceived as longer and more accurately than visual stimuli (Penney, Gibbon, and Meck, 2000). This auditory dominance is even present when audio-visual durations are used (Burr, Banks, Morrone, 2009; Chen and Yeh, 2009). In the context of internal clock which integrates temporal pulses to provide an estimates of elapsed time, auditory dominance is believed to result from the enhanced pacemaker rate, something that could be reflected in a build up of sustained magnetic fields (Sieroka et al., 2003) . Alternatively, duration and accuracy of temporal performance could be reflected in a pre- and pos- interval neural dynamics (Mayo and Sommer, 2013). Given that the neural signatures of multisensory duration perception have been rarely investigated, we asked participants to discriminate subsecond intervals marked by Audio, Visual, and Audio-Visual stimuli. This study was also designed to address how expectancy of a certain modality can modulate duration perception and how this expectancy changes pre-interval dynamics. To this end, participants were cued with cues that were predictive of interval modality. We will demonstrate how interval modality and cue predicting interval modality impacts subjective timing. Moreover, the role of ramping activity (Wittman, 2013) and oscillatory dynamics in duration discrimination will be discussed (Kosem, Gramfort, and Van Wassenhove, 2014).

Disclosures: **T.W. Kononowicz:** None. **L. Lecoutre:** None. **V. van Wassenhove:** None.

Poster

556. Human Cognition: Timing and Temporal Processing

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 556.22/TT66

Topic: F.01. Human Cognition and Behavior

Title: Paced auditory stimuli with distinct characteristics affect differently the clock-like neural process?

Authors: *D. MINCIACCHI, E. QUARTA, E. J. COHEN, R. BRAVI
Dept. of Exptl. and Clin. Med., Univ. of Florence, Florence, Italy

Abstract: In millisecond timing research, two forms of timing are distinguished: event-based and emergent timing (Spencer and Ivry, Brain Cogn, 2005, 58, 84-93). The essential difference between the two timing modes is considered to be consisting in the involvement or noninvolment of a clock-like neural process, i.e., an abstract effector-independent representation of the time intervals to be produced (Wing and Kristofferson, Percept Psycholphys, 1973, 14: 5-12). The

character of movements in a rhythmic motor task is considered a key factor for eliciting a specific mode of timing. The class of discrete movements, defined as having a clear-cut beginning and end, shown to engage the involvement of clock-like neural process (Huys et al., 2008, PLoS Comput Biol, 4: e1000061.10.1371). Discrete movements are not the only ones favoring exploitation of the event-based timing. Recent studies have demonstrated that salient auditory markers, such as streams of clicks (Torre and Delignières, 2008, Biol Cyb, 99: 159-170) and tactile feedback (Studenka et al., 2012, Q J Exp Psychol 65, 1086-1100.10.1080), are also able to elicit the event-based timing. Here, we investigated whether simple and complex paced auditory stimuli, as streams of clicks and excerpts of music, influence differently the processes for temporal regulation. Particularly, we wanted to study if music, when compared with clicks, has a different power in encouraging the event-based timing. Also, since Zelaznik and Rosembaum (2010, J Exp Psychol H Percept Perfor, 36: 1565-1575) provided evidence that external auditory event representation of movement favors event-based timing, we decided to explore whether the recall of an auditory stimulus, where auditory imagery is involved and is simulating an internal auditory representation, might influence timing control processes. In order to answer to these questions, subjects have participated in a session in which sets of repeated isochronous wrist's flexion-extensions were performed under various auditory conditions and during their recall. Kinematics was recorded and temporal parameters were extracted and analyzed. Results indicate that streams of clicks and music do affect differently the timing processes, with music having only a minor role in provoking event-based timing, especially evident at high ranges of tempi. In addition, the auditory experience, constructed of components drawn from memory in the absence of direct sensory instigation of experience, favors the involvement of event-based timing.

Disclosures: **D. Minciacchi:** None. **E. Quarta:** None. **E.J. Cohen:** None. **R. Bravi:** None.

Poster

556. Human Cognition: Timing and Temporal Processing

Location: Halls A-C

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Program#/Poster#: 556.23/TT67

Topic: F.01. Human Cognition and Behavior

Title: The role of perceptual and semantic processing on visually distracted time reproduction

Authors: *K. FOLTA
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Abstract: Question In humans, valence, temporal location and perceptual complexity of distracters have been demonstrated to modulate the perception and precise reproduction of physical time intervals in the seconds to minute range (interval timing). However, perceptually complex distracters might also be semantically more complex. This study aimed to further investigate whether perceptual and/or semantic complexity of distracters significantly contributed to the magnitude of distracter-induced temporal distortions. Methods Human participants were trained to a fixed target interval, before they reproduced the memorized duration in trials with and without presentation of distracting visual stimuli. Distracters varied according to their arousal, perceptual and semantic complexity, as well as their location within the to-be-timed interval. Possible effects of distracter-induced saccadic eye-movements were taken into account by complementing psychophysical measurements with high-speed eye-tracking recordings. Results In line with previous studies, we observed relatively large overproductions of the trained target duration, when distracters were presented near the end of the critical interval. In addition, a significant interaction between distracter characteristics of arousal and semantic complexity was observed. High arousing distracters induced longer time reproductions, when semantically complex (vs. less complex) distracters were presented, whereas low arousing distracters induced longer time reproductions in trials with presentation of semantically less complex distracters. However, no correlations between the number of saccades and the magnitude of temporal misperceptions had been observed. Conclusion Within an internal clock framework, our results can best be explained by a time- or resource-sharing mechanism that becomes modulated by the expectancy, arousal, and semantic complexity of visual distracters. The importance of our data will be discussed in the context of psychiatric disorders and involved symptoms of temporal misperceptions.

Disclosures: K. Folta: None.

Poster

556. Human Cognition: Timing and Temporal Processing

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

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Topic: F.01. Human Cognition and Behavior

Support: Elizabeth H. Solomon Center for Neurodevelopmental Research

NSF Grant SBE-0542013

Title: Spatial and temporal neural dynamics in infant development with early auditory experience

Authors: *G. MUSACCHIA[1,2], S. ORTIZ-MANTILLA[2], N. CHOUDHURY[2,3], T. REALPE-BONILLA[2], H. LOVERING[1], R. SHELL[1], A. A. BENASICH[2]
[1]Ctr. for Mol. & Behavioral Neurosci., Montclair State Univ., Bloomfield, NJ; [2]Ctr. for Molec. and Behav. Neurosci., Rutgers Univ., Newark, NJ; [3]Neuroscience, Psychology, Ramapo Col. of New Jersey, Ramapo, NJ

Abstract: Language acquisition relies on accurate neuronal representations of the rapid acoustic changes in the sounds we hear. Precise neuronal synchrony, and refinement of tonotopic and phonetic maps in the auditory system are fundamental to acquiring these representations. In support of this theory, previous data have shown that event-related potentials (ERPs) to rapid acoustic change can predict language outcomes from as early as 6 months-of-age. However, the hemispheric and temporal dynamics of development, and how auditory experience impacts the dynamic interplay are only beginning to be understood. To investigate this, we examined ERPs and source-localized brain activity to rapidly presented tone-pairs at 4 and 7 months in typically developing infants. Two groups were recruited: 1) The Active Experience (AEx) group received interactive auditory experience between 4 and 7 months, and 2) 7-month-olds who had no such experience (Naïves). EEG responses were recorded to frequency-invariant (STD) and frequency-variant (DEV) tone pairs in an oddball paradigm. Acoustic change in the tone-pair approximated formant transition timing in consonant-vowel phonemes during clear speech. Infants were fitted with an 128-sensor net, seated in the caregiver's lap, and entertained for the duration of the experiment. Individual ERPs were averaged offline (MATLAB) and submitted to mass univariate analyses in order to 1) compare current data to previous findings and 2) understand standard vs. deviant differences. ERPs showed no significant difference between STD and DEV responses at the first positive peak ("P1") and marked differences at the second negative peak ("N2*"). To investigate maturation and training effects, co-registered, age-appropriate brain templates were then used to model a two-dipole source solution (BESA, Inc.) for each individual ERP at P1 and N2* (N2 on the deviant wave). P1 and N2* peak values were submitted to significance testing with ANOVAS and t-tests (when appropriate). MATURATION: Shorter latencies and smaller amplitudes were seen bilaterally to sound onset (e.g. P1) and frequency change (N2*) as a function of age. TRAINING: AEx responses trended towards faster and more symmetric left/right activation to sound onset (P1). N2* latency was earlier than at 4 months and earlier on the right. AEx amplitude at N2* was smaller, compared to 4 months and to the Naïve 7-month counterparts. This implies a maturational increase in synchrony and decreased recruitment of neurons from 4-7 months and that auditory training at this age enhances neuronal synchrony to rapid frequency change, particularly in right auditory cortex.

Disclosures: G. Musacchia: None. **S. Ortiz-Mantilla:** None. **N. Choudhury:** None. **T. Realpe-Bonilla:** None. **H. Lovering:** None. **R. Shell:** None. **A.A. Benasich:** None.

Poster

556. Human Cognition: Timing and Temporal Processing

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 556.25/TT69

Topic: F.01. Human Cognition and Behavior

Title: Effect of Transcranial Magnetic Stimulation on Long Range Temporal Correlations in the motor cortex of the human brain

Authors: *E. BLAGOVECHTCHENSKI[1], M. NAZAROVA[2], V. NIKULIN[2]
[1]Higher Sch. of Econ., Moscow, Russian Federation; [2]Natl. Res. Univ. "Higher Sch. of Economics", Moscow, Russian Federation

Abstract: Long Range Temporal Correlations (LRTC) is one of the methods for studying the processes associated with neurodynamics of the brain networks that is becoming more popular. As shown by the model , this method of analysis of electrical activity can show the ratio of excitation and inhibition in the study of neural populations. We applied this method of electroencephalogram (EEG) analysis in the primary motor cortex during and after TMS . TMS is not nature characteristic effects on the nervous system, so it is interesting to evaluate how such effects can shift the ratio of inhibition and excitation and how such changes permanent . Primary motor cortex was chosen because in this case we can compare the effects shown in LRTC and in evoked motor responses (EMR). Furthermore, we can estimate the duration of effects (LHTC and EMR). We applied different protocols of TMS : like single pulse and double pulse stimulation up to rTMS and theta TMS . We got significant differences in changes LRTC primary motor and premotor cortex. Depending on the protocol of TMS, we obtained various deviations. We have also discovered that the duration TMC effects not directly correlated with the effects evoked motor responses .

Disclosures: E. Blagovechtchenski: None. **M. Nazarova:** None. **V. Nikulin:** None.

Poster

556. Human Cognition: Timing and Temporal Processing

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Program#/Poster#: 556.26/TT70

Topic: F.01. Human Cognition and Behavior

Support: Santa Fe Institute Consortium

Elizabeth H. Solomn Center for Neurodevelopmental Research

National Science Foundation Grant SMA-1041755 to the Temporal Dynamics of Learning Center

Title: Neural dynamics of perceptual narrowing for phonemes in infants

Authors: *S. C. ORTIZ-MANTILLA[1], J. A. HÄMÄLÄINEN[2], A. A. BENASICH[1]
[1]Rutgers The State Univ. of New Jersey, NEWARK, NJ; [2]Univ. of Jyväskylä, Jyväskylä, Finland

Abstract: During the first months of life, infants process phonemic elements from all languages similarly. This ability fades by 12 months-of-age as the brain constructs language-specific phonetic maps in acoustic cortex and as preference for the infant's native language increases. This ontogenetic process, known as perceptual narrowing, supports formation of specific neural representations and promotes more efficient processing of sensory information. To explore the oscillatory mechanisms underlying perceptual narrowing during early language acquisition, 6- and 12-month-old infants born into English monolingual families were presented with an oddball paradigm comprised of a native English and a non-native Spanish syllable contrast that varied in voice onset time. Dense array EEG/ERPs were collected with a 62-channel EGI sensor net and mapped onto age-appropriate brain templates. Source modeling placed dipoles for both conditions in right and left auditory and frontal cortices. Temporal-spectral analyses were conducted using a brain region montage consisting of 15 regional sources. The frequency range examined was 2-50 Hz at -300 to 930 ms, with 1Hz wide frequency bins and time resolution of 50 ms. Changes in frequency band amplitude, as a function of time relative to stimulus presentation, and consistency of phase alignment across trials were evaluated using TSE (temporal spectral evolution) and ITPL (inter-trial phase locking) measures. Permutation testing in combination with data clustering analyses followed by repeated measures ANOVA showed an age by stimulus interaction in the left posterior temporal region. At 6 months-of-age, a phase synchronization increase at 2-4Hz between 250 and 400 ms characterized infant's evoked responses to both, native and non-native syllable contrasts. In contrast, at 12 months-of-age, a decrease in ITPL was observed for non-native but not for native contrasts. It is assumed that phase synchronization would increase with age due to increasing myelination and more coherent neuronal connections (synaptogenesis and pruning). We found that in 12- as compared to 6-month-old infants, non-relevant sensory (phonemic) information does not induce increased phase synchronization in left auditory cortex. This suggests that changes in the magnitude of phase

synchronization may play a supporting role in the infant phonemic perceptual narrowing that favors processing of native over non-native syllable contrasts.

Disclosures: **S.C. Ortiz-Mantilla:** None. **J.A. Hämäläinen:** None. **A.A. Benasich:** None.

Poster

556. Human Cognition: Timing and Temporal Processing

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Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 556.27/TT71

Topic: F.01. Human Cognition and Behavior

Support: JSPS KAKENHI 23680029

Title: Long-term recalibration of neural time lag in audiovisual temporal order judgment

Authors: ***S. YAMAMOTO**, K. MORI
AIST, Tsukuba, Japan

Abstract: The brain is able to determine the temporal order of multiple sensory signals; this is a key factor in reconstructing the external world. However, the existence of two types of time lag, physical (from the event to receptors) and neural (from receptors to a comparator, if any), makes temporal ordering theoretically indeterminate. One possible solution that may promote the adjustment of these time lags would be to take past experiences into account. When a single source of sound and light is located close to an individual, the final time lag at the comparator should be primarily due to neural lag. In this case, the final arrival time of the sound signal (relative to that of the light signal) should be earlier compared to the situation where the source is located far from the individual. Thus, we hypothesized that 1) the brain can adjust neural lag such that the final time lag at the comparator in the case that the arrival time of the sound signal (relative to that of light signal) would be the earliest is equal to the size of the neural lag, and that 2) this adjustment is slow and stable, because the neural lag is normally supposed to change only during development and aging. To test these hypotheses, we performed a multiple-day experiment in which subjects judged the temporal order of pairs of audiovisual stimuli (a white noise burst and a red light-emitting diode flash). Stimulus onset asynchronies (SOAs) were sampled from -220 (light-first) to +220 ms (sound-first) in 40 ms steps (mean = 0 ms). A single experimental session consisted of 72 trials; there were 12 sessions on each experimental day, and subjects were tested for a total of 8 days with an interval of less than 7 days. In the first session on the first day, the point of subjective simultaneity (PSS) was slightly biased towards the light-

first direction (mean: -13.3 ms), but it gradually shifted towards the sound-first direction within and across days. In the final session on the eighth day, the PSS was as large as 69.3 ms on average. Furthermore, when subjects attended 1-day sessions using the same protocol more than 1 month after the eighth day, the PSS remained as large as that on the eighth day. Taken together, these results support our hypotheses, that is, neural lag was adjusted by taking long-term past experiences into consideration. This slow change in subjective temporal order with retention was termed "zero-adjustment". The zero-adjustment may contribute to a reconstruction of the temporal structure of the external world together with another type of quick and flexible temporal recalibration (lag adaptation), which is considered to facilitate the adjustment of the physical lags.

Disclosures: S. Yamamoto: None. **K. Mori:** None.

Poster

557. Executive Function: Models of Disorders I

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 557.01/TT72

Topic: F.02. Animal Cognition and Behavior

Support: CIHR MOP-133579

Title: A comparison of the effects of different pharmacological stressor and restraint stress on effort-based decision-making

Authors: C. A. BRYCE[1], *S. B. FLORESCO[2]
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Abstract: Decision-making involves choosing between several alternative possibilities after evaluation of the relative costs and benefits. Increasing the amount of effort required to obtain a reward is one type of cost that can diminish the subjective value of objectively larger rewards. Repeated episodes of stress can result in depressive symptoms including anergia, which may reduce the tendency to exert effort to obtain rewards. The goal of the present study was to compare the effects of restraint stress to different types of pharmacological stressors on effort-based decision-making. Using an operant chamber assay, rats were required to choose between a low effort/low reward lever (LR; 2 pellets), and a high effort/high reward lever (HR; 4 pellets), with the effort requirement increasing over trial blocks (2, 5, 10 and 20 presses). Acute restraint stress, but not injection of corticosterone, causes rats to choose the HR option less compared to

baseline performance. In a subsequent study, we assessed the effects of the alpha 2 adrenoreceptor antagonist yohimbine (1-3 mg/kg, IP), which mimics increased noradrenaline transmission induced by acute stress. Contrary to our expectation, yohimbine increased preference for the HR option compared to vehicle treatment. This suggests that yohimbine and restraint stress produce divergent effects on some decision-making tasks, highlighting the fact that different types of stressors can induce opposing effects on behavior. However, in a second experiment, we assessed how effort-related choice may be affected by increased corticotropin-releasing factor (CRF), a stress hormone and centrally acting excitatory neurotransmitter, as CRF is known to induce anxiolytic behavior in certain assays. Indeed, we found that microinfusions of CRF (3 ug/ul ICV) decreased HR choice and increased response latency, mimicking the behavioral profile of restraint stress on effort-related choice behavior. This identifies CRF as a potential mediator of restraint stress on decision-making ability. Whether blockade of CRF transmission will ameliorate the effects of restraint stress on effort discounting is a topic for future research.

Disclosures: **C.A. Bryce:** None. **S.B. Floresco:** None.

Poster

557. Executive Function: Models of Disorders I

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 557.02/TT73

Topic: F.02. Animal Cognition and Behavior

Support: CIHR MOP-130393

Title: Prefrontal GABAA receptor regulation of working memory assessed with a delayed non-match to position task

Authors: *M. AUGER, S. B. FLORESCO
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Abstract: Perturbations in prefrontal GABA function are thought to contribute to the cognitive symptomatology of schizophrenia. However, there has been surprisingly few studies assessing the role of GABA in cognitive functions carried out by the frontal lobes. Recent work from our group has shown that blockade of prefrontal GABA receptors leads to a constellation of cognitive abnormalities that resemble those observed in schizophrenia. However, intra-PFC infusions of a GABAA antagonist did not impair working memory accuracy assessed with a

delayed-response version of a radial arm maze task. Notably, performance on this task was self-paced, while cognitive tasks used with patients often present multiple trials in quick succession, placing higher demands on attention. As such, the goal of this study was to assess the contribution of prefrontal GABA signaling to working memory in these conditions. Male Long Evans rats were trained in a delayed non-match to position task. The task consists of a sample phase in which one of two levers is extended, and a choice phase that requires the selection of the opposite lever, separated by a variable delay (1-24 s). Well-trained rats received counter-balanced intra-medial PFC infusions of saline or the GABAA antagonist, bicuculline (50 ng). Prefrontal GABA receptor blockade induced a delay-independent impairment in performance. While this effect was significant, the magnitude of the impairment was relatively small in comparison to effects induced by prefrontal GABA blockade in forms of cognition, such as cognitive flexibility or spatial reference memory. As such we are currently assessing the contribution of other transmitter systems relevant to schizophrenia to the performance of the delayed non-match to position task, including NMDA GluN2B mediated glutamate transmission. These findings indicate that prefrontal GABA transmission plays a role in working memory processes executed by the frontal lobes, and diminished PFC GABA function can lead to cognitive abnormalities relevant to schizophrenia.

Disclosures: M. Auger: None. **S.B. Floresco:** None.

Poster

557. Executive Function: Models of Disorders I

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 557.03/TT74

Topic: F.02. Animal Cognition and Behavior

Support: CIHR

NSERC #261543-13

Title: Alterations in probabilistic learning, testosterone and the prefrontal cortex during aging

Authors: R. J. TOMM, C. Q. MA, M. T. TSE, M. M. GRIST, S. B. FLORESCO, *K. K. SOMA
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Abstract: During aging, changes in the mesocorticolimbic dopamine system may lead to impairments in decision making and executive function. In addition, circulating levels of testosterone (T) decrease during aging, and T regulates dopamine synthesis and/or action in the ventral tegmental area (VTA), nucleus accumbens (NAc) and prefrontal cortex (PFC). In the present study, we used a rat model and compared young adult (3-5 months) and aged (20-22 months) male rats (Fischer 344/Brown Norway hybrid). To measure cognitive flexibility, we used a probabilistic reversal task. Rats were trained to choose between a correct lever (80% probability of food reward) and an incorrect lever (20% probability of food reward). After 8 consecutive correct choices, the contingencies of the correct and incorrect levers were switched, and this continued over the 200 trials of a daily session. Rats were trained on the task for 16 days. Over the last 5 days of training, when performance was asymptotic, aged rats showed increased win-stay behavior, relative to young adults (i.e., selecting the correct lever after a rewarded correct choice on the previous trial). Aged rats also showed increased lose-shift behavior (i.e., selecting the incorrect lever after a non-rewarded correct choice). Thus, aged rats displayed an increased sensitivity to both rewards and negative feedback, and instead of using a longer-term representation of action-reward history, they were driven by more immediate feedback. The increase in reward sensitivity suggests impaired medial PFC functioning. Furthermore, there was a significant decrease in circulating T levels in aged rats. Testosterone may modulate the PFC via either dopaminergic projections from the VTA or androgen receptors in the PFC. Presently, we are measuring tyrosine hydroxylase immunoreactivity in the PFC and VTA of these subjects. These data will further our understanding of the neurochemical mechanisms that underlie cognitive changes during aging.

Disclosures: R.J. Tomm: None. **C.Q. Ma:** None. **M.T. Tse:** None. **M.M. Grist:** None. **S.B. Floresco:** None. **K.K. Soma:** None.

Poster

557. Executive Function: Models of Disorders I

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Program#/Poster#: 557.04/TT75

Topic: F.02. Animal Cognition and Behavior

Support: MRC CASE award G1001602

Title: Predictive validity of the neurokinin-1 knockout mouse model of ADHD

Authors: *K. PILLIDGE[1], A. PORTER[1], Y.-C. TSAI[1], T. VASILI[1], D. HEAL[2], C. STANFORD[1]
[1]Univ. Col. London, UCL, London, United Kingdom; [2]RenaSci Ltd, Nottingham, United Kingdom

Abstract: Attention Deficit Hyperactivity Disorder (ADHD) is a heterogeneous psychiatric disorder which presents as different subtypes according to the primary characteristic (i.e. predominantly hyperactive/impulsive or inattentive). The disorder is highly heritable; recent evidence suggests that four single-nucleotide polymorphisms within the tachykinin1 (TACR1) gene are associated with ADHD (Sharp et al., 2009, Neuropharmacology, 57:590-600). Mice with functional ablation of the murine equivalent of the TACR1 gene, neurokinin 1 (NK1R-/-), display locomotor hyperactivity, impulsivity and inattentiveness. Moreover, the psychostimulant treatments for ADHD (d amphetamine and methylphenidate) paradoxically alleviate the hyperactivity of this mouse. However, d amphetamine does not alleviate the inattention/impulsivity displayed by these mice in the 5 Choice Serial Reaction-Time Task (5 CSRTT). Our aim in these experiments was to further validate the NK1R-/- mouse in preclinical studies of ADHD-like behaviour. We tested non-stimulant treatments (atomoxetine; a preferential noradrenaline reuptake inhibitor and guanfacine; an alpha2A-adrenoceptor agonist) in wildtype and NK1R-/- mice in the 5-CSRTT. Wildtype and NK1R-/- mice were trained to perform the 5-CSRTT as previously described (Yan et al., 2011, PLoS One, 6, e17586). Separate cohorts of mice were tested with either atomoxetine (1, 3 and 10mg/kg) or guanfacine (0.1, 0.3 and 1mg/kg). Mice were tested once weekly, using a variable inter-trial interval (VITI) after reaching the criteria for a stable baseline of performance. Each treatment was given to each mouse once, 30 min before testing and treatments were assigned in counterbalanced order. Behaviour specific, and genotype dependent, effects were found: guanfacine (0.1mg/kg) improved attention in NK1R-/- mice only, but caused a non-specific blunting of behaviour, which reduced impulsivity, at a high dose (1mg/kg). This resembles the clinical profile of guanfacine in ADHD. Atomoxetine (10mg/kg) selectively reduced impulsivity in NK1R-/- mice only, without affecting attention, and thus may be a preferential treatment for the predominantly hyperactive/impulsive subtype of ADHD. The results further validate the use of NK1R-/- mice to study ADHD-like behaviour expressed by patients with TACR1 polymorphisms.

Disclosures: **K. Pillidge:** None. **A. Porter:** None. **Y. Tsai:** None. **T. Vasili:** None. **D. Heal:** F. Consulting Fees (e.g., advisory boards); Shire. **C. Stanford:** None.

Poster

557. Executive Function: Models of Disorders I

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Program#/Poster#: 557.05/TT76

Topic: F.02. Animal Cognition and Behavior

Support: G1001602

Title: The brain renin angiotensin system influences the locomotor hyperactivity and impulsivity of neurokinin-1 receptor 'knockout' mice

Authors: *A. J. PORTER[1], K. PILLIDGE[2], T. VASILI[2], C. STANFORD[2]
[1]Neuroscience, Physiol. and Pharmacol., [2]Univ. Col. London, London, United Kingdom

Abstract: Mice with functional ablation of the substance P-preferring neurokinin-1 receptor gene (NK1R-/-) display behavioural abnormalities that resemble Attention-Deficit Hyperactivity Disorder (ADHD), namely: hyperactivity, impulsivity and inattentiveness (Yan et al., 2011, PloS ONE, 6:e17586). The brain renin angiotensin system (BRAS) influences both motor activity and cognition (see: Wright et al., 2008, JRAAS, 9(4):226). Angiotensin converting enzyme (ACE) hydrolyzes substance P (Yokosawa et al. 1985, J Biochem, 98:1293), and there is a striking overlap between the distribution of substance P and angiotensin binding sites in the brain (Diz et al., 1986, J Hypertens Suppl., 4:S468). Here, we tested the effects of the ACE inhibitor, captopril, and the angiotensin receptor (ATR) antagonists, losartan (ATR1) and PD123319 (ATR2), on the locomotor hyperactivity, impulsivity, and inattentiveness of NK1R-/- mice using the light/dark exploration box (LDEB) and 5-Choice Serial Reaction Time Task (5-CSRTT). In the LDEB, following 90 min habituation to a 'dark zone', animals were transferred to a 'light zone' and their free movement across both zones recorded for 30 min. During habituation, animals received an i.p. injection of (dose; time before recording): vehicle (saline; 30 min), captopril (10 and 25 mg/kg; 30 min), losartan (10 and 25 mg/kg; 60 min), or PD123319 (1 and 3 mg/kg; 30 min). In the 5-CSRTT, a separate batch of animals was trained to baseline performance criteria and tested, once weekly, using both a variable intertrial interval (2, 5, 10 and 15 s) and a long intertrial interval (10 s). In both tests, animals received either no injection or an i.p. injection of vehicle or captopril (5, 10 and 25 mg/kg). Every animal experienced each drug treatment once for each test, in a counterbalanced sequence. Data were analysed using either single-measures (LDEB) or repeated measures (5-CSRTT) ANOVA, with post hoc (LSD) pairwise comparisons. Captopril reduced the locomotor hyperactivity of NK1R-/- mice in the LDEB (10 and 25 mg/kg: $P<0.05$ and $P<0.001$) and reduced their impulsivity in the 5-CSRTT (10 mg/kg: $P<0.05$), but did not affect the wildtypes in either test. By contrast, both ATR antagonists caused an apparent increase in locomotor activity in NK1R-/- mice. These findings point to an interaction between ACE activity in the BRAS and activation of NK1R that regulates motor activity and impulsivity. The possibility that ACE inhibitors provide a novel therapeutic treatment for ADHD (particularly the Predominantly Hyperactive/Impulsive subtype) warrants further investigation.

Disclosures: **A.J. Porter:** None. **K. Pillidge:** None. **T. Vasili:** None. **C. Stanford:** None.

Poster

557. Executive Function: Models of Disorders I

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Topic: F.02. Animal Cognition and Behavior

Support: NIH/NCI/MSKCC #U54CA137788/CCNY#U54CA132378

NCRR 5G12RR003060-26

NIMHHD 8G12MD7603-28

Title: Chemotherapy reduces locomotor activity, impairs working and spatial memory and decreases BDNF, but does not affect anxiety or visual memory

Authors: *D. WOO[1], C. RICHARDSON[2], C. STREET[3], K. URIBE[2], M. QADRI[3], N. TALUKDER[3], G. DEJESUS[3], S. PEREZ[4], T. AHLES[5], K. HUBBARD[2], K. Y. SALAS-RAMIREZ[3]
[1]Physiology, Pharmacol. and Neurosci., Sophie Davis Sch. of Biomed. Education, CCNY, New York, ; [2]The City Col. of New York, New York, NY; [3]The Sophie Davis Sch. of Biomed. Education, CCNY, New York, NY; [4]Lehman College, CUNY, Bronx, NY; [5]Mem. Sloan Kettering Cancer Ctr., New York, NY

Abstract: Breast cancer is the most common cancer among women impacting over 100,000 women a year and has the second highest mortality rates among the cancers women suffer. A growing body of work has shown that chemotherapy can have profound cognitive effects on patients up to ten years after exposure, specifically on memory function and executive function. This study aimed to determine whether chemotherapeutic agents, commonly used in breast cancer, results in cognitive impairments. Forty Sprague Dawley female rats, half intact and half overiectomized (OVX), were treated intravenously with either saline or a combination of cyclophosphamide (40mg/kg) and doxorubicin (4 mg/kg) once a week for three weeks. One week after the last chemo treatment, all subjects were tested for anxiety (elevated plus maze), locomotor activity (open field), working (y-maze), visual (object recognition) and spatial (object placement) memory. After all behavioral assessments were performed, animals were decapitated and trunk blood was collected. Although anxiety and visual memory remained intact,

chemotherapy significantly decreased locomotor activity. More significantly, it decreased working memory and spatial memory in female rats, independent of their hormonal status. In addition, we determined that brain derived neurotrophic factor in the blood plasma was significantly decreased by treatment. The cognitive deficits observed are hippocampal dependent, hence confirming other findings that cyclophosphamide and doxorubicin impact synaptic plasticity molecules that regulate learning and memory function. Further studies will focus on understanding additional aspects of neural plasticity that can be affected by chemotherapy that could result in cognitive deficits. We hypothesize that chemotherapy reduces neurogenesis and synaptic communication of hippocampal neurons resulting in cognitive dysfunction.

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Poster

557. Executive Function: Models of Disorders I

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Topic: F.02. Animal Cognition and Behavior

Support: NIMH 1F32MH087076

NINDS 5R01NS024760-25

NIMH 5R01MH057414-14

NSF

CELEST

Title: Amygdalar pathways target excitatory and distinct inhibitory mechanisms in mnemonic cortices of the primate medial temporal lobe

Authors: *J. G. BUNCE, H. BARBAS
Neural Systems Lab., Boston Univ., Boston, MA

Abstract: Signals of affective value from the amygdala play a prominent role in the neural processes underlying learning and memory. The recent confirmation that amnesic patient H.M. had severe damage to the amygdala while much of his hippocampus was spared, has refocused attention on the interaction between the amygdala and other mnemonic structures in the medial temporal lobe. Information flow to and from the hippocampus is gated by the rhinal cortices (areas 28 and 35) and is largely segregated by layers. The upper layers direct projections to the hippocampus while the deep layers receive hippocampal output. Signals arriving in the rhinal cortices must overcome the "wall of inhibition" to propagate to and from the hippocampus, though the anatomical substrate is unclear. Here we examined the organization and features of the amygdalar pathway to excitatory and inhibitory post-synaptic targets in entorhinal (area 28) and perirhinal (area 35) cortices in the rhesus monkey (N=5) using anterograde tracers and standard immunohistochemistry techniques. We mapped and measured the distribution of amygdalar bouton terminals and their interaction with neurochemical classes of inhibitory post-synaptic targets labeled for parvalbumin (PV), calbindin (CB), and calretinin (CR). Analysis using unbiased stereological techniques, showed that the amygdalar pathway to area 28 was most dense in the upper layers (68.6%) compared to the deep layers (31.4%). In contrast, amygdalar terminations in area 35 were more evenly distributed in the upper (51.8%) and deep (48.2%) layers. We measured the size of amygdalar bouton terminals in areas 28 and 35 and used a k-means cluster analysis to classify them into large and small populations based on a criterion threshold of 1.2 μm for area 28 and 1.1 μm for area 35. Amygdalar projections to both the upper and deep layers of areas 28 and 35 terminated in small and large boutons with a ratio that exceeded 3:2. Confocal analysis at the fluorescence microscope of neurochemically distinct inhibitory post-synaptic targets showed that the amygdalar pathway was preferentially in apposition with CR (5.5%) neurons in the upper layers and PV (3.8%) neurons in the deep layers of area 28. In area 35, amygdalar boutons were in apposition most frequently with PV (4.8%) inhibitory neurons in the upper layers and CR (3.8%) inhibitory neurons in the deep layers. These findings suggest that the amygdala is positioned to transfer affective information to memory related rhinal cortices that may overcome the rhinal "wall of inhibition" via interactions with mostly excitatory post-synaptic targets as well as CR inhibitory neurons which disinhibit the local network.

Disclosures: J.G. Bunce: None. **H. Barbas:** None.

Poster

557. Executive Function: Models of Disorders I

Location: Halls A-C

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Topic: F.02. Animal Cognition and Behavior

Support: 5R01NS024760-25 (NINDS)

5R01MH057414-14 (NIMH)

CELEST (NSF)

Title: Context and emotion: Computational modeling of interactions between hippocampus and amygdala

Authors: *Y. J. JOHN[1], D. BULLOCK[2], J. G. BUNCE[1], B. ZIKOPOULOS[1], H. BARBAS[1]
[1]Neural Systems Lab., [2]Psychological and Brain Sci., Boston Univ., Boston, MA

Abstract: An important function of cognitive-emotional interactions is to allow an organism to discern the affective values of perceived cues or contexts. The emotional value of recognized cues or contexts can then be used to adjust decisions and behavior. Contextual learning, which is distinct from focal cue learning, allows assignment of affective value to a context or a spatial location. For example, animals exposed to an aversive stimulus such as footshock in a particular context form an association which leads them to respond fearfully when reintroduced to that context. Experimental data indicate that the hippocampus is required for the spatial and contextual aspects of such learning, and that the amygdala is required for the affective associations as well as the expression of fearful behavior. Further evidence suggests that the role of the hippocampus in emotion extends beyond spatial processing: it has been implicated in stress, anxiety and depression. Uncovering the precise role of the hippocampus in emotion requires understanding its interactions with the amygdala. The network that links the hippocampus and the amygdala may serve as a basis for the formation of an “emotional space”. In such a space, external contextual information becomes labeled or “colored” according to affective significance. Our computational model of interactions between the hippocampal formation and amygdala explicates network features that enable learned associations of contexts with appetitive or aversive events. Simulations also distinguish mechanisms by which dysfunctions in the hippocampus-amygdala network can lead to mislabeling or overgeneralization of emotional contexts. This insensitivity or overgeneralization may become manifest as an emotional disorder. For instance, some forms of depression may involve an inability to distinguish between aversive, neutral and positive contexts. Similarly, post-traumatic stress disorder (PTSD) may involve a form of overgeneralization that leads to the conflation of a neutral or pleasant context with that of a previously experienced traumatic episode. Our computational model can help generate hypotheses regarding the origins of such disorders and may point to possible treatment strategies.

Disclosures: **Y.J. John:** None. **D. Bullock:** None. **J.G. Bunce:** None. **B. Zikopoulos:** None. **H. Barbas:** None.

Poster

557. Executive Function: Models of Disorders I

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Support: 1R01MH101209

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5R01MH057414-14

NSF CELEST

Title: Common architecture of subgenual anterior cingulate cortices and white matter pathways in human and non-human primates

Authors: *B. ZIKOPOULOS, M. Á. GARCÍA-CABEZAS, H. BARBAS
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Abstract: Anterior cingulate cortices and in particular areas 25 and 32, ventral to the genu of the corpus callosum (referred to as sgACC), are consistently affected in various neuropsychiatric and neurodevelopmental disorders, including depression, schizophrenia, and autism. Diverse, but often discordant, findings correlate structural changes in the grey matter volume, cellular content, and integrity of underlying white matter pathways of sgACC with hyper- or hypo-activity of this frontal region in diverse diseases. This makes it hard to discern patterns of systematic variation in key anatomical/molecular substrates that can predict normal or abnormal connectivity and function. To address this issue, we examined and compared the laminar density and morphology of neurons, glia, and myelinated axons in the grey and white matter of sgACC from post-mortem normal adult human and rhesus monkey brains at very high resolution. Our goal was to establish a framework that can be used to integrate and correlate functional/neuropathological data from human studies with fine details of pathway connectivity from primate studies. The features and organization of the grey and white matter were similar in both primate species. Although both areas are dysgranular, individual layers in area 32 were overall better delineated than in area 25. The density of neurons was higher in area 25, especially

in the middle/deep layers, but the density of glia was similar throughout sgACC, with a tendency for fewer oligodendrocytes in area 25, leading to a significantly lower glia to neuron ratio. Levels of myelinated axons, which were mostly small and medium in diameter, were moderate in layer 1, dropped in the other layers, and got progressively higher through the transition from layers 5-6 to the white matter, where axons made up about 40% of the volume, with the rest of the space occupied by glia. Interestingly, area 25 had overall the lowest levels of myelin and myelinated axons within sgACC, especially in the middle/deep layers of the cortex and the superficial white matter. Our findings highlight key loci in the primary output layers (middle/deep) of sgACC, and the superficial white matter below, which contains mostly short/medium-range pathways, connecting these areas with their neighbors. In these loci, we pinpoint differences in: a) glia/neuron ratio and b) myelin content that may underlie, on one hand, the remarkable potential of these limbic cortices for plasticity and, on the other hand, their disproportionate vulnerability in disease.

Disclosures: **B. Zikopoulos:** None. **M.Á. García-Cabezas:** None. **H. Barbas:** None.

Poster

557. Executive Function: Models of Disorders I

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Support: NINDS Grant 5R01NS024760-25

NIMH Grant 5R01MH057414-14

Title: A novel pathway from the anterior cingulate to the premotor cortex may link cognitive control and movement

Authors: O. K. SWANSON, *M. GARCIA-CABEZAS, H. BARBAS
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Abstract: The prefrontal cortex has a key role in flexible behavior and decision for purposeful movement, yet the circuits linking this cortex and effector motor areas are understudied. Within the prefrontal cortex, the anterior cingulate cortex (ACC) is a unique node for cognitive processing: the ACC has strong and widespread connections with other prefrontal areas, such as dorsolateral areas 9 and 46, and is activated during tasks involving high cognitive load, including

those that demand selective attention or elicit response conflict. To determine whether the ACC directly influences motor areas, we mapped pathways after injection of neural tracers into ACC area 32 in rhesus monkeys. We found a novel pathway from ACC area 32 that terminates across all layers of dorsorostral area 6 (6DR) of the premotor cortex. A majority of axon boutons from ACC area 32 formed synapses with spines of excitatory neurons in area 6DR; only approximately 10% of the total number of boutons formed synapses with neurochemically and functionally specialized classes of inhibitory interneurons. Area 6DR is distinguished from other premotor cortices by having fewer direct spinal projections and more widespread connections with lateral prefrontal areas associated with cognitive processes. We found that the same sites of area 32 that project to area 6DR also receive pathways from lateral prefrontal areas 46 and 9. This evidence suggests that serial pathways from the lateral prefrontal cortex influence ACC area 32, which has a role in attention and a direct output to the premotor cortex. These connections, together with projections to other motor cortices, place area 6DR in an ideal position to interface between areas associated with cognition and motor control. The novel pathway that links ACC area 32 and premotor area 6DR provides a circuit through which the prefrontal cortex may mediate decision for movement in goal-directed behavior.

Disclosures: O.K. Swanson: None. **M. Garcia-Cabezas:** None. **H. Barbas:** None.

Poster

557. Executive Function: Models of Disorders I

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Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant MH091630 (JMF)

Department of Veterans Affairs (RWG)

Title: Effects of ventral medial-prefrontal-cortex NMDAR-NR1 subunit deletion on a spatial reference memory radial maze task in adult mice

Authors: R. M. WESTON[1], *M. J. MANA[1], A. M. SCHILLER[1], T. V. NGUYEN[1], M. M. GORHAM[1], R. F. KYDD[1], R. W. GREENE[2], J. M. FINLAY[1]
[1]Dept of Psychology, Western Washington Univ., Bellingham, WA; [2]Psychiatry, UTSW & Dallas VAMC, Dallas, TX

Abstract: Dysfunction of glutamate *N*-methyl-D-aspartate (NMDA) receptors may contribute to cognitive deficits in schizophrenia. In the present study, we examined the effects of chronic NMDA receptor dysfunction in the ventral medial prefrontal cortex (VmPFC) on acquisition of a spatial reference memory radial maze (SRM) task as employed by Niewoehner et al. (2007). Localized NR1 gene deletions were induced in the VmPFC of floxed NR1 mice (DEL, n=10) using an AAV-Cre vector; Control mice (CON, n=10) received sham deletions. In the SRM task, food was placed in 3 arms of a 6-arm radial maze at the start of each trial; the location of the food was consistent for each mouse across all trials, but randomly varied between mice. At the start of each trial, each mouse was placed in the central chamber of the maze and allowed to enter any of the 6 arms; once an arm was visited and a mouse returned to the central chamber, the entrance to each of the 6 arms was blocked for 10 sec. At the end of each 10 sec timeout, the arms that had not yet been visited during the trial were made accessible and a mouse could choose to enter one of the remaining unvisited arms. Thus, this task only assessed reference memory errors (RME); that is, entries to arms that were never baited. Mice were tested in 4 trials/each daily session. We found that NMDA receptor dysfunction in the VmPFC had no effect on acquisition of the SRM task. There were no differences in behavior observed in the first session (mean arms visited, DEL = 5.3 arms and CON = 5.2 arms; mean baited arms visited DEL and CON = 3.0; mean unbaited arms visited, DEL = 2.3 arms and CON = 2.2 arms). Mice in both the groups acquired the SRM task over 24 sessions (mean arms visited, DEL = 3.5 arms and CON = 3.6 arms; mean baited arms visited DEL and CON = 3.0; mean unbaited arms visited (that is, RME), DEL = 0.5 errors and CON = 0.6 errors). After the 24-session Acquisition Phase, we rotated the extra-maze spatial cues to confirm the use of such cues in SRM performance by both groups (mean RME during the Rotation session, DEL and CON = 1.8 errors). The lack of significant between-group differences in the SRM task in our study contrasts with recent reports that $Grin^{1DGCA1}$ mice with NMDA receptor deletions in the dentate and CA1 regions of the hippocampus show impaired acquisition in this task (Bannerman et al., 2013), reflecting different contributions of the VmPFC and hippocampus in the task. Given the well-known contribution of the PFC to working memory, we are currently assessing PFC NR1-deleted mice in a working memory variant of this task.

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Poster

557. Executive Function: Models of Disorders I

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Support: NIH Grant MH091630 (JMF)

Department of Veterans Affairs (RWG)

Title: Effects of ventral medial prefrontal cortex NMDAR-NR1 subunit deletion on attention and response inhibition in adult mice

Authors: H. G. MANNING[1], M. A. MAYNES[1], R. K. FLYNN[1], R. M. WESTON[1], R. W. GREENE[2], *J. M. FINLAY[1]
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Abstract: Dysfunction of prefrontal cortex (PFC) glutamate N-methyl-D-aspartate receptors (NMDARs) is implicated in cognitive impairments associated with schizophrenia. NMDAR antagonists administered directly into the medial PFC (mPFC) of adult rats have been found to impair cognitive processes involved in both attention and response inhibition. Recent work in our lab indicates that chronic NMDAR-NR1 subunit deletion in the mouse dorsal mPFC, while not affecting attention, results in deficits in response inhibition that are manifest as increased compulsive behavior. We have now extended this work to examine the effects of chronic NMDAR-NR1 deletion in the mouse ventral mPFC on attention and response inhibition. NMDAR-NR1 dysfunction was induced on postnatal day 70 by bilateral infusions of adeno-associated virus Cre-recombinase into the ventral mPFC of targeted knock-in mice with loxP sites flanking exons 11-22 of the NR1 gene. Attention and inhibitory response control were assessed using a 5-choice serial reaction time task (5CSRTT) in which mice exhibit a nosepoke in response to a brief, random illumination of one of five apertures. Premature responses, responses occurring during an intertrial interval (ITI), and perseverative responses, multiple responses to a single stimulus, were assessed as measures of inhibitory response control. Baseline conditions consisted of a 0.8 s stimulus duration (SD) and a 5.0 s ITI. On baseline trials, deleted and control mice exhibited comparable accuracy (all data are represented as mean ± SEM; 90 ± 1.6% and 88 ± 1.4%, respectively), premature responses (1.5 ± 0.4 and 1.6 ± 0.3), and perseverative responses (13.1 ± 1.7 and 11.8 ± 1.5). Upon acquisition of baseline responding, we examined whether differences between deleted and control mice would emerge under conditions of increased demand, achieved by testing under shorter and longer ITIs, reduced SD and stimulus intensity, and white noise. In a test session consisting of variable longer ITIs (randomly presented 5, 6, 7, and 8 s ITIs), control mice exhibited progressively more premature responding as the ITI increased such that premature responding increased from 0.3 ± 0.1 response during a 5 s ITI to 3.8 ± 0.9 responses during an 8 s ITI. This effect was exacerbated in deleted mice, with this group exhibiting premature responding of 0.4 ± 0.2 responses during a 5 s ITI and 5.1 ± 1.1 responses during an 8 s ITI. Together with our previous

study, these findings suggest that the NMDARs in the dorsal mPFC play a role in inhibition of compulsive behavior whereas those in the ventral mPFC play a role in inhibition of impulsive behavior.

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Poster

557. Executive Function: Models of Disorders I

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 557.13/TT84

Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant 1R15MH097223-01

Middlebury College Undergraduate Research Office

Title: The neurosteroid pregnenolone sulfate interacts with the NMDA receptor antagonist MK-801 to impair cognitive flexibility and spatial working memory in a rat model of schizophrenia

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Abstract: Schizophrenia is a chronic disorder characterized by debilitating cognitive impairments. Most affected are the 'executive functions' crucial for the temporal organization of goal-directed behaviors. These cognitive abilities, which include working memory and cognitive flexibility, require brain networks that share as a common node the prefrontal cortex (PFC). Recent research has linked these cognitive deficits to reduced PFC inhibitory signaling by the neurotransmitter GABA, leading to the hypothesis that reduced inhibitory regulation of glutamatergic principle neurons results in disorganized information processing and cognitive impairments. We used male Sprague-Dawley rats to examine the cognitive effects of pregnenolone sulfate, a neurosteroid that modulates GABA-A receptors to reduce GABA-mediated inhibition, and MK-801, an NMDA glutamate receptor antagonist commonly used to induce schizophrenia-like cognitive deficits in rodents. MK-801 is believed to act, in part, through inhibitory actions on GABA neurons. Rats were tested for extradimensional (ED) set-shifting ability, a measure of cognitive flexibility, and spontaneous alternation behavior (SAB), a measure of spatial working memory. Rats received bilateral intra-medial PFC injections (0.5

uL/hemisphere) 20 min prior to the ED shift phase of the set-shift task or the single SAB session. Injections consisted of a vehicle solution, MK-801 (0.3 or 3 μg/hemisphere), or pregnenolone sulfate (0.05, 0.5 or 5.0 ng/hemisphere), alone or in select combinations. We observed that MK-801 alone at 3.0 ug but not 0.3 ug impaired cognitive performance relative to vehicle-injected controls, in the set-shift task by increasing the trials required to reach a learning criterion and increasing the amount of perseverative responding to the first discrimination rule learned, and in the SAB task by decreasing the percent alternation score. Pregnenolone sulfate also dose-dependently impaired task performance. In the set-shift task, the highest dose of pregnenolone sulfate produced significant impairments; in the SAB task, the intermediate dose significantly impaired. Co-administration of individually ineffective doses of MK-801 and pregnenolone sulfate impaired task performance relative to vehicle-injected controls in the set-shift task. This pattern of results, in which two neuromodulatory compounds known to act by decreasing GABA-mediated inhibitory tone interact to produce performance deficits on behavior tasks dependent on prefrontal function, provide further support for the hypothesis that aberrant GABAergic signaling underlies schizophrenia-associated cognitive deficits.

Disclosures: **M.R. Stefani:** None. **J.K. Gehrmann:** None. **E.D.H. Barnard:** None. **D.M. Prior:** None.

Poster

557. Executive Function: Models of Disorders I

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Topic: F.02. Animal Cognition and Behavior

Support: NIH 1R01 NS063009

Title: Cerebellar pathology influences cerebellar-nucleus accumbens and striatum pathways in modulating dopamine release: Relevance to Autism-related behavioral disorders

Authors: E. MCKIMM[1], D. M. COOMES[1], M. A. CALTON[1], D. GOLDOWITZ[2], G. MITTLEMAN[1], *C. D. BLAHA[1]
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Abstract: Autism is characterized by at least two neural abnormalities: cerebellar pathology that includes Purkinje cell loss or functional dysregulation as well as compensatory changes in

prefrontal cortex (PFC) dopamine (DA) transmission. The inter-relationship between these two abnormalities is unknown. However, we have previously shown that PFC DA release evoked by electrical stimulation of the deep cerebellar dentate nucleus (DN) is attenuated in Lurcher (Lc) mutant mice lacking Purkinje cells (Mittleman et al. 2008). A similar attenuation in DN stimulation-evoked PFC DA release was also observed in Fragile X (Fmr1) mutant mice showing cerebellar neuropathology involving Purkinje cells. Our previous research has also shown disruptions in both the cerebello-thalmocortical and cerebello-ventral tegmental pathways in Lc mutant mice mediating both PFC DA (Rogers et al. 2011) and glutamate (McKimm et al. 2013) release. Pastura et al. (2011) and Vaidya (2012), have also shown that decreased volume in the cerebellum influences the striatum and nucleus accumbens (NAc). Here we explore the potential influence that cerebellar neuropathological abnormalities have on DA release in both the NAc as well as the dorsal striatum, while stimulating the DN. We used fixed potential amperometry in combination with carbon fiber electrodes in urethane anesthetized (1.5g/kg i.p.) wildtype Fmr1 mice. We stimulated the DN (50 pulses; 50 Hz every 60 sec) and recorded NAc and striatal DA release. Results indicated that there were comparable levels of stimulation-evoked DA in both the NAc and striatum (60 and 40 nM), respectively. Further exploration of the current research is expected to show similar deficits in DA release in Lc and Fmr1 mutant mice compared to their corresponding wildtype strains, as seen in previous research involving strains with cerebellar neuropathological abnormalities. These studies indicate an influence of the cerebellum on NAc and striatal DA transmission. In addition to cognitive and social deficits, autism patients also show motor and reward deficits. Given the well-known involvement of the striatum and NAc in, respectively, motor function and reward, these results certainly suggest that developmental cerebellar neuropathology could also result in deficits in these abilities.
Mittleman, G et al., 2008. Synapse 62, 544-550. Rogers, TD et al., 2011. Synapse 65, 1204-1212. McKimm, E et al., 2014. Cerebellum 13, 346-353. Pastura, G et al., 2011. Arquivos de Neuro-Psiquiatria 69, 242-252. Vaidya, CJ, 2012. Current Topics in Behavioral Neurosciences 9, 49-66.

Disclosures: E. McKimm: None. **D.M. Coomes:** None. **M.A. Calton:** None. **D. Goldowitz:** None. **G. Mittleman:** None. **C.D. Blaha:** None.

Poster

557. Executive Function: Models of Disorders I

Location: Halls A-C

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Topic: F.02. Animal Cognition and Behavior

Support: MRC Grant

Title: Does lack of CaMKII autophosphorylation model a neuropsychiatric disorder?

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Abstract: Neuropsychiatric disorders, such as autism and schizophrenia, are accompanied by spine abnormalities and behavioural abnormalities including deficits in social interaction, impaired communication abilities, and ritualistic-like repetitive behaviors. The alpha-isoform of Ca2+/calmodulin-dependent protein kinase II (CaMKIIα) is a major synaptic kinase, which is expressed exclusively in glutamatergic neurons. Upon activation via the NMDA receptor CaMKIIα autophosphorylates at T286, which prolongs CaMKII activity for about one minute after the Ca2+ stimulus has declined to baseline. Additionally, the T286 autophosphorylation may alter the interaction of CaMKIIα with other proteins in the post-synaptic density. The T286 autophosphorylation is fundamentally important for the induction of NMDA receptor-dependent synaptic potentiation (with the exception of LTP in dentate gyrus) and memory formation, as demonstrated with T286A mutant mice (targeted point mutants where T286 is changed to A286, which cannot be phosphorylated). T286A mutants also have aberrant dendritic branching and sensory inputs at least in spinal cord (Pattinson at al., MCN 33, 88-95, 2006). Therefore, T286A mutant line could be an animal model of neuropsychiatric disorders. Since mouse models of neuropsychiatric disorders are characterized by disruptions in social and repetitive behaviors, we studied behaviors of T286A mutants in such tasks. T286A mutants displayed significantly less repetitive digging behavior in the marble-burying test compared with wild-type littermate controls. The impairment was age- and sex-dependent. T286A males, but not females, were impaired at 6 months of age. However, T286A females were also impaired at 12 months of age. Sociability and preference for social novelty, using the three-chamber paradigm, did not differ between T286A mutants and wild-type littermates at 6 months of age. However, in the buried food test T286A mutants differed from wild-type littermates. The latency to find the food was longer in T286A mutants. Therefore, the mutants appeared less motivated than the wild-type mice. Taken together, our data are in support of the idea the loss of T286 autophosphorylation causes some neuropsychiatric endophenotypes.

Disclosures: K. Mizuno: None. **K. Giese:** None.

Poster

557. Executive Function: Models of Disorders I

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Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant K08MH081190

NIH Grant T32MH14185

Title: Behavioral assay of the lack of the SLITRK1 gene in C57BL/6 mice

Authors: *J. M. ANDRE[1], S. NISHIMURA[2], A. LOUVI[2,3], C. PITTENGER[1,4,5,6]
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Abstract: Tourette syndrome (TS) is a neurodevelopmental disorder that affects approximately 0.5% of the population, with a peak onset in preadolescence with symptoms ranging from mild to disabling. The underlying mechanisms of the disorder are incompletely understood. A better appreciation for the neurochemical substrates will set a path for developing new pharmacological treatment. Data from a recent genetic study found a connection between mutations in the slit and trk-like family member 1 (SLITRK1) gene and TS. Although some research into the family of SLITRK genes has found an association between these genes and neuronal growth, guidance, and branching, the exact function of this gene in a healthy population and after development is still being uncovered. We have developed a floxed SliTrk1 mouse, which permits global or regional inactivation of the gene; we produced global knockouts by crossing this mouse with actin-CRE transgenic mice. We assessed the phenotype of knockout, heterozygous, and control littermates in several behavioral experiments. The mice were examined for startle and prepulse inhibition, in an open field and rotarod for locomotion, in the elevated plus maze and light/dark box for anxiety, the forced swim test for helplessness, and spray-induced grooming and amphetamine-induced stereotypy for tic-like or compulsive behaviors. There was a significant effect of genotype on prepulse inhibition, with the knockout mice showing a deficit compared to the controls and the heterozygous mice in an intermediate position. The knockout mice also spent less time in the open arm of the plus maze. Further analyses are ongoing. A behavioral classification of the phenotypes involved in knocking out the SLITRK1 gene can further our general understanding of its role in development and more specially how mutations may contribute to the symptoms of Tourette syndrome.

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Poster

557. Executive Function: Models of Disorders I

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Topic: F.02. Animal Cognition and Behavior

Support: CMSB/NCSB 050-060-409

EU EUROHEADPAIN 602633

Title: Familial hemiplegic migraine type 1 mutant mice show cognitive impairment in hippocampal memory tasks

Authors: *A. M. LENSELINK[1], T. HOUBEN[2], E. DILEKÖZ[3], C. AYATA[3], M. FERRARI[2], A. B. SMIT[1], A. M. J. M. VAN DEN MAAGDENBERG[2], S. SPIJKER[1]
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Abstract: Familial hemiplegic migraine (FHM) type 1 is a monogenic subtype of migraine with aura that is caused by mutations in the CACNA1A gene, which encodes the alpha1 subunit of voltage-gated CaV2.1 Ca2+ channels. Because transgenic knock-in mice that express gain-of-function FHM1 mutations (R192Q and S218L) in the Cacna1a gene exhibit a synaptic phenotype, including an increased cortical glutamatergic neurotransmission, we investigated whether these mutations would lead to hippocampal plasticity changes that are accompanied by cognitive and memory phenotypes. In learning and memory tests, FHM1 mice showed significantly impaired expression of the memory compared with controls, with S218L mice being more affected. This genotype difference fits with the notion that patients and mice with the S218L mutation have more pronounced clinical and neurobiological phenotypes than those with the R192Q mutation. Anxiety-related behavior appeared reduced in mutant mice , and was mildly confounded by increased locomotor activity, as observed in the open field. Furthermore, hippocampal LTP was augmented by two-fold in FHM1 knock-in mice, whereas LTD was unchanged compared with wild-type. Taken together, mutations causing a gain-of-function of CaV2.1 channels impair learning and memory, while enhancing hippocampal excitatory transmission and LTP. Our data suggest that abnormally enhanced hippocampal neuroplasticity can be as unfavorable to learning as reduced plasticity.

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Poster

558. Decision Making I

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Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

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Topic: F.02. Animal Cognition and Behavior

Support: CIHR Doctoral Research Award

Title: Nicotine's effects on cognitive effort are dependent upon individual differences: acetylcholine manipulation on a rodent cost/benefit decision-making task

Authors: *J. G. HOSKING[1], F. C. W. LAM[2], C. A. WINSTANLEY[2]
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Abstract: All individuals are confronted with choices that require weighing potential benefits against their associated costs. Animal models of such cost/benefit decision making have typically focused on dopamine's contributions to choice. Acetylcholine is heavily interconnected with the dopaminergic system, and has also been demonstrated to be essential in many cognitive processes. Despite this, its role in cost/benefit decision making remains poorly understood, especially within the realm of effort. Here we utilize a rat Cognitive Effort Task (rCET) to probe acetylcholine's contribution to effortful decision making. In the rCET, animals can choose either an easy trial, where the visuospatial attentional demand is low but the potential reward (sugar) is small, or a difficult trial on which both the attentional demand and available reward are greater. Following the establishment of baseline behavior, four drug challenges were administered via intraperitoneal injection: nicotine, mecamylamine, scopolamine, and oxotremorine (saline plus three doses for each). As per previous rCET studies, animals were divided by their baseline preferences, with "workers" choosing high-effort/high-reward options significantly more than "slackers". Nicotine dose-dependently caused slackers to choose even fewer high-effort trials than at baseline, but had no effect on workers' choice, which was near ceiling. Despite slackers' decrease in willingness to expend effort, nicotine modestly improved all animals' ability to perform the task, by decreasing nosepoke omissions. Nicotine also increased measures of motor impulsivity in all animals. In contrast, scopolamine dose-dependently decreased all animals' choice of high-effort trials, while oxotremorine decreased motor impulsivity for all animals without affecting choice. In sum, it appears both nicotinic and muscarinic cholinergic systems contribute to decision making, and in part their contributions can be understood as a function of individual differences. While nicotine has been considered as a cognitive enhancer, these data suggest that its modest benefits to attention may be coupled with impulsiveness and decreased

willingness to work hard, especially in individuals who are particularly sensitive to effort costs (i.e. slackers).

Disclosures: J.G. Hosking: None. **F.C.W. Lam:** None. **C.A. Winstanley:** None.

Poster

558. Decision Making I

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

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Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant R01MH092868

NIH Grant R21MH099534

Title: Noradrenergic regulation of optimal decision making

Authors: *E. M. VAZEY, G. ASTON-JONES
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Abstract: Decision making goes awry in many psychiatric disorders. Cortical function during decision processing is heavily influenced by several ascending monoamines, including norepinephrine (NE). Locus coeruleus (LC) provides the vast majority of NE to the cortex. Our lab and others have previously shown LC-NE neurons respond phasically during optimal decision processing in several cognitive tasks including two alternative forced choice (2AFC) tasks. NE release is posited to act as a temporal filter for integrating task relevant information and facilitating decision execution. We tested a range of pharmacological compounds to identify potential mechanisms of noradrenergic influence in optimal decision performance in a 2AFC task. We trained male Long-Evans rats to perform a 2AFC task in which one of two adjacent central cue lights (red/green) illuminated on every trial to indicate which of the two laterally-located levers would be rewarded. Rats self-initiated cue presentation by nose-poking in front of the cue lights, and performed 249 trials per session with each trial a 50% probability of either cue presentation. Correct responses were rewarded with 100µl of 15% sucrose. The α2-noradrenergic agonist guanfacine, or the noradrenergic reuptake inhibitor atomoxetine, both increased accuracy of 2AFC performance. However, this effect was restricted to animals that had <75% accuracy on vehicle, indicating a ceiling effect in the cognitive enhancement with these compounds. Guanfacine and atomoxetine also increased reaction times, possibly indicating an effect on the

response criterion (β in signal detection theory). The α2 antagonist atimpamezole produced no clear effects on either accuracy or reaction time. The α1 antagonist prazosin did not alter accuracy but caused significant increases in reaction time, indicating a possible arousal or motor effect. The β noradrenergic antagonist propranolol strongly reduced accuracy in all subjects; however, propranolol caused no change in reaction time, indicating a role for β noradrenergic signaling in cognitive processing. These results have implications for the development of cognitive enhancers and highlight intricacies of noradrenergic function during optimal decision processing that require further investigation.

Disclosures: E.M. Vazey: None. **G. Aston-Jones:** None.

Poster

558. Decision Making I

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Topic: F.02. Animal Cognition and Behavior

Support: F32 MH 088272

P20 GM 12345

R01 MH 09286

Title: Locus coeruleus norepinephrine neurons are phasically activated during behavioral response inhibition

Authors: *M. D. RIEDY, G. ASTON-JONES
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Abstract: Response inhibition is a central element in the executive control of behavior. Humans suffering from attention deficit hyperactivity disorder (ADHD) and various cognitive pathologies show impaired performance on go/nogo (GNG) and stop-signal (SS) tasks, clinical measures of behavioral response-restraint and response-cancellation. Importantly, some behavioral impairment is recovered in humans by treatment with the selective norepinephrine (NE) reuptake inhibitor atomoxetine. As the locus coeruleus (LC) is a primary endogenous source of NE, these data bolster findings indicating an important role for LC-mediated regulation of behavioral response inhibition. We analyzed the activity of LC-NE neurons in rats performing GNG and SS tasks. Rats were rewarded with aqueous sucrose (15%), punished with a lights-off delay, and

instilled with a pre-potent go response via pseudorandom control of trial ratio (75:25; Go:NoGo/Stop). In our versions of these tasks, rats were trained to maintain a lever-hold. The shaped behavior then was cue-contingent and rapid lever-release (<600 msec), which constrained the execution of alternate behaviors and permits analysis of neural activity strictly associated with inhibition of the pre-potent response. Upon reaching performance criterion (≤15% premature releases; ≥80% correct trials; ≈300 msec mean reaction time), rats were unilaterally implanted with drivable stereotrode arrays in LC, static electrodes in orbitofrontal (OFC) and anterior cingulate cortices (ACC), skull surface electroencephalogram (EEG), and dorsal cervical electromyogram (EMG) electrodes. Putative LC single units were identified in awake rats by their long-duration waveforms (>1 msec), low tonic firing rate (1-3 Hz), and neutral tone-evoked phasic bursts. Anodal current (20 μA 10 sec) was passed through a single steel wire of the array, and rats were perfused and processed for histological validation of recording sites. Results to date demonstrate that LC neurons exhibit greater cue-elicited phasic activation associated with successful vs. failed response inhibition trials. These ongoing studies promise to reveal properties of LC function important for response inhibition behavior and the associated cognitive functions.

Disclosures: **M.D. Riedy:** None. **G. Aston-Jones:** None.

Poster

558. Decision Making I

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Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant MH092257

Title: Genetics and pharmacogenetics of simple vertebrate decision-making

Authors: ***R. JAIN**[1], K. MARSDEN[1], M. WOLMAN[2], H. BELL[1], K. HAYER[1], J. HOGENESCH[1], M. GRANATO[1]
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Abstract: The nervous system constantly integrates sensory information to select appropriate, context-dependent behavioral responses. Selecting one out of several potential responses to a given situation is called decision-making. While decision-making can involve complex cognitive processing, it has become clear that even simple reflexes are dynamically biased and modulated,

representing a more tractable system to study the mechanisms of decision-making. We have developed a simple decision-making paradigm in larval zebrafish using the evolutionarily conserved acoustic startle response. Larvae perform 2 kinematically and neuronally distinct forms of the startle response: a Short-Latency C-bend (SLC) initiated 4-15 ms post-stimulus, or a less vigorous Long-Latency C-bend (LLC) initiated 20-80 ms post-stimulus. Individual larvae can respond to acoustic stimuli with either behavior, yet bias their responses toward SLCs following intense (26dB) stimuli and toward LLCs following weak (13dB) stimuli. Importantly, individuals incorporate prior experience in selecting their behavioral output, shifting their response bias from SLCs to LLCs following repeated strong stimuli. Thus, the basic dynamic aspects of complex cognitive decision-making are present in the simple SLC/LLC decision-making paradigm. To identify genes and pathways critical for the development and function of startle decision circuits, we performed a small molecule screen and a forward genetic screen. The small molecule screen results demonstrate that as in more complex cognitive assays, serotonergic modulation is critical for decision-making. Through our forward genetic screen we identified 11 mutants with specific defects in SLC/LLC bias, the first vertebrate mutants specifically isolated based solely on decision-making deficits. Using whole-genome sequencing we have identified mutations in both the calcium-sensing receptor (CaSR) gene and a regulator of CaSR trafficking. We will present functional neural imaging data to elucidate how serotonergic signaling and CaSR gene function influence simple decision-making.

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Poster

558. Decision Making I

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Topic: F.02. Animal Cognition and Behavior

Support: IBS Grant HQ1401

Title: Choice of strategies is influenced by emotionality in a repeated prisoner's dilemma in mice

Authors: *K. KIM[1], I. CHOE[2], I. KIM[1], S. PARK[1], H. SHIN[1]
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Abstract: The repeated prisoner's dilemma (RPD) is an established paradigm to explore animal behaviors upon various strategies involved in consecutive economic decision-making. We investigated whether individuality affects the choice of strategies in the RPD in inbred mice. We developed a new system for robust operant training using a brain stimulation reward via wireless intra-cranial stimulation to the medial forebrain bundle, a pleasure center in the brain. This new system overcomes the disadvantages of using natural rewards which is known to induce unstable behavioral results dependent on the level of deprivation. Prior to the RPD game, we measured general locomotor behavior of each mouse in the open field test (OFT). A 3-day operant training test and a 5-day reversal learning test in a modified T-maze were used to screen for the mice with sufficient learning ability and flexibility. Subsequently, each mouse performed an RPD game against a virtual, tit-for-tat partner for 12 days. Two groups received two different 'reward' payoffs for the mutual cooperation choice. One group (A) received a 'reward' equal to the 'predicted' value, the same amount of reward used in the preceding operant trainings, whereas the other group (B) received a lower 'reward' than the 'predicted' value. We found that the choice frequency of certain strategies became heterogeneous among the mice in the Group B while it remained homogeneous in the Group A. Further analysis of the results of the Group B revealed a strong correlation between the choice frequency of a certain strategy and the level of anxiety-related emotion of an individual mouse, as measured in the OFT, and the choice of the strategy directly affected the level of benefit to the mouse. These findings suggest that an individual's emotionality is an important factor involved in the cognitive process of economic decision-making.

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Poster

558. Decision Making I

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Topic: F.02. Animal Cognition and Behavior

Support: UCLA Division of Life Sciences Recruitment and Retention Fund to Izquierdo

Title: Altered performance on a novel effortful maze task following methamphetamine in rats

Authors: *A. STOLYAROVA, A. DE LA TORRE, A. B. THOMPSON, A. IZQUIERDO
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Abstract: One of the fundamental components of adaptive decision making is an ability to assess the value of rewards in relation to the effortful costs required to obtain them, and to use this information to guide choices. Effort-based task performance is heavily modulated by distributed dopaminergic and glutamatergic signals, primarily in striatal and prefrontal cortical regions of the brain (Floresco et al., 2008; Assadi et al., 2009). Given that frontostriatal neurotransmission is vulnerable to the effects of methamphetamine (mAMPH) exposure and that psychostimulant abuse has been linked to alterations in cost/benefit decision making (Floresco and Whelan, 2009), we hypothesized that effortful reward magnitude choices would be altered in animals treated with mAMPH. Work-aversion on an effortful t-maze task following binge doses of mAMPH has been reported previously (Kosheleff et al., 2012), yet work-aversion was not observed following an escalating mAMPH dose regimen on the same task (unpublished). In the present experiment we employed a novel maze task procedure with three possible courses of action, each associated with different effort requirements and reward magnitudes. One arm of the maze was randomly designated as a low reward (LR), another as a medium reward (MR), and the third as a high reward (HR) arm. The LR arm was unimpeded by a barrier, but in order to obtain the medium or high reward, rats were required to climb a 20 cm or 30 cm barrier, respectively. The arm assignment was counterbalanced across animals, and held constant between sessions. Once baseline arm preference was established and stable across 3 subsequent sessions, rats were treated with 4 weeks of saline (SAL) or escalating mAMPH, followed by reassessment of effortful choice behavior after an acute withdrawal period (7 d). Following treatment, mAMPH-treated animals increased their choices of the MR option over the LR option compared to SAL, and were indistinguishable from SAL in their choice of the HR option. The shift away from the LR was present from the beginning of the post-treatment testing suggesting increased sensitivity to differences in reward magnitude following mAMPH. In contrast, MR choice preference developed with repeated training, possibly as a function of reward history for that arm. MAMPH animals did not take any more days than SAL to establish stable performance and treatment groups were not different in weight or food intake. These findings suggest that mAMPH exposure has long-lasting effects on effortful decision-making and reward choice preferences. Ongoing studies are aimed at determining the dopaminergic and glutamatergic changes that underlie these altered reward choices.

Disclosures: **A. Stolyarova:** None. **A. De La Torre:** None. **A.B. Thompson:** None. **A. Izquierdo:** None.

Poster

558. Decision Making I

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 558.07/UU3

Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant 030646

Title: Effects of nicotine exposure on probability discounting in rats

Authors: *C. REICH[1], J. BURK[2]
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Abstract: Nicotine administration has been shown to affect impulsivity in multiple paradigms, including delay discounting. Probability discounting tasks are used as a measure of impulsive choice with aspects of risky behavior. We conducted two experiments to assess the effects of nicotine on probability discounting. First, we examined the effects of repeated exposure, abstinence, and re-exposure to nicotine on a probability-discounting task in rats. Rats received nicotine (0.4 mg/kg, ip) or saline twice per day for four days prior to probability discounting task performance. All rats then received challenge doses of 0.1 mg/kg or 0.4 mg/kg nicotine on Days 5, 8 and 12 and saline injections prior to task performance on all intervening days. The probability of receiving the large reward during the first block was 100% and then was decreased to 33% and then to 17% in subsequent blocks within each testing session. We observed a main effect of nicotine pre-exposure, but no interaction with administration day. Rats that were pre-exposed to nicotine continued to enter the port that allowed access to the larger, uncertain reward more frequently than the saline pre-exposed rats. In Experiment 2, we tested whether the α4β2 nicotinic acetylcholine receptors were necessary for the effects of nicotine on probability discounting. Rats were trained in the same probability discounting task as in Experiment 1. All rats then received nicotine (0.4mg/kg) and/or the α4β2 receptor antagonist, dihydro-β-erythroidine, along with appropriate vehicle control injections, prior to task performance. Similar to Experiment 1, nicotine-treated animals did not show a significant change in choice behavior when the probability of receiving the large reward was decreased. Blockade of α4β2 nicotinic receptors did not impact the effects of nicotine on probability discounting. We conclude that nicotine administration affects probability discounting performance. Nicotine may have increased perseverative behavior or decreased sensitivity to contingencies. The effects of nicotine on probability discounting do not appear to be solely mediated by α4β2 nicotinic receptors.

Disclosures: C. Reich: None. **J. Burk:** None.

Poster

558. Decision Making I

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 558.08/UU4

Topic: F.02. Animal Cognition and Behavior

Support: KAKENHI 23120007

Title: Action-dependent state prediction in the parietal cortex of mouse during a virtual navigation task

Authors: *A. FUNAMIZU, B. KUHN, K. DOYA
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Abstract: Model-based decision making requires representation of predicted states that are updated by action-dependent state transition models. To investigate their neural implementation, we conducted a virtual sound navigation task with mice and recorded the neuronal activities of posterior parietal cortex (PPC) with 2-photon microscopy. A mouse was head restrained for 2-photon imaging and maneuvered a spherical treadmill. 12 speakers around the treadmill provided a virtual sound environment. The direction and the amplitude of sound pulses emulated the location of the sound source, which was moved according to the mouse's locomotion on the treadmill. When the mouse reached the sound source and licked a spout, it got a water reward. The task consisted of two conditions: continuous condition in which the guiding sound was presented continuously and intermittent condition in which the sound was presented intermittently. In both conditions, mice increased the licking as they approached the sound source. This indicates that (i) mice recognized the sound-source position in the virtual environment and (ii) they predicted the reward given at the sound source. In the intermittent condition, the anticipatory licking was increased even when the sound was omitted, suggesting that mice updated the predicted sound-source position without auditory feedback based on their own actions. We optically recorded calcium transients of layer 2/3 neurons in the PPC with the genetically encoded indicator GCaMP6f transfected by AAV. The neural activities during the task were recorded on 23 days (sessions) from 3 mice; each session targeted a different coordinate of PPC. The PPC contained neurons with ramping activities whose slopes correlated with the distance to the sound source rather than the licking frequency or locomotion. The sound-source distance was then decoded from the population activities of PPC by the least absolute shrinkage and selection operator (LASSO). The decoder trained with the data in continuous condition could successfully decode the sound-source distance during no-sound periods in intermittent condition. We also trained a decoder of time to reach the sound source and found that the PPC neurons better represented the distance than the timing in all the sessions (two-sided t-test, $p = 2.29E-13$ - $1.09E-172$). LASSO extracted the $39.9 \pm 2.7\%$ of all neurons for distance coding; the neurons were homogeneously distributed in the PPC. These results suggest that the

PPC neurons represent and update the distance of sound source not only from present auditory inputs but also by dynamic update of the estimate using an action-dependent state transition model.

Disclosures: **A. Funamizu:** None. **B. Kuhn:** None. **K. Doya:** None.

Poster

558. Decision Making I

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Program#/Poster#: 558.09/UU5

Topic: F.02. Animal Cognition and Behavior

Support: KAKENHI 26119530

JSPS DC 282824

Title: Emergence of abstract knowledge that guides decision making in rats

Authors: ***S. TERADA**[1,2,4], H. NAKAHARA[3], S. FUJISAWA[2]
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Abstract: Decision making or selection of actions is often guided by prediction of outcome such as reward. Both reward prediction and action selection need to be acquired (learning by reinforcement) and generated (decision making process) on neuronal representation that reflects sensory inputs or information of the environment. Critically, the representation should not be merely a collection of sensory inputs but instead, should be organized to encode and abstract information required for prediction and selection. So far mostly using a simple sensory cue, the prediction and selection have been studied in the field of value-based decision making, however, surprisingly little is known about the neural representations for the abstraction of the required information during the acquisition and generation, For this purpose, we have newly developed a behavioral task for rats, called cue-combination task. Central for this task is that the correct choice of action (and possibly reward prediction) should be determined by a combination of two cues (sound and odor cues); they cannot be determined by either of cues alone. In each session, sound and odor cues will be presented simultaneously for a couple of seconds to the rat, followed by a choice period, i.e., right or left lever to pull for getting a reward. Here the correct choices

will be determined by the combination of the sound and odor cues. Once the rat pulls the correct lever, then a drop of water will be provided as a reward from the tip of the spout lever, otherwise a reward will be omitted. This is a simplest circumstance that requires abstracting appropriate information of the environment for value-based decision making. After four weeks training, the rats were able to perform up to 200 trials of this task per session with 80~90% correct answer ratios. Combining a large-scale extracellular recording techniques it will be a powerful paradigm for studying neuronal representations of abstract knowledge.

Disclosures: **S. Terada:** None. **H. Nakahara:** None. **S. Fujisawa:** None.

Poster

558. Decision Making I

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Program#/Poster#: 558.10/UU6

Topic: F.02. Animal Cognition and Behavior

Support: DA029330

Title: Addition of numerical quantities in working memory by rhesus monkeys

Authors: ***B. MASSI**, H. SOHN, N. CENERI, D. LEE
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Abstract: Animals must often estimate and arithmetically combine quantities of various objects in the environment, even when direct sensory inputs from the objects are not available. For example, an animal preparing for an upcoming winter may estimate the total quantity of food previously stored in several caches to decide whether or not to continue foraging. In fact, previous studies have demonstrated that humans and non-human primates might share a similar cognitive mechanism for manipulating estimated quantities, such as the approximate set size of a visible collection of objects. However, despite its ecological importance, the behavioral characteristics and neural mechanisms of arithmetic operations remain poorly understood. As a first step to neurophysiological studies on arithmetic, we developed an oculomotor choice task that requires macaque monkeys to add two separate quantities. During each trial, the animal fixated a central target while three clusters of dots sequentially appeared perifoveally. After a short delay, each cluster entered one of the two peripheral target areas, which was followed by another delay before the presentation of the next cluster of dots. The first cluster of dots (the augend) remained hidden until the end of the trial after it entered the target area. The second

group of dots (the addend) appeared centrally and moved to the same target area containing the augend, but remained visible. Finally, the third group of dots (the singleton) appeared and moved to the other target area and also remained visible. After another delay, the animal was required to shift its gaze to one of the target areas and received a juice reward with a magnitude proportional to the number of dots at the chosen target. Thus, the animals were encouraged to add the remembered augend with the visible addend and then compare the resulting sum to the visible singleton to maximize reward. We found that two animals reliably performed this task with over 80% accuracy. The animals performed above chance level even in trials where the magnitude of the visible quantities did not determine the correct choice. Crucially, both animals exhibited similar performance on novel trials, suggesting that the animals might use a generalizable addition rule. Consistent with existing theories of internal number representation, we found that the animals' performance depended on the ratio of the sum and singleton. Our results provide evidence that macaque monkeys can add in working memory, and future work will characterize the underlying neurophysiology.

Disclosures: **B. Massi:** None. **H. Sohn:** None. **N. Ceneri:** None. **D. Lee:** None.

Poster

558. Decision Making I

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Program#/Poster#: 558.11/UU7

Topic: F.02. Animal Cognition and Behavior

Support: Longwood PRISM Program

Longwood University Faculty Research Grant

Title: Maternal rats use frontal cortex to discriminate between own and alien pups

Authors: *A. FRANSSEN[1], A. J. HAUVER[1], J. LAFEVRE[1], M. CLASEN[3], K. GAFFNEY[3], A. PARDES[3], R. PRUETT[3], H. UYGUNER[3], M. VASSALLO[3], C. L. FRANSSEN[2,3]
[1]Biol. and Envrn. Sci., [2]Psychology, Longwood Univ., Farmville, VA; [3]Psychology, James Madison Univ., Harrisonburg, VA

Abstract: One intriguing behavior of mother rats is the care and nurturing of pups that are not her own. Previous research has demonstrated that mother rats are able to identify their own pups (OWN) versus those from another mother (ALIEN) through olfactory and auditory cues (e.g.,

Smotherman, 1974); yet somewhat surprisingly, mother rats eventually care for ALIEN pups once her natural pups are attended to (Beach and Jaynes, 1956). However, the underlying neural basis for the responses of mothers to pups is unclear and may aid in understanding triggers of the maternal response more generally. Here, we attempt to elucidate the neurological underpinnings of the decision to care for both OWN and ALIEN pups. We compared three groups of female Sprague-Dawley rats (Rattus norvegicus): Mothers with OWN pups; Mothers with OWN and ALIEN pups (creating a MIXED group); and Mothers with ALIEN pups. Pup exposures were video-recorded for analysis of behaviors including latency to retrieve and grooming. Rats were then sacrificed and neural tissue examined through immunohistochemistry for differences in c-fos in several regions of the brain. Behavioral results indicate that mothers will care for both OWN and MIXED pup groups more quickly than a completely ALIEN group. Expression patterns of c-fos suggest that while identification may occur in sensory-associated areas, such as the insular cortex (highest fos expression in response to OWN, lowest to ALIEN), the decision to care for pups may involve the prefrontal cortex (highest c-fos expression in response to MIXED). Data from additional brain regions will be discussed in the context of a maternal recognition circuit.

Disclosures: **A. Franssen:** None. **A.J. Hauver:** None. **J. LaFevre:** None. **M. Clasen:** None. **K. Gaffney:** None. **A. Pardes:** None. **R. Pruett:** None. **H. Uyguner:** None. **M. Vassallo:** None. **C.L. Franssen:** None.

Poster

558. Decision Making I

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Topic: F.02. Animal Cognition and Behavior

Support: NIEHS R01 ES015295

Title: Low level lead exposure differentially impairs performance of an attentional set shifting task depending on developmental period of exposure

Authors: *L. S. NEUWIRTH, D. W. ANDERSON, J. S. SCHNEIDER
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Abstract: Exposure to low levels of lead (Pb) impairs a variety of cognitive processes. Although children exposed to lead developmentally present with a variety of cognitive impairments that

include deficits in learning, memory, language, and executive functioning, experimental work on Pb toxicity in rats has focused mostly on learning and memory deficits and less on executive functions. However, detrimental effects on executive functioning could lead to or even underlie a variety of other cognitive problems attributed to Pb exposure. In this study, we examined the ability of Long Evans rats (control and Pb-exposed: 150ppm Pb-acetate in food given perinatally (gestation through weaning) or early postnatally (EPN, birth through weaning)) to acquire and perform an attention set shifting test (ASST) that requires animals to locate a food reward based on discriminating between digging materials and odors. The task consisted of simple (SD) and compound (CD) discriminations and reversals and intra-dimensional (ID) and extra-dimensional (ED) shifts followed by reversals. Pb-exposed animals performed the task different than controls. Rats with EPN Pb exposure were unable to learn an odor-based SD. Perinatally exposed rats learned the odor SD but had significant numbers of errors at most task levels with particular difficulty in performing the ID shift. Perinatally exposed rats also exhibited very short response latencies suggesting impulsive responding. Additionally, the more complex the stimuli presented during testing the greater the number of trials needed to reach criterion and the greater the numbers of errors made, suggesting that Pb exposure disrupted cognitive information processing when animals attempted to associate reinforcement with odor and/or digging material. These data suggest that low level Pb exposure results in significant executive dysfunction and in particular, may impair the ability to form, maintain, and shift response sets and may result in impulsivity and problems with cognitive flexibility.

Disclosures: L.S. Neuwirth: None. **D.W. Anderson:** None. **J.S. Schneider:** None.

Poster

558. Decision Making I

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Program#/Poster#: 558.13/UU9

Topic: F.02. Animal Cognition and Behavior

Title: Ipsilateral ventral striatal lesions disrupt reward prediction error signals in rat dopamine neurons

Authors: *Y. K. TAKAHASHI[1], G. SCHOENBAUM[2]
[1]NIDA/NIH, BALTIMORE, MD; [2]NIDA/NIH, Baltimore, MD

Abstract: Midbrain dopamine neurons have been shown repeatedly to signal errors in reward prediction. The ventral striatum has been proposed as a major source of the predictions upon

which these errors are based, even serving the role of "Critic" in some accounts. Here we tested this hypothesis by recording from putative dopamine neurons in ventral tegmental area in rats with sham or neurotoxic lesions of ipsilateral ventral striatum. Recordings were made in a simple odor-guided choice task, which we have used previously to demonstrate prediction error signals in rat dopamine neurons. In this task, different odor cues signal that a sucrose reward is available in one of two nearby fluid wells. During recording, we independently manipulate the timing or size of reward across blocks of trials to induce both positive and negative reward prediction errors. Consistent with our prior work in this task, dopamine neurons in sham rats exhibited robust error signals; activity in both single units and across the population showed a phasic increase to unexpected reward and this firing declined with learning. After learning, the same neurons also showed phasic activity to the predictive cues that differed according to their value and suppressed firing upon omission of the expected reward. By contrast, although the ipsilateral ventral striatal lesions had no apparent effect on learning or performance in the task, dopamine neurons recorded in the lesioned hemisphere failed to show this pattern. In preliminary analyses, these neurons appear to still fired strongly to unexpected rewards, however this activity does not change with learning, nor is there any evidence of suppression on omission or transfer to the reward-predicting cues after learning. These results are consistent with the proposal that that input from ventral striatum is an important source of the predictions that midbrain dopamine neurons use to calculate reward prediction errors.

Disclosures: Y.K. Takahashi: None. **G. Schoenbaum:** None.

Poster

558. Decision Making I

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Topic: F.02. Animal Cognition and Behavior

Support: Brain and Behavior Research Foundation

Title: Neuronal activity-dependent BDNF alterations in corticostriatal circuits during flexible decision-making

Authors: *R. COLE, P. J. PATEL, P. N. OSUAGWU, V. PARIKH
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Abstract: Brain-derived neurotrophic factor (BDNF) exerts neuromodulatory effects on synaptic transmission, and is essential for actively regulating learning, memory, and motivational processes. Recent evidence suggests that exogenous BDNF administration into the dorsal striatum regulate cognitive flexibility by altering cortiostriatal glutamatergic transmission (D'Amore et al., 2013). However, the role of endogenous BDNF signaling in flexible decision-making remains unknown. Activity-dependent alterations in BDNF expression is considered to be a key event in synaptic plasticity and cognition. As frontostriatal circuits involving discrete regions of the prefrontal cortex (PFC) and orbitofrontal cortex (OFC), and striatum, are critical in maintaining different forms of cognitive flexibility such as extradimensional shifting and reversal learning, we hypothesized that shifting to new strategies would produce neuronal activity-dependent alterations in BDNF expression in these circuits. Male C57BL/6J mice were trained in an operant task that required the animals to shift a visual cue-based strategy to an egocentric spatial response-based strategy. Another cohort of mice were trained on reversal learning where they initially acquired a response-based discrimination and then required to flexibly adapt to the reversal of stimulus-response contingencies. Brains were removed either during the initial learning phase or after complete acquisition of the new strategy to conduct quantitative immunohistochemical examination of BDNF and c-fos (a marker of neuronal activation) expression in the regions of interest. During the early learning phase, the counts of BDNF-positive cells and BDNF-c-fos co-labelled cells were elevated in the OFC and prelimbic/infralimbic areas of the PFC irrespective of the shift-type compared to discrimination control groups. However, the dissociation between region-specific changes in BDNF expression became apparent only after acquisition of specific task type. Acquisition of reversal learning was only associated with increased BDNF expression in the OFC while higher c-fos-BDNF colabelled neurons were restricted to PFC after attainment of strategy shifting. Increased neuronal activation in the dorsal striatum occurred regardless of the task type. Collectively, these data suggests that flexible decision-making produces temporal alterations in corticostriatal BDNF expression that depend upon region-specific neuronal activity. Moreover, cortical BDNF plays a critical role in modulating striatal activity to maintain shifts in strategies with changing environmental contingencies.

Disclosures: **R. Cole:** None. **P.J. Patel:** None. **P.N. Osuagwu:** None. **V. Parikh:** None.

Poster

558. Decision Making I

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Topic: F.02. Animal Cognition and Behavior

Support: Ville de Paris. HABOT Project.

Title: The role of temporal variability during time-constrained motor sequences: A computational study

Authors: *I. COS-AGUILERA[1], P. RUEDA-OROZCO[2], D. ROBBE[2], B. GIRARD[1]
[1]ISIR, Univ. Pierre et Marie Curie, Paris, France; [2]Inst. de Neurobiologie de la Mediterranee (INMED), Marseille, France

Abstract: The study of decision-making of motor actions with rodents is mainly based on tasks in which animals perform specific action sequences to attain reward [1]. Here we were interested in understanding the behavioural organization of rats during time-constrained, reward driven, locomotion tasks. Experimental evidence suggests that whenever the motor responses are time-constrained to obtain reward, the resulting strategy reflects a compromise between the motor factors relevant to the task and the timely requirements to attain the goal. To investigate this, we took advantage of a novel behavioural protocol in which rats running on a treadmill were required to estimate a fixed-temporal interval to obtain reward [2]. Interestingly, rats became proficient by developing stereotyped running sequences through a trial and error learning process that extended 2-3 months, attaining success rates of ~70-80% per session. We hypothesized that the behaviour was habitual [4] and consistent with a principle of reward maximization that traded-off effort and temporal discount. Moreover, because of the motor and temporal constraints of the task, this had to be equally constrained by the ability of the CNS to estimate time intervals [6]. Two complementary methods were used to estimate this hypothesis: our first analyses showed of the habitual motor strategy was consistent with an optimization process of each behavioural phases as a function of effort and discount, but also of the temporal variability associated to each phase of their behaviour. For example, the rat's behaviour was less variable at constant acceleration than at constant speed, and the duration of their phases of movement was consistent with an overall strategy to minimize temporal variability over the entire movement. Second, we built a continuous time and space RL model that could accommodate the statistics of Weber's law for time estimation [5], as well as learn locomotion sequences by optimizing reward. This model yielded behaviours that reproduced the statistics of the rat's by optimizing the trade-off between discounted reward and effort. Remarkably, the optimal behaviour also yielded the motor sequences with least overall temporal variability, therefore suggesting that the estimates of time intervals and the trade-off between reward and effort are intrinsically related.
1.Barnes TD, Kuboda Y, Hu D, Yin DZ, Graybiel AM. Nature 2005 437:1158-1161. 2.Rueda-Orozco P, Robbe D. 2014 Submitted. 3.Desmurget M, Turner RS. J Neurophysiol 2008, 99:1057-1076. 4.Shmuelof L, Krakauer JW, Mazzoni P. J Neurophysiol 2012, 108(2), 578-594. 5.Hazeltine E and Ivry R 1995 Trends in Cognitive Sciences, 1(5):163-169.

Disclosures: **I. Cos-Aguilera:** None. **P. Rueda-Orozco:** None. **D. Robbe:** None. **B. Girard:** None.

Poster

558. Decision Making I

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Topic: F.02. Animal Cognition and Behavior

Support: NSERC Create Grant 372305

Alberta Innovates Health Solutions

Alberta Gambling Research Institute

Title: Investigating the neural mechanisms of disordered gambling using the N-Arm Bandit Task

Authors: ***C. S. LASKOWSKI**[1], R. WILLIAMS[2], A. GRUBER[3], K. MARTENS[4], D. EUSTON[3]
[2]Fac. of Hlth. Sci., [3]Dept. of Neurosci., [1]The Univ. of Lethbridge, Lethbridge, AB, Canada; [4]Dept. of Psychology, The Univ. of British Columbia, Vancouver, BC, Canada

Abstract: INTRODUCTION: Damage to the medial prefrontal cortex (mPFC) often leads to problems characteristic of addiction, such as impulsivity and insensitivity to future consequences. To learn more about the role of this region, we studied the effects of mPFC lesions in rats on decision making processes related to behavioral addiction. We hypothesized that rodents with mPFC lesions would be less flexible when faced with changing task contingencies resulting in a diminished ability to obtain as much reward over time compared to control animals. METHODS: 23 male Long Evans rats (10 lesion; 13 sham) were tested on a novel version of the N-arm Bandit Task. Each rat was given a choice between 3 food ports which delivered a different amount of liquid Ensure (i.e. high, medium or low reward). Each port maintained this amount of food for a given amount of trials at which time the amounts associated with each port changed abruptly. The rats then had to disengage from the port that was previously highly rewarding and searched for the new high reward port. RESULTS: We found that damage to the mPFC made rats much more likely to perseverate on ports that were previously rewarding and were much less sensitive to outcomes. Lesioned animals achieved less net reward and were slower to adjust to changes in reward contingency. CONCLUSIONS:

Rodents with mPFC damage display less flexible goal-directed behaviour. These data support a role for the mPFC in behavioural addictions. In humans, this effect may contribute to some of the difficulties individuals experience when trying to disengage from activities related to substance or behavioural addictions. This region may be a useful target for research relating to the treatment of addictions.

Disclosures: C.S. Laskowski: None. **R. Williams:** None. **A. Gruber:** None. **K. Martens:** None. **D. Euston:** None.

Poster

558. Decision Making I

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Program#/Poster#: 558.17/UU13

Topic: F.02. Animal Cognition and Behavior

Support: NIMH MH051383

Title: The nematode *C. elegans*: A new model organism for economic decision making

Authors: *A. W. KATZEN[1], W. T. HARBAUGH[2], S. R. LOCKERY[3]
[2]Econ., [3]Biol., [1]Univ. of Oregon, Eugene, OR

Abstract: Investigation of the neuronal basis of economic decisions would be accelerated by establishing decision making paradigms in simple, genetically tractable organisms, such as the nematode *Caenorhabditis elegans.* For an organism to be a valid model of economic decision making, it must satisfy two conditions: (i) the organism must be capable of distinguishing between high and low quality goods, and (ii) its decision must be sensitive to the prices of those goods. Previous work has shown that the nematode worm *C. elegans* quickly learns to prefer those foods (species of bacteria) that promote higher rates of growth and reproduction. Preference is manifested by the amount of time the worm spends foraging in patches of good bacteria (high worm growth rate) versus mediocre bacteria (moderate growth rate) when equally abundant. Until now, however, it has not been possible to precisely manipulate both the quality and cost of food during choice presentation We have developed an electro-microfluidic device in which a semi-restrained worm forages between contiguous yet discrete fluid streams containing good and mediocre quality food. Electrodes inserted into the device monitor muscular impulses associated with individual swallowing events. Relative consumption of good and mediocre food is measured by counting the number of swallowing events in the respective fluid streams. The

fraction of total swallowing events in good vs mediocre food serves as an index of food preference. Importantly, we can alter the effective prices of the two foods by adjusting the concentration of the bacteria, with price being inversely related to concentration. We find that worms exposed to the two species of bacteria at equal prices prefer good bacteria, indicating that feeding preferences are normal in the device. However, when mediocre food is made much less expensive than good food, worms prefer mediocre food. We conclude that *C. elegans* is sensitive to both quality and cost when deciding where to forage in the microfluidic device. Thus, the new device enables investigation of the neuronal and genetic basis of economic decisions in *C. elegans*. Because of the extensive genetic and neurochemical similarities between the nervous system of *C. elegans* and its mammalian counterparts, the present approach is well positioned to provide novel insights into how economic decisions are generated in a range of medically relevant nervous systems.

Disclosures: A.W. Katzen: None. **W.T. Harbaugh:** None. **S.R. Lockery:** None.

Poster

558. Decision Making I

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Title: Variability of perceptual decision-related activity differs between the frontal eye field and caudate nucleus

Authors: J. A. CABALLERO, J. I. GOLD, *L. DING
Dept of Neurosci., Univ. of Pennsylvania, Philadelphia, PA

Abstract: Variability in neuronal activity is widely observed in the brain. Although it is often treated as a nuisance, neuronal variability can be modulated by task context (e.g., in songbirds) and provide clues about a given neuron's computations (e.g., [1, 2]). Previously we reported that neurons in the frontal eye field (FEF) and caudate nucleus (CD) show different patterns of mean

firing rate in a random-dot visual motion discrimination saccade task, in which macaques report their choices with saccades to fixed target locations [3, 4]. In this study, we compared several measurements of firing-rate variability in the same samples to provide a more complete characterization of the decision-related activity patterns in the two brain regions. We focused on the task period from stimulus onset to saccade onset and included only neurons with choice- and motion strength-dependent activity (n = 34 and 48 for FEF and CD, respectively). We estimated several variability-related quantities using a sliding window (100 ms) and tested for choice and/or coherence (motion strength) dependence using a combination of linear regression analyses and t-tests (criterion: p = 0.05). Our three main findings are as follows. First, firing-rate variance followed choice-, motion strength- and time-dependent trajectories akin to those of mean firing rates in both CD and FEF. Second, the average Fano factor was largely unmodulated in CD. In contrast, in FEF it decreased gradually for both choices after stimulus onset and more rapidly and in a choice-dependent manner prior to saccade onset. This pre-saccadic decrease was earlier and deeper for saccades toward the neurons' response field (IN) than for opposite saccades (OUT). Third, the average coefficient of variation squared (CV2) was larger for OUT than IN saccade trials in both CD and FEF. During early motion viewing, coherence modulation of CV2 was observed for both IN and OUT trials in CD neurons, but only for IN trials in FEF neurons. Closer to saccade onset, coherence modulation of CV2 was observed only for OUT trials in FEF, but not for either choice in CD. These differences in neuronal variability may support different computational roles of FEF and CD in perceptual decision making. References [1] Churchland et al, Nat Neurosci, 13(3):369-378, 2010 [2] Churchland et al, Neuron, 69(4):818-831, 2011 [3] Ding and Gold, J Neurosci, 30(47):15747-15759, 2010 [4] Ding and Gold, Cerebral Cortex, 22(5):1052-1067, 2012

Disclosures: **J.A. Caballero:** None. **J.I. Gold:** None. **L. Ding:** None.

Poster

558. Decision Making I

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 558.19/UU15

Topic: F.02. Animal Cognition and Behavior

Support: DA011717

DA027844

Title: Dorsomedial striatum lesions disrupt the balance between model-free and model-based learning in a multi-stage decision-making task in rats

Authors: *S. M. GROMAN[1], L. CHEN[1], N. J. SMITH[1], D. LEE[2], J. R. TAYLOR[1]
[1]Psychiatry, [2]Neurobio., Yale Univ., New Haven, CT

Abstract: Reinforcement learning processes are impaired in individuals with psychiatric disorders, such as addiction and schizophrenia. Although a large body of work indicates that model-free (MF) learning is altered in psychiatric disorders, emerging evidence suggests that model-based (MB) learning strategies may also be impaired. However, the neural mechanisms mediating MF and MB learning processes are not well understood. Here, we developed a rodent version of a multi-stage decision-making (MSDM) task to determine the role of the dorsomedial (DMS) striatum in MF and MB learning strategies. Rats received either bilateral lesions of the DMS striatum or sham surgery and were trained on a MSDM task analogous to that used in humans (Daw et al., 2011). In this task, rats made decisions between two options in two successive trial stages. In the first stage, rats are presented with two levers. Rats respond on a single lever to probabilistically illuminate two noseports (NPs): responses on one lever result in the presentation of noseports A and B 70% (common) of the time and noseports C and D 30% (rare) of the time; responses on the other lever resulted in the opposite configuration. In the second stage, rats chose between the two illuminated noseports that were differentially reinforced. MF and MB learning models posit that reinforcement in the second stage should differentially impact first-stage choices. To examine the contributions of MF and MB learning strategies, we calculated the probability that rats would stay on the first-stage option based on the trial outcome (win/lose) and trial type (common/rare). Similar to humans, rats use both MF and MB learning strategies (main effect of trial outcome: $p=0.001$; trial outcome by trial type interaction: $p<0.001$). Furthermore, there was a significant interaction with lesion type ($p=0.04$): although sham rats displayed evidence of both MF and MB learning (main effect of trial outcome: $p=0.04$; trial outcome by trial type interaction: $p<0.001$), lesion rats only displayed evidence of MF learning (main effect of trial outcome: $p=0.006$; trial outcome by trial type interaction: $p=0.27$). These data indicate that rats, similar to humans, use both MF and MB learning strategies to guide their decision-making processes and that the DMS striatum plays a critical role in MB learning. By using a MSDM task with high translation value, our ongoing studies will disentangle the neural mechanisms mediating MF and MB learning strategies to provide insight into the neuroanatomical basis of decision-making impairments in psychiatric disorders.

Disclosures: S.M. Groman: None. **L. Chen:** None. **N.J. Smith:** None. **D. Lee:** None. **J.R. Taylor:** None.

Poster

558. Decision Making I

Location: Halls A-C

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Topic: F.02. Animal Cognition and Behavior

Support: CIHR MOP-133579

Title: Neural correlates of risk/reward decision making in nucleus accumbens neurons

Authors: *J. D. LARKIN, S. B. FLORESCO
Psychology, Univ. of British Columbia, Vancouver, BC, Canada

Abstract: Many of the choices we make in everyday life have ambiguous or uncertain outcomes. Choosing the most appropriate course of action to optimizing potential outcomes is mediated by distributed neural circuits incorporating the prefrontal cortex, amygdala and in particular, the nucleus accumbens (NAc). The NAc is thought to play a critical role in determining how an animal prioritizes responses, particularly *in situ*ations where reward contingencies may vary. Inactivation of this nucleus reduces preference for larger, uncertain rewards, most prominently when riskier options have greater long-term utility. To further clarify how NAc neurons encode different types of choices during risk/reward decision making, the present study used moveable tetrode arrays to obtain extracellular electrophysiological recordings from NAc neurons of rats performing a probabilistic discounting task conducted in an operant chamber. Rats were well trained on a task where they choose between a 'risky' lever that delivered a larger, 3-pellet reward at varying probabilities, and a 'certain' lever that always delivered a smaller, 1 pellet reward. For the first 40 trials of a session (20 forced/20 free-choice) the risky option delivered the large reward 70% of trials and during a subsequent 40 trial block, the odds of obtaining the larger reward decreased to 10%. Our analyses focused primarily on differential patterns of activity occurring prior to choices of the risky versus certain option. Preliminary analyses revealed that pre-choice activity of some NAc neurons appear to encode choice relative to utility of the two options. We have found that NAc neurons fire differentially to 'risky' and 'safe' options depending on the probability of large reward. Specifically, some cells displayed differential changes in firing prior to risky versus certain choice when the odds of obtaining the larger reward were high, but this differential encoding was not apparent when reward probabilities were low. In some cells, this effect was dependent on whether animals were forced to choose an option or free to choose between them. Other cells differentially encoded the outcome of choices (eg; firing more or less after a non-rewarded vs rewarded choice). Collectively, these data suggest that NAc neurons integrate information about actions, outcomes

and reward expectation during risk/reward decision making, which in turn may enable a decision maker to update choice behavior *in situ*ations where reward probabilities are volatile.

Disclosures: J.D. Larkin: None. **S.B. Floresco:** None.

Poster

558. Decision Making I

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 558.21/UU17

Topic: F.03. Motivation and Emotion

Title: Distinct mechanisms of reward that underlie decision-making in mice

Authors: *C. F. ROBLES, J. ROTONDO, A. GRANT, A. W. JOHNSON
Neurosci., Michigan State Univ., East Lansing, MI

Abstract: Intact decision-making results in the ability to choose appropriately between distinct courses of action. To characterize mechanisms of decision-making in rodents, we developed a task in which CD1 mice were trained to respond separately on two distinct levers ('high' and 'low') for the delivery of different reinforcers and auditory stimuli. Responses on the 'high' lever led to the delivery of 20% sucrose and contemporaneous presentation of an auditory cue (e.g., noise), whereas 'low' lever responses led to 2% sucrose and presentation of a second discriminable cue (e.g., tone). Subsequently mice received a choice test with access to both levers. The results revealed significant variability across mice in preferences for the 'high' lever. To examine whether lever preferences may be accounted for by differences in value attributed to the sucrose reinforcers or the auditory cues, we conducted tests of licking microstructure and conditioned reinforcement, respectively. Analyses of licking microstructure revealed that high (but not low) palatability responses for 20% sucrose was predictive of responding on the 'high' lever. By comparison, high levels of conditioned incentive value to the cue associated with 2% sucrose was predictive of responding on the 'low' lever. Finally, to evaluate this behavioral difference neurobiologically, prior to a second choice test, mice were treated with the D2 receptor antagonist, Haloperidol. This influenced responding to a greater extent in mice where conditioned reinforcement performance predicted responding on the 'low' lever. These results suggest dissociable mechanisms of reward that may influence (and predict) decision-making performance.

Disclosures: C.F. Robles: None. **J. Rotondo:** None. **A. Grant:** None. **A.W. Johnson:** None.

Poster

558. Decision Making I

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 558.22/UU18

Topic: F.02. Animal Cognition and Behavior

Support: R01 MH084911

T32 EY07143

Title: Reward timing in primary sensory cortex during multi-modal intertemporal decision-making

Authors: *J. M. LEVY[1], M. G. SHULER[2]
[1]Johns Hopkins, Baltimore, MD; [2]Johns Hopkins Univ., Baltimore, MD

Abstract: Primary visual cortex (V1) has traditionally been thought to exclusively represent low-level, physical attributes of visual stimuli. This canonical view has been challenged by the discovery that V1 can represent an interval between a stimulus and a predicted reward, a phenomenon known as reward timing. The discovery of reward timing in V1 suggests that, beyond merely sensing the visual world, primary visual cortex may relay expectation signals--vital components of reinforcement learning models--and, thereby, contribute to prospective decision-making. To test this hypothesis, we have trained wild type and ChAT-ChR2 mice on an intertemporal decision-making task in which they are required to lick at one of two reward ports, each yielding water after the same delay, depending on whether a visual or auditory cue is presented. After performing this task with high accuracy, mice are exposed to choice trials in which both cues are presented simultaneously and the animal is allowed to choose whichever port he prefers. Recordings from V1 during this task reveal that temporal expectation signals are only present following the visual, but not auditory, cue. Furthermore, this expectation signal does not modulate with the animal's choice on free choice trials. When an earlier water reward is introduced following the visual stimulus, this reward time also comes to be represented in V1, and the animal's choice behavior comes to be biased toward this port. We attempt to manipulate choice behavior of the transgenic mice by introducing an earlier, fictive reward representation in V1 following the visual stimulus through optogenetic hijacking of basal forebrain inputs. This manipulation has a similar effect in V1 as introducing an earlier water reward and preliminary evidence suggests that it may bias an animal's choice behavior in this intertemporal decision-making task.

Disclosures: J.M. Levy: None. **M.G. Shuler:** None.

Poster

558. Decision Making I

Location: Halls A-C

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Program#/Poster#: 558.23/UU19

Topic: F.02. Animal Cognition and Behavior

Support: NIH F32 EY023523

NIH R01 EY007023

NIH R01 MH085802

Title: Optical dissection of cortical circuits underlying short-term memory

Authors: *M. GOARD, G. PHO, M. SUR
MIT, Cambridge, MA

Abstract: Short-term memory, the ability to hold information in mind over short timescales, is a fundamental cognitive process underlying an array of complex abilities. Short-term memory is associated with sustained neural activity in cortical (and subcortical) structures, but it is not established how mnemonic information is maintained within distributed cortical regions. Here we developed a memory-guided visual discrimination task (with stimulus, delay, and choice epochs) for head-fixed mice, enabling large-scale 2-photon calcium imaging and targeted optogenetic perturbation of sensory, association, and motor cortices during STM. High-speed volume scanning was used to simultaneously image the neural activity of large populations (hundreds to thousands of cells) of GCaMP6s-expressing cortical neurons in visual, parietal, and frontal motor cortices. Visual cortex (V1) neurons primarily responded during the stimulus epoch whereas posterior parietal (PPC) and frontal motor cortical (fMC) neurons exhibited complex responses across all task epochs, including steady-state delay-period activity. To test the functional role of each region during task performance, we used VGAT-ChR2-EYFP mice (expressing channelrhodopsin in inhibitory interneuron types) to inactivate defined regions of cortex in a spatially and temporally restricted manner. Optogenetic inhibition of bilateral V1 or PPC disrupted behavioral performance only during the stimulus epoch, revealing that PPC was not necessary for STM maintenance despite exhibiting prominent delay-period activity. Surprisingly, photoinhibition of fMC disrupted performance not only during choice, but also during stimulus and delay epochs. These results indicate that in a memory-guided behavior, task-relevant information can be rapidly transmitted from sensory to association to frontal motor cortices, where steady-state activity is essential for STM maintenance.

Disclosures: **M. Goard:** None. **G. Pho:** None. **M. Sur:** None.

Poster

558. Decision Making I

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 558.24/UU20

Topic: F.02. Animal Cognition and Behavior

Support: NIH

UCLA Academic Senate

Title: Dysfunctional learning: what happens in the dentate nucleus when one gets the answer to an acoustically cued question wrong?

Authors: *C. D. WOODY
UCLA Med. Cntr. NPI 58-258, Los Angeles, CA

Abstract: Can patterns of neuronal response in subcerebellar nuclei distinguish correct from incorrect learned motor responses to acoustic stimuli? To examine this spike activity was recorded from 204 single units of the dentate nuclei of awake cats trained to perform discriminatively conditioned blink responses (CRs) to a 70 dB click CS versus a 70 dB hiss DS. Concurrent bipolar recordings of EMG activity from the orbicularis oculi muscles during each trial of CS presentation allowed objective assessment of the presence or absence of CRs while remaining blind to the associated spike activity. The trials were separated into those with absent CRs (failed to exceed 3 sd of baseline activity and no apparent CR-like increase >2 sd), weak CRs (exceeded 2 but not 3 sd of baseline), and strong CRs (exceeded 3 sd of baseline activity). Averages were then made of PSTHs of spike activity associated with each classification and the concurrent EMG activity. The overall averages of EMG activity showed a peak response 12 Z above baseline in the trials with strong CRs, a response 8 Z above baseline in the trials with weak CRs, and a response 3 Z below baseline in the trials with putatively absent CRs. There was a separate group of cells in which each trial showed a strong CR (EMG mean peak response 157 Z above baseline). The PSTHs of corresponding CS-evoked spike activity showed peak discharges 25 Z above baseline in the cells with each trial showing a strong CR, 21 Z in the group with strong CRs, discharges 13 Z in the group with weak CRs, and discharges 12 Z in the group with putatively absent CRs. Baseline spike activity was highest in the group with putatively absent CRs. The results demonstrate that the signal:noise ratio of spike activity

elicited by the CS in dentate nucleus is reduced when performance of the CR is deficient and that the coding matters. Early latency (4-12 ms) spike responses were less well correlated with changes in EMG activity than slightly later (12-24 ms) responses, illustrating the dual functions of the dentate (see refs.). Apparently the dentate nucleus is involved directly or indirectly with production of dysfunctional learned motor responses to an acoustic CS, and enhancement of spike activity plus reduction of baseline noise may help reduce such errors. Data from: Woody, C.D. Database of recordings of spike activity. http://repositories.cdlib.org/mrrc/1 2005. Also see: Wang, X.F. et al. NeuroReport, 2:361-364, 1991; Woody, C.D. et al. NeuroReport 5:513-515, 1994; Xi, M.-C. et al. NeuroReport 5:1567-1570, 1994; Woody, C.D. et al. Brain Res. 789: 74-83, 1998; and Woody, C.D. In: Acoustical Signal Processing in the Central Auditory System, J. Syka (Ed.), New York: Plenum, pp. 501-511, 1997.

Disclosures: C.D. Woody: C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); NIH, UCLA Academic Senate.

Poster

558. Decision Making I

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 558.25/UU21

Topic: F.02. Animal Cognition and Behavior

Support: Research, Innovation and Graduate Education at the University of Oregon

Title: Adaptive interpretation of sounds without the auditory cortex

Authors: T. L. GIMENEZ[1], M. LORENC[2], *S. JARAMILLO[1]
[1]Univ. of Oregon, Eugene, OR; [2]Cold Spring Harbor Lab., Cold Spring Harbor, NY

Abstract: A defining feature of adaptive behavior is our ability to change the way we interpret sensory stimuli depending on context. We designed a two-alternative choice sound-categorization task for rats to study the neuronal mechanisms underlying this flexibility in behavior. Animals were required to associate low frequency sounds with a left reward port, and high-frequency sounds with a reward port on the right. Each behavioral session was divided into blocks of 200 trials, and the boundary that separated the two acoustic categories changed from one block to the next. These shifts in the boundary resulted in changes in the rewarded action for a subset of stimuli. Rapid adaptation in behavior has usually been attributed to cortical circuits. In this study we tested if the auditory cortex was necessary for succesful performance of the

flexible categorization task. We found that extensive lesions of the auditory cortex did not impair the ability of rats to switch between categorization contingencies, and sound discrimination was minimally impaired as measured by psychometric curves under each contingency. Similar results were obtained after reversible inactivation of the auditory cortex with muscimol. In contrast, lesions of the auditory thalamus largely impaired discrimination performance, and as a result, the ability to modify behavior across contingencies. Thalamic lesions did not impair performance of a visual discrimination task, indicating that the effects were specific to audition and not to motor preparation or execution. These results suggest that subcortical outputs of the auditory thalamus can mediate adaptive interpretation of sounds.

Disclosures: T.L. Gimenez: None. **S. Jaramillo:** None. **M. Lorenc:** None.

Poster

558. Decision Making I

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 558.26/UU22

Topic: F.02. Animal Cognition and Behavior

Support: NIMH Intramural Research Program

Title: Early termination of drift diffusion trajectories in decision making in monkeys

Authors: *S. CHANDRA, M. A. G. ELDRIDGE, B. RICHMOND
NIMH, Lab. of Neuropsychology, NIH, Bethesda, MD

Abstract: Monkeys can learn to classify whether images morphed from cat to dog, i.e., images of cats and dogs that are blended in different proportions, are more cat-like or dog-like. In the experiments that we are modeling here, monkeys were required to identify whether the morph being presented is closer to a cat or a dog in a sequential two-alternative forced choice task. The monkeys were trained to touch a bar to initiate a trial, and release the bar during one of two intervals; early (during the presence of a red central target) if they identified the stimulus as more cat-like, or late (following the transition of the central target to green) if the stimulus was more dog-like. Rewards were only delivered for correctly identifying a dog. In summary, if cat, release on red to skip the trial and move on to the next, if dog, release on green to get a reward. We measured the percentage of trials and reaction times when the monkey correctly indicated cat. As the morph level changes from pure cat towards dog, the number of times the monkey indicated a cat decreased sigmoidally. The sigmoid was also shifted toward dog, indicating a bias toward

choosing dog - when very unsure, guessing dog prevents missing a potential reward, with a penalty of having to wait through the whole trial for nothing if the stimulus should have been identified as cat. As the morph progresses from all cat to equal cat/dog, the monkeys take longer to make a decision, i.e., the reaction times become longer. We model this behavior as a drift diffusion process. A point starts midway between 2 boundaries, and takes a random walk until it hits a boundary, which represents a decision of cat or dog. The drift velocity depends on the morph level presented. The model has an analytical solution. Here we use the experimentally measured frequency with which the monkey identifies a given morph as dog to determine the drift velocity, which is then used to predict the mean reaction time. We find that for morph levels close to a pure cat the experimental mean reaction times are similar to the theoretical ones. When the morph level is close to a 50:50 mixture, the experimental mean reaction times are faster than the theoretical predictions. It appears that for the easy morph levels the monkey reaches the boundary quickly and decides. For more ambiguous morphs the monkey takes more time to accumulate evidence. If reaching a bound takes too long, the monkey stops accumulating evidence and indicates dog - not wanting to miss a potential reward. The question now is whether there is another process apart from drift diffusion, also stochastic, governing how long the monkey will wait to accumulate evidence before simply terminating the trial by guessing dog.

Disclosures: **S. Chandra:** None. **M.A.G. Eldridge:** None. **B. Richmond:** None.

Poster

558. Decision Making I

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 558.27/UU23

Topic: F.03. Motivation and Emotion

Support: NIDA IRP

Title: VTA neurons exhibit associative activity to cues based on inferred value

Authors: *B. F. SADACCA, G. SCHOENBAUM
Natl. Inst. On Drug Abuse, Baltimore, MD

Abstract: In recent years, the application of reinforcement learning models to neuroscientific data has led to an understanding that multiple decision making systems do, in fact, coexist in the brain, with two of these described in terms of ‘model-free’ and ‘modal based’ decision making.

Dopamine (DA) released from the ventral tegmental area (VTA) has often been related to model-free learning; actions that occur just prior to increases in DA release are more likely to occur again, and cues that occur just prior to increases in DA release are more likely to be sought. However, it is unclear if dopamine neurons of the VTA have access to information about cues whose relationship to reward is inferred. To test if VTA neurons can predict this value, rats were run in a sensory pre-conditioning task, while the activity of VTA neurons was recorded extracellularly. In this task, rats first learn a timing relationship within two pairs of cues in the absence of reward (A before B, and C before D). Rats then learn that one of the cues (B+) predicts the availability of reward while another (D-) predicts the absence of reward. In a final phase, rat behavior is monitored while cues A and C occur; here rats infer the learned relationship between cues A and B, and try to access reward in the presence of cue A alone. In the test, neurons showed early phasic responses to cues with inferred value mimicking the responses to cues explicitly paired with reward, clearly demonstrating that the VTA can access values beyond the model-free systems of the brain. Further, beyond this reward-related activity, during initial pre-conditioning VTA neurons show a diverse array of evoked responses to cues not yet paired with reward. Taken together, these finding demonstrate a role for the VTA beyond simply driving learning based on immediate experiences, and suggests that there may be further effects of drug-induced plasticity in VTA beyond changes to simple cue-reward learning.

Disclosures: B.F. Sadacca: None. **G. Schoenbaum:** None.

Poster

558. Decision Making I

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 558.28/UU24

Topic: F.02. Animal Cognition and Behavior

Title: Different effects of dopamine D1 and D2 receptor knock-out on performance in a dynamic and uncertain environment

Authors: *S. KWAK[1], N. HUH[2], J.-S. SEO[3], J.-E. LEE[3], P.-L. HAN[3], M. W. JUNG[4,5]
[1]Ctr. for Synaptic Brain Dysfunctions, Inst. of Basic Sci., Systems Neurosci. Lab., Deajeon, Korea, Republic of; [2]Ctr. for Synaptic Brain Dysfunctions, Inst. of Basic Sci., Deajeon, Korea, Republic of; [3]Dept. of Brain and Cognitive Sciences, Ewha Womans Univ., Seoul, Korea, Republic of; [4]Ctr. for Synaptic Brain Dysfunctions, Inst. for Basic Scinece, Daejeon, Korea,

Republic of; [5]Dept. of Biol. Sceince, Korea Advanced Inst. of Sci. and Technol., Daejeon, Korea, Republic of

Abstract: Since the finding that midbrain dopamine neurons signal the difference between actual and predicted outcomes (reward prediction error), dopamine has been postulated to play an essential role in updating values according to reward prediction error as postulated by reinforcement learning theory. However, the extent and nature of dopamine roles in reward-based learning are still under debate. Also unknown are specific roles of different dopamine receptor subtypes in this process. In order to investigate roles of dopamine D1 and D2 receptor subtypes in reward-based learning, we examined choice behavior of dopamine D1 and D2 receptor-knockout (D1R-KO and D2R-KO, respectively) mice in an instrumental learning task with progressively increasing reversal frequency and a dynamic foraging task. Performance of D2R-KO mice was progressively impaired in the reversal task as the frequency of reversal increased and severely impaired in the dynamic foraging task even with prolonged training. By contrast, D1R-KO mice showed only minor performance deficits in these tasks. The animal's choice behavior was well explained by a hybrid model including win-stay-lose-switch and simple reinforcement learning terms. A model-based analysis revealed increased win-stay, but impaired value updating and decreased value-dependent action selection in D2R-KO mice, which would be detrimental to making optimal choices based on the history of past rewards in the dynamic foraging task. Our results indicate an important role of dopamine D2 receptors in learning from the history of past choices and their outcomes for optimizing choice strategy in a dynamic and uncertain environment.

Disclosures: S. Kwak: None. **N. Huh:** None. **J. Seo:** None. **J. Lee:** None. **P. Han:** None. **M.W. Jung:** None.

Poster

558. Decision Making I

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 558.29/UU25

Topic: F.02. Animal Cognition and Behavior

Support: NIMH IRP

Title: Information processing in temporal lobe area TE and in rhinal cortex during a visual categorization task

Authors: *K. LOWE, M. A. G. ELDRIDGE, R. C. SAUNDERS, B. J. RICHMOND
Lab. of Neuropsychology, NIH, Bethesda, MD

Abstract: Single unit recordings in regions of inferior temporal cortex--specifically area TE and rhinal cortex (Rh)--have implicated these regions in late-stage visual processes, such as categorization and stimulus-reward association, respectively. We have previously reported (SFN, 2012) that TE-lesioned monkeys are only mildly impaired in a perceptually difficult test of visual categorization (employing dog-cat morphs), and that the performance of Rh-lesioned monkeys is indistinguishable from that of controls. Now, we asked whether decreasing the information available by shortening the stimulus duration would affect any of our study groups: controls, or monkeys with either TE or rhinal removals. We trained the monkeys to touch a bar to initiate a trial, and release the bar during one of two intervals; early (during the presence of a red central target) if they identified the stimulus as more cat-like, or late (following the transition of the central target to green) if the stimulus was more dog-like. A correct response resulted in the delivery of a fixed-size liquid reward, an incorrect response led to a punishment time-out. We presented the morphed stimuli for durations of 25, 50, 100, 250 or 500 ms in an interleaved design. The stimuli appeared on a background of black and white noise. When the stimuli were removed, the background immediately reappeared; the reappearance of the visual noise appeared to mask the after-image. The accuracy with which control monkeys categorized the stimuli decreased as the stimulus presentation became shorter. The reaction times of control monkeys were indistinguishable across the different stimulus durations. Bilateral TE and Rh cortex removals had different effects: TE-lesioned monkeys made more incorrect categorization judgments, but made their decisions as quickly as controls. Rh-lesioned monkeys categorized as accurately as controls, but took longer to respond, that is, their reaction times were longer. The mild impairment in the performance of TE-lesioned monkeys is consistent with our previous results, and may reflect compromised perceptual or associative processes. The slower reaction times of the Rh-lesioned monkeys could be related to the decision process in some as yet undefined manner.

Disclosures: K. Lowe: None. **M.A.G. Eldridge:** None. **R.C. Saunders:** None. **B.J. Richmond:** None.

Poster

559. Memory Consolidation and Reconsolidation: Neural Mechanisms

Location: Halls A-C

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Program#/Poster#: 559.01/UU26

Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant R01AA016852

Tab Williams Family Fund

Title: Optogenetic induction of fast-gamma and sharp-wave ripples *in vivo*

Authors: *D. C. KLORIG[1], G. ALBERTO[2], M. MASICAMPO[3], D. W. GODWIN[3]
[1]Neurosci. Program, Wake Forest Hlth. Sci., Winston-Salem, NC; [2]Neurosci., [3]Neurobio. and Anat., Wake Forest Univ. Hlth. Sci., Winston-salem, NC

Abstract: Sharp-wave ripples (SWRs) are naturally occurring high-frequency (140-200 Hz) oscillations thought to be involved in memory reconsolidation. SWRs are found in the pyramidal cell layer of hippocampal area CA1 during quiescent and sleep states, associated with strong depolarizing inputs from CA3. Weaker depolarizing inputs to CA1 produce similar but distinct oscillatory activity in the fast-gamma range (90-150 Hz). Optogenetic light-ramp stimuli have been demonstrated to induce gamma oscillations in cortex and CA3, despite conveying no oscillatory content themselves. Using chronically implanted transgenic mice expressing Channelrhodopsin 2, we found that depolarization of CA1 pyramidal neurons using light ramp stimuli was sufficient to induce high-frequency oscillations (HFOs) in the fast-gamma to ripple range (80-200 Hz) (n = 5). We also observed that the frequency of the evoked oscillation was tightly correlated with the amplitude, but not the slope, of the depolarizing input and was reduced by potentiation of GABAa receptors. Evoked oscillation frequency and magnitude did not vary with behavioral state and could be held for the longest duration tested (10 sec). These results support the hypotheses that: 1) HFOs in the fast-gamma and ripple ranges are a robust intrinsic property of the CA1 local circuit; 2) SWRs and fast-gamma are paced by GABAergic interneurons and share similar mechanisms; and 3) CA1 functions as a voltage controlled oscillator.

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Poster

559. Memory Consolidation and Reconsolidation: Neural Mechanisms

Location: Halls A-C

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Topic: F.02. Animal Cognition and Behavior

Support: ATIP - Avenir Program

Emergence - Paris

ITMO - Neurosciences - Neurologie - Psychiatrie

ANR Astrosleep 12-BSV4-0013-01

Title: Role of astrocyte connexins in the regulation of sleep oscillatory patterns

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Abstract: Coordination across brain structures is thought to be crucial for the appropriate consolidation of memory trace. Cortical slow oscillations orchestrate the timing of hippocampal ripples (supporting neuronal reactivations), cortical delta waves and cortical spindles. Our project aims at challenging the classical "neurocentric" view of brain rhythms regulation during sleep. Indeed, recent evidences showed that astrocytes regulate cortical slow oscillations during sleep and are involved in brain processes related to memory deficits induced by sleep deprivation (Halassa et al. 2009). We therefore investigated the role of astrocytic connexins (main constituent of gap junctions and hemichannels) in the regulation of network oscillations, by multi-site recordings in mice double knock-out for astrocytic connexins Cx43 and Cx30 (dKO) (Wallraff et al. 2006). Our results show that sleep slow oscillations are decreased in cortical structures and that the coordination between spindles and ripples are impaired in this dKO model. Moreover, we found that the slow rhythm associated to breathing was also impaired in the olfactory bulb of dKO mice, confirming the results obtained *in vitro* by Lisa Roux. Olfactory bulb slices indeed exhibit spontaneous oscillations very similar to the slow oscillations recorded during sleep in cortical cells, and those oscillations are impaired in dKO mice. Therefore here we suggest that astrocytes could be involved in the fine regulation of sleep oscillatory patterns.

Disclosures: M.M. Lacroix: None. **K. Benchenane:** None. **L. Roux:** None. **C. Giaume:** None.

Poster

559. Memory Consolidation and Reconsolidation: Neural Mechanisms

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Program#/Poster#: 559.03/UU28

Topic: F.02. Animal Cognition and Behavior

Support: ANR - ASTROSLEEP (12-BSV4-0013-01)

ATIP - AVENIR

GRANT ITMO : Neurosciences, Neurologie et psychiatrie

Title: Sleep-scoring in mice using gamma frequency in the olfactory bulb

Authors: *S. BAGUR[1], M. LACROIX[2], K. BENCHENANE[2]
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Abstract: The growing interest in the physiology of various behavioral states demands reliable and systematic methods of wake and sleep stage scoring. Traditional methods identify sleep by monitoring movement or muscular activity, however if sleep and wake are truly different brain states the two should be identifiable using information on neuronal activity alone. It has been shown that gamma oscillations in the olfactory bulb (OB) of the mouse are strongly reduced during sleep (Manabe et al. 2013). Here, we show that low gamma power in the 50-70Hz band recorded from the OB shows a bimodal distribution that allows to separate sleep and waking states. Moreover rapid-eye-movement (REM) and non-REM (NREM) sleep can be distinguished using theta-band power (6-10Hz) recorded in the hippocampus (HPC). We therefore present a method of constructing a two-dimensional phase space allowing to distinguish between waking, REM and NREM sleep that relies exclusively on LFP recordings from the HPC and the OB and can be automatically calibrated. This technique allows for identification of brief periods of arousal and dozing and is therefore a promising tool for the fine study of sleep microstructure.

Disclosures: S. Bagur: None. **K. Benchenane:** None. **M. Lacroix:** None.

Poster

559. Memory Consolidation and Reconsolidation: Neural Mechanisms

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Support: FRM - DEQ20120323730

ANR - REG-071220-01-01

ATIP-Avenir Program

Emergence Paris

Neuro-IC

ITMO neurosciences, neurology, psychiatry

Title: Explicit memory creation during sleep: The causal role of place cell on navigation

Authors: *G. DE LAVILLEON[1,2], M. M. LACROIX[1], L. RONDI-REIG[2], K. BENCHENANE[1,2]
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Abstract: It is now widely accepted that sleep is important for the consolidation of preexisting memory traces, and hippocampus is thought to be a critical player is this process. Here we show that a place preference task can be learned during sleep without prior waking experience, using spontaneous neuronal reactivations. We design a protocol to trigger during sleep, intracranial rewarding stimulations by the action potentials of a unique hippocampal place cell. At waking, animals went and stayed within the associated place field. These results show that it is possible to create an artificial explicit memory during sleep and that this memory trace is used during subsequent waking period to drive a goal directed behavior. Moreover, it demonstrates that place cells activity is functionally significant for navigation, and that place cells still conveys spatial information during sleep.

Disclosures: G. De Lavilleon: None. **M.M. Lacroix:** None. **L. Rondi-Reig:** None. **K. Benchenane:** None.

Poster

559. Memory Consolidation and Reconsolidation: Neural Mechanisms

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 559.05/UU30

Topic: F.02. Animal Cognition and Behavior

Support: ONR MURI (N00014-12-1-0850)

Title: Sleep and memory consolidation of auditory classification learning in European starlings

Authors: *T. P. BRAWN, H. C. NUSBAUM, D. MARGOLIASH
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Abstract: Memory consolidation strengthens newly encoded memory traces and stabilizes them against interference, a process widely believed to benefit from sleep. Behavioral evidence of sleep-dependent memory consolidation is well established in human memory tasks but is limited in adult animals. We previously demonstrated sleep-dependent memory benefits in European starlings that were trained on a Go/No-Go task to classify pairs of 5-second segments of novel starling songs. Classification accuracy improved after retention intervals that included sleep but not after waking retention (Brawn et al., *Journal of Neuroscience*, 2010). Furthermore, learning two similar classification tasks (tasks A and B) interfered with each other during the day, resulting in performance impairments for both tasks after waking retention. Yet, performance on both tasks improved after sleep (Brawn et al., *Psychological Science*, 2013). Here we aim to characterize patterns of sleep in starlings and to correlate post-training changes in sleep structure with post-sleep memory improvements on an auditory classification task. In an initial behavioral experiment, starlings in three conditions were trained on classification task A and received an immediate post-training test followed by posttests in the evening and next morning. The Control (no interference) condition only learned task A. The New-Interference condition learned classification task B (with novel stimuli) after task A. The Old-Interference condition also completed task B but with a previously learned stimulus set. Whereas performance in the Control and Old-Interference conditions remained stable across wakefulness, the New-Interference condition expressed significant performance impairments after waking retention, suggesting that the formation of interference requires the learning of a new classification task. Nonetheless, classification accuracy in each condition improved after sleep. In the current study, new starlings implanted with 4 EEG electrodes in each hemisphere complete the same three conditions while undergoing 56 hours of chronic EEG and video recording. Additionally, each bird will undergo 48 hours of chronic baseline EEG and video recordings prior to and after the completion of the three behavioral conditions. Though data analysis of sleep states has not begun, the behavioral results demonstrate a robust system of sleep-dependent memory consolidation in European starlings. We are currently attempting to use EEG recordings to connect the behavioral benefits of sleep to changes in slow wave sleep, intermediate (or transition) sleep, and rapid-eye movement sleep.

Disclosures: **T.P. Brawn:** None. **H.C. Nusbaum:** None. **D. Margoliash:** None.

Poster

559. Memory Consolidation and Reconsolidation: Neural Mechanisms

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Program#/Poster#: 559.06/UU31

Topic: F.02. Animal Cognition and Behavior

Support: ERC-2010-Ad6-268800-Neuroschema

Title: Selective intervention in hippocampal and cortical consolidation: Impact of novelty and sleep

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Abstract: BACKGROUND: For memory traces to be successfully recalled, they need to be consolidated. Different factors are thought to influence this process including (1) novelty exposure that enhances the persistence of a hippocampal trace via neuromodulation; and (2) sleep that aids systems consolidation and thus a cortically based memory. Do such hippocampal and cortical and traces differ qualitatively with respect to their behavioural expression, or their persistence over time? To investigate this, in Expts 1 and 2 rats were trained on two competing memories with training to one followed by sleep deprivation plus novelty (hippocampal) and the other by sleep (cortical). Further, in Expt 3, we compare IEG expression in the hippocampus and prefrontal cortex in association with retrieval between animals given each of the conditions. METHODS: In Expts. 1 and 2, rats were trained to learn two opposite escape locations in a watermaze over 2 sessions, with novelty + sleep deprivation, or sleep following session 1 and the alternative intervention after session 2 (counterbalanced). Probe trials without any platform present were conducted at varying times afterwards (Expt 1: 24h, 7d and 21d (within-subjects); Expt 2: 7d and 21d). In Expt. 3, rats were taught only one escape location and were then divided into two groups with novelty + sleep deprivation, or sleep, following the encoding session. Probe trials were performed for Expt 3a at 24h, 7d and for Expt 3b at 7d. 30 min after the 7d probe the animals were sacrificed for qPCR analysis. RESULTS: In probe tests at 24h, the rats remembered both escape locations in the watermaze, but displayed a preference for the location whose encoding was followed by novelty (hippocampal trace dominating). This hippocampal memory trace did not survive to 7d at which time the cortical trace dominated. In Expt 2 without the 24h probe, the novelty-enhanced memory trace did survive, suggesting the expression of a hippocampal trace can contribute to its demise. Performance was at chance in all conditions by 21d. Expt. 3 confirmed this pattern of results in the between subject design, but further showed that cortical memory at 7d can paradoxically benefit from the 24h probe trial without feedback,

i.e. under conditions where there is no competition between the hippocampal and cortical trace. qPCR analysis of IEG is underway. CONCLUSION: These data contribute to other findings suggesting a dynamic interplay of hippocampal and cortical memory. They indicate that cortical memory is more resistant to interference, but at the cost of being less exact and vivid, while hippocampal memory can enable a stronger behavioural response soon after memory encoding.

Disclosures: L. Genzel: None. **J. Rossato:** None. **R. FItzpatrick:** None. **R.G.M. Morris:** None.

Poster

559. Memory Consolidation and Reconsolidation: Neural Mechanisms

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Support: RFBR Grant 13-04-01892A

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Title: The neural basis of complex associative memory in tone-light compound fear conditioning in mice

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Abstract: Cellular mechanisms of associative memory are commonly studied in Pavlovian fear conditioning to a single discrete stimulus. Much less is known about neuronal mechanisms engaged in formation and retrieval of complex associative memories composed of stimuli from several modalities. In the present study we used mouse fear conditioning to a compound cue consisted of auditory (tone, T) and visual (blinking light, L) stimuli. First, we showed that mice could successfully establish fear learning to compound T+L stimulus, along with discrete T or L stimuli. In retrieval session 7 days after the training mice, conditioned to compound cue were presented either with the entire compound cue, or with its discrete T or L components. Mice, that were conditioned to one of cues (T or L) were presented with the relevant cue in the retrieval

session. Active control animals were presented with T+L compound stimulus, without footshock during training session and with T+L, T or L stimuli during retrieval session. To evaluate patterns of brain regions involved into memory formation and retrieval by entire compound cue or its discrete components we performed c-Fos mapping in associative and sensory cortices (frontal, prelimbic, cingular, retrosplenial, parietal, primary and secondary visual, primary and secondary auditory), hippocampal areas (CA1, CA3, dentate gyrus) and amygdala nuclei (basolateral, lateral and central). First, we showed the specific involvement of prelimbic and frontal associative cortices along with amygdala nuclei in associative memory formation, both to compound or single cues. Next, we compared patterns of c-Fos expression after memory retrieval by the entire compound cue or by its discrete components in compound cue conditioned-mice and by relevant cue in single cue-conditioned animals. Retrieval of complex memory by light or tone component was accompanied by specific activation of the prelimbic, parietal, primary visual, mediolateral area of the secondary visual cortex and amygdala nuclei. Hippocampus was specifically involved neither in formation nor in the retrieval of associative memory. Taken together our data suggests that complex associative memory is supported by distinct cortical mechanisms that affect both sensory and associative cortical areas. Moreover, brain regions that shape neural basis of complex associative memory formation and its retrieval are different.

Disclosures: **O.I. Ivashkina:** None. **M. Roshchina:** None. **K. Toropova:** None. **K. Anokhin:** None.

Poster

559. Memory Consolidation and Reconsolidation: Neural Mechanisms

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Topic: F.02. Animal Cognition and Behavior

Support: KAKENHI 24116008

KAKENHI 23300120

KAKENHI 24650172

Title: Erasure of recent and remote hippocampus-dependent fear memory by enhancing memory forgetting through increase in adult hippocampal neurogenesis

Authors: *R. ISHIKAWA[1], P. FRANKLAND[2], S. KIDA[1,3]
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Abstract: Erasure of fear memory is thought to be a therapeutic target for emotional disorders such as post-traumatic stress disorders (PTSD). Memantine (MEM) is a noncompetitive N-methyl-D-aspartate (NMDA) glutamate receptor antagonist and has known to enable to increase in adult hippocampal neurogenesis. Previous study has shown that increasing adult hippocampal neurogenesis enhanced forgetting of hippocampus-dependent memory formed recently. In this study, we tried to examine effects of MEM treatment on forgetting of hippocampus-dependent fear memory. Mice were contextual fear-conditioned with an electrical footshock and then received systemic injections of MEM (50 mg/kg body weight) once a week for 4 weeks. We found that MEM-treated group displayed disruption of contextual fear memory following the MEM treatment, while control group treated with saline displayed normal contextual fear memory. Importantly, MEM-treated group did not show spontaneous recovery of fear memory even a month after the MEM treatment. Moreover, we observed similar effects of MEM treatment on inhibitory avoidance memory, another type of hippocampus-dependent fear memory. In contrast, MEM-treated group showed normal amygdala-dependent cued fear memory. We next examined the relationship between erasure of hippocampus-dependent memory and enhancement of adult hippocampal neurogenesis by MEM. Mice received an injection of MEM followed by injections of 5-bromo-2-deoxyuridine (BrdU; 50 mg/kg body weight) 2 days later, and then were contextual fear-conditioned. Interestingly, there was a significant negative correlation between the number of BrdU-positive cells increased by MEM and differences of freezing scores before and after the MEM treatment. These observations suggest that MEM treatment enables to erase hippocampus-dependent fear memory by enhancing memory forgetting through the increase in adult hippocampal neurogenesis. Thus our findings suggest a possible therapeutic treatment to weaken traumatic memory. We are now examining effects of the MEM treatment on remote fear memory.

Disclosures: R. Ishikawa: None. **P. Frankland:** None. **S. Kida:** None.

Poster

559. Memory Consolidation and Reconsolidation: Neural Mechanisms

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Topic: F.02. Animal Cognition and Behavior

Support: 24116008

Title: Up-regulation of CREB activity in forebrain enhances working-like memory and increases spine density in the hippocampus

Authors: *T. SERITA[1], H. FUKUSHIMA[1,2], S. KIDA[1,2]
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Abstract: Transcription factor cAMP response element-binding protein (CREB) plays essential roles in memory formation through activation of gene expression. Our previous study indicated that loss-of-CREB function leads to an impairment of long-term memory (LTM), but not short-term memory (STM). To further understand roles of CREB in learning and memory, we have examined effects of gain-of-CREB function on memory formation by generating transgenic mice expressing a constitutively active CREB mutant (CREB DIEDML) in the forebrain. Interestingly, these transgenic mice displayed enhancement of not only LTM but also STM, suggesting that CREB positively regulates both LTM and STM. These findings raise the possibility that CREB plays critical roles in learning processes. To ask this, we first examined the ability of temporal association learning and spatial working memory in DIEDML mice. In the trace fear conditioning task, mice learn an association between the conditioned stimulus (CS; tone) and the unconditioned stimulus (US; footshock) separated by the trace interval. During the training that consists of 8 CS-trace-US-intertrial interval, mice were assessed freezing responses during each trace interval. DIEDML mice learned trace fear conditioning significantly faster and better compared to wild-type (WT) mice. We next performed working memory version of Morris water maze task in which mice were given training with 4 trials (trial 1-4) per day and the position of platform was changed every day. DIEDML mice showed significantly more decreases in escape latencies to the platform at the trial 2-4 compared to WT mice although these mutant mice displayed normal escape latency at the trial 1. These results indicated that DIEDML mice showed enhancement of working memory. Thus our results suggest that up-regulation of CREB activity improves working-like memories. Furthermore, morphological analysis of dendritic spine of hippocampus revealed that DIEDML mice showed significant increase in dendritic spine density compared to WT mice. These changes in dendritic spine density or morphology of neurons observed in DIEDML mice may contribute to improvement of learning process.

Disclosures: **T. Serita:** None. **H. Fukushima:** None. **S. Kida:** None.

Poster

559. Memory Consolidation and Reconsolidation: Neural Mechanisms

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Topic: F.02. Animal Cognition and Behavior

Support: KAKENHI 23300120

KAKENHI 24116008

Title: Poly ADP-ribosylation is required for reconsolidation and extinction of contextual fear memory

Authors: *H. INABA[1], A. TSUKAGOSHI[1], S. KIDA[1,2]
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Abstract: Retrieval of contextual fear memory by re-exposure to conditioned stimulus (CS; context) initiates new gene expression-dependent processes; reconsolidation and extinction. Our previous study showed that short (3 min) re-exposure to the CS (context) inducing memory reconsolidation activated new gene expression in the hippocampus and amygdala, while long (30 min) re-exposure inducing memory extinction activated it in the medial prefrontal cortex and amygdala (Mamiya et al., 2009). Poly (ADP-ribose) polymerase-1 (PARP-1) has been known to covalently modify nuclear proteins including histones and transcription factors through poly ADP-ribosylation. Interestingly, recent studies have suggested that poly ADP-ribosylation by PARP-1 plays critical roles in long-term neuronal plasticity and formation of long-term memory in *Aplysia* and mammals (Cohen-Armon et al., 2004; Hernandez et al., 2009; Fontan-Lozano et al., 2010). To understand roles of poly ADP-ribosylation in the regulation of contextual fear memory after retrieval, we have examined effects of inhibition of poly ADP-ribosylation in the hippocampus and medial prefrontal cortex in reconsolidation and extinction in mice. Mice were micro-infused a PARP-1 inhibitor (3-aminobenzamide (3AB) or PJ34) into the hippocampus or medial prefrontal cortex 5 min before or immediately after the re-exposure for 3 or 30 min. This inhibition of poly ADP-ribosylation in the hippocampus impaired reactivated contextual fear memory when tested 24 hrs, but not 2 hrs, after the re-exposure for 3 min, suggesting that poly ADP-ribosylation in the hippocampus is required for reconsolidation of contextual fear memory. Additionally, the inhibition of poly ADP-ribosylation in the medial prefrontal cortex, but not the hippocampus, blocked long-term extinction of contextual fear memory, suggesting that poly ADP-ribosylation in the medial prefrontal cortex is required for consolidation of extinction memory. Our findings suggest that poly ADP-ribosylation in the hippocampus and medial prefrontal cortex plays essential roles in reconsolidation and extinction, respectively, of contextual fear memory.

Disclosures: H. Inaba: None. **A. Tsukagoshi:** None. **S. Kida:** None.

Poster

559. Memory Consolidation and Reconsolidation: Neural Mechanisms

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Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant MH097111

Title: Posttraining optogenetic control of basolateral amygdala projections to the ventral hippocampus modulates the consolidation of emotional, but not contextual, learning in rats

Authors: *M. L. HUFF, R. T. LALUMIERE
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Abstract: The basolateral amygdala (BLA) modulates memory consolidation for multiple types of learning, including contextual fear conditioning (CFC), whereas other brain regions appear to play more specific roles in different kinds of learning. However, whether BLA projections to such regions influence memory consolidation in a learning-specific manner has never been directly investigated. One of the BLA's efferent targets, the ventral hippocampus (vHPC), has previously been suggested to selectively process emotion-related aspects of learning, yet whether the BLA->vHPC pathway modulates memory consolidation, and does so in a manner specific to the type of information being learned, is unknown. Therefore, the present study used an optogenetic approach to modulate activity in the BLA->vHPC pathway during the consolidation period immediately following training in a modified CFC task that permits separation of the context and footshock learning. Male Sprague-Dawley rats received intra-BLA injections of an adeno-associated virus that produced expression of either the light-sensitive membrane-bound cation channel channelrhodopsin-2 [ChR2(E123A); to permit high-frequency stimulation] or the outward proton pump archaerhodopsin (eArchT3.0; to permit inhibition). After allowing ~5 weeks for opsin expression, fiber optic probes were implanted immediately dorsal to the vHPC to provide later illumination of the BLA axonal fibers innervating this region. For the CFC task, rats received 3 min of pre-exposure to the apparatus on day 1. On day 2, rats were placed into the apparatus, received an immediate footshock, and were then quickly removed. Retention was tested on day 4. Results indicate that optical stimulation of the BLA->vHPC pathway following context pre-exposure had no effect on retention regardless of the stimulation frequency. In contrast, optical stimulation of the BLA->vHPC pathway following footshock training, using trains of 40, but not 20 or 80, Hz light pulses, enhanced retention. Similar light pulses given to eYFP-control rats following footshock training had no effect on retention. In rats that did not receive context pre-exposure, optical stimulation of this pathway did not affect retention.

Preliminary work also suggests 15 min of optical inhibition of this pathway following footshock training impairs retention. These findings provide direct evidence that BLA projections to other brain regions modulate memory consolidation differently depending on separable components of a task. Specifically, BLA->vHPC projections influence the consolidation for the footshock, but not context, learning for a modified CFC task.

Disclosures: M.L. Huff: None. **R.T. LaLumiere:** None.

Poster

559. Memory Consolidation and Reconsolidation: Neural Mechanisms

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Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant R00 AG039511

Title: Retrieval of contextual fear memory enhances the post-burst afterhyperpolarization in burst-spiking cells in the subiculum

Authors: *K. A. HOPE, S. M. NEUNER, L. A. WILMOTT, C. C. KACZOROWSKI
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Abstract: Changes in intrinsic neuronal excitability following a learning task have been demonstrated in many brain regions, especially the hippocampus. The subiculum is the major output pathway of the hippocampus and contains two distinct pyramidal cell types: burst-spiking and regular-spiking neurons (also referred to as early-bursting and late-bursting neurons, respectively). It is generally thought that burst-spiking neurons convey spatial information, while regular-spiking neurons convey non-spatial information. Despite the importance of the subiculum for hippocampal output, learning related changes have remained largely unexplored in this key brain region. Here we used a hippocampus-dependent contextual fear conditioning task to examine changes of intrinsic excitability in burst-spiking and regular-spiking neurons of the subiculum in the mouse. We found that the medium component of the afterhyperpolarization (mAHP) was significantly enhanced in burst-spiking neurons after fear conditioning and retrieval of contextual fear memory (-3.75±0.21 mV) compared to mice that either underwent fear conditioning without retrieval (-2.61±0.36 mV), context exposure without shock (-3.07±0.39 mV), or home cage controls (-2.16±0.45 mV). Interestingly, regular-spiking neurons showed no change in AHPs in any treatment group. Moreover, the slow component of the AHP was

unaffected relative to conditioning groups and across cell types. We hypothesize that the cell-type specific enhancement of the mAHP in burst-spiking neurons following retrieval of fear memory may facilitate extinction of conditioned contextual fear memory. Ongoing studies in our laboratory aim to identify the molecular mediators of AHP plasticity in burst-spiking neurons during the retrieval process and assess their role in the extinction process. Mechanisms underlying this plasticity may be particularly relevant to understanding dysfunction of fear-related disorders including post-traumatic stress disorder.

Disclosures: K.A. Hope: None. **S.M. Neuner:** None. **L.A. Wilmott:** None. **C.C. Kaczorowski:** None.

Poster

559. Memory Consolidation and Reconsolidation: Neural Mechanisms

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Topic: F.02. Animal Cognition and Behavior

Support: NASA Grant NNX13AB73G.

Title: Acute effects of exposure to space radiation on CNS function and cognitive performance

Authors: *B. M. RABIN[1], B. SHUKITT-HALE[2], K. L. CARRIHILL-KNOLL[1], D. F. BIELINSKI[2], S. M. POULOSE[2], N. A. HEROUX[1], C. BAXTER[1]
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Abstract: On exploratory class missions, such as a mission to Mars, astronauts will be exposed to types and doses of radiation (cosmic rays) that are not experienced in low earth orbit where the Space Shuttle and International Space Station operate. Exposure to cosmic rays produces changes in neuronal function and in cognitive performance. While the changes in neuronal function are observed as shortly as 36 hr following irradiation, it is not known whether or not there are similar acute effects of exposure on cognitive performance and how the behavioral changes may relate to the changes in neuronal function. The present experiment was designed to determine the acute effects of exposure to space radiation on cognitive performance (novel object recognition) and the relationship to radiation-induced changes in neuronal function. Conditioning occurred either 3-6 hr before (memory) or 18 hr after (learning) exposure to 56Fe or 16O particles (600 MeV/n). Exposure to either particle disrupted the recall of a previously

acquired task (memory), but did not affect the acquisition (learning) of the task. Following behavioral testing rats were euthanized; their brains removed and selected areas analyzed for oxidative stress. Increases in the levels of NOX2 were observed in several brain regions including hippocampus, frontal cortex, cortex, striatum and cerebellum following irradiation. Changes in oxidative status varied as a function of whether or not there was an effect of irradiation on cognitive performance, as NOX2 levels were different between the rats exposed using the memory design and those exposed using the learning design. These results suggest that exposure to the types of radiation encountered in space may affect the recall of recently acquired material (memory) but may not have immediate effects on the acquisition of new material (learning). The results also suggest that exposure to space radiation has widespread effects on neuronal function throughout the brain, and some of these changes in neuronal function may be related to the radiation-induced changes in cognitive function.

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Poster

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SRFDP/RGC grant 2900703

Title: Layer specific inputs to layer V primary motor cortex exhibit different profiles of training-induced synaptic plasticity

Authors: *Q. LI[1], H. KO[1], C. W. CHAN[1], G. ARBUTHNOTT[2], Y. KE[1], W. H. YUNG[1]
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Abstract: Functionally distinct streams of information are conveyed by synapses that terminate at different dendritic sites of cortical pyramidal neurons. As the major output neuron of neocortex, how layer V pyramidal neurons integrate widespread synaptic inputs to both proximal

basal dendrites and distal apical tuft dendrites and shape the learning-related cortical plasticity in intact brain is an important but unanswered question. As a first step to address this issue, in the present study, we examined the properties of physiologically induced plasticity in these layer-specific synaptic inputs by chronic field potential recordings from the primary motor cortex of rats undergoing forelimb-reaching task. A 16-channel microwire recording array and 2 pairs of monopolar stimulating electrodes were implanted into MI forelimb territory, with recoding site at layer V and stimulating sites at both layer V and layer I. Different synaptic inputs targeting basal and apical dendrites were induced by stimulating the layer V local circuit and superficial layer I respectively, which were further confirmed with the aid of current source density analysis. The potentiation level of synaptic plasticity was evaluated by the increment of the stimulation evoked field potential amplitude. In the initial learning phase (day1 to day3), rats exhibited rapid improvement in single success rate (baseline in naïve subjects: 8.74± 3.0%; end of day1: 31.3±3.1%; end of day3: 42.9±1.1%; n=6). This fast, motor skill acquisition stage was accompanied by substantial learning-related synaptic potentiation on both local basal dendrites (day1: 26.2±5.3%; day3: 35.4±3.2%, n=4) and distal apical tuft dendrites (day1: 28.2±4.1%; day3:27.7±4.8%, n=5). As training continued, motor skill was consolidated gradually, and the single success rate reached a plateau (day7: 44.4±2.4%, n=6). Intriguingly, only the potentiated synaptic input targeting the basal dendrites could be sustained till day 7 (34.2±5.6%, n=4), but not those targeting the apical tufts dendrites (7.2±3.2%, n=5). The consolidated synaptic potentiation on local basal dendrites could be impaired by locally depleting the mesocortical dopaminergic innervation, which also compromised overnight retention of newly learned skill. Our results suggest that motor learning-related synaptic potentiation in basal and apical dendrites of layer V pyramidal neuron exhibit different coupling profiles, implying different functions in motor memory formation. Dopamine appears to be essential for long-term consolidation of synaptic plasticity mediated by local basal dendritic circuit.

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Poster

559. Memory Consolidation and Reconsolidation: Neural Mechanisms

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 559.15/UU40

Topic: F.02. Animal Cognition and Behavior

Support: The National Natural Science Funds (No. 81171031)

Title: The effect of subhypnotic doses of propofol on spatial memory retrieval and the phosphorylation of glycogen synthase kinase-3β in the hippocampus

Authors: H. LIU[1], *X. LIU[2], Y. LI[1]
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Abstract: Objective: Propofol administration affects multiple stages of memory processes in both humans and animals. Many researches focused their attention on the influence of propofol on memory encoding and consolidation while leaving the unexplored area of propofol's effect on memory retrieval. Glycogen synthase kinase 3β (GSK3β) is a serine/threonine protein kinase and GSK3β has been suggest to implicate in synaptic plasticity and memory formation. Inhibition of GSK-3β induced by SB216763 was recently shown to weaken memory retrieval. The current experiments were designed to examine the effects of subhypnotic doses of propofol on memory retrieval in rats, and, more precisely, whether the glycogen synthase kinase GSK3β signaling pathway was involved in mediating the effects of pre-testing propofol administration on Morris water maze retention. Methods: Adult male Sprague-Dawley rats were trained on a water maze task in which they subjected eight trials during a single acquisition session. After 24 hours a probe test was processed to examine the memory retrieval effects of subhypnotic doses of propofol(10 or 25 mg/kg) administered intraperitoneally 5 minutes before test. Animal' hippocampi were isolated to measure the levels of different forms of GSK-3β. In addition, the behavior of 25mg/kg propofol in the open filed was observed to excluding the probable effects on spontaneous locomotor. Results: Subhypnotic doses of propofol (25mg/kg) administered 5 minutes before test significant decreased the time percentage spent in the target quadrant(24.6±2.3%,mean ± SEM) compared with the vehicle group(37.3±4.0%).Meanwhile, the impairment of memory retrieval by propofol is not due to the alteration of locomotor activity. On GSK- 3β assessment: memory retrieval is accompanied with inhibition of the Serine-9 residue phosphorylation of GSK-3β rather than the activation of Tyrosine- 216 residue, and propofol could reverse the increasing activity of GSK-3β induced by retrieval. Conclusion: A subhypnotic doses of propofol (25mg/kg) injected 5 minutes before Morris water maze test impaired memory retrieval. In addition, our results suggest that the amnestic effects of propofol might be mediated by weaken GSK-3β signaling in the hippocampus. [Key words] Propofol; Memory retrieval; hippocampus; glycogen synthase

Disclosures: H. Liu: None. **X. Liu:** None. **Y. Li:** None.

Poster

559. Memory Consolidation and Reconsolidation: Neural Mechanisms

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 559.16/UU41

Topic: F.02. Animal Cognition and Behavior

Support: NSF CRCNS

Title: Neural mechanisms of strategy change during conditioned learning

Authors: R. KOZMA[1], *M. H. MYERS[2], W. J. FREEMAN[3]
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Abstract: Aim: Analyze neural dynamics underlying conditioned learning in gerbils using multichannel recordings in the auditory cortex and ventral striatum. Introduction: Conditioned learning is usually described as a monotonic optimization processes that can be well described in reinforcement learning framework. Experimental evidence, however, points to the existence of clearly defined moments of sudden changes in neural processes underlying learning [1, 2]. We hypothesize that the moments of abrupt changes indicate that the system gives up its existing behavioral strategy and transitions to the next state of cognition. Methods: We describe phase transitions in distributed systems to provide a suitable theoretical framework to model neuronal processes underlying behavioral strategy changes. We analyze multichannel recordings in the auditory cortex and ventral striatum in gerbils making the transition from naïve to avoidance behavior. Phase transitions in neuropercolation models [3] are used to interpret experimental findings. Results: We developed statistical tools to discriminate spatiotemporal activity patterns in cortical structures during the different phases of learning. We described the emergence of the attractor landscape of brain states, as well as state transitions in each action-perception-cycle that were analyzed in already trained animals. Conclusions: Cognitive abstraction from learned stimulus enables rodents to categorize previously unknown stimuli. The abstraction is described through the emergence of classifiable metastable spatiotemporal patterns. These patterns can be viewed as dynamically enacting symbols executing brain commands, rather than passive representational symbols [3]. 1. Ohl FW, Scheich H, Freeman WJ (2001) Nature 412: 733-736. 2. Deliano M, Ohl FW (2009) New Mathematics and Natural Computation 5: 61-81 3. Kozma, R., W.J. Freeman (2009) Neural Networks, 22(3), pp. 277-285.

Disclosures: R. Kozma: None. **M.H. Myers:** None. **W.J. Freeman:** None.

Poster

559. Memory Consolidation and Reconsolidation: Neural Mechanisms

Location: Halls A-C

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Topic: F.02. Animal Cognition and Behavior

Support: Intramural Research Program of NIDA

Title: Median raphe nucleus regulates hippocampal ripple oscillation and memory consolidation

Authors: *D. V. WANG, H.-J. YAU, C. J. BROKER, J.-H. TSOU, A. BONCI, S. IKEMOTO
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Abstract: Sleep promotes memory consolidation, a process known as reorganization and progressive integration of newly acquired information into pre-existing neural networks for long-term storage. Hippocampal sharp-wave ripples that occur primarily during slow-wave sleep are believed to play an essential role in the memory consolidation process. Ripples are thought to reactivate newly-formed hippocampal-neocortical neural ensembles and gradually transform them into neocortical memory traces. However, as ripples are intrinsically generated in the hippocampus, little is known about how they are regulated by other brain regions. Here we provide the first direct evidence that the median raphe nucleus, a midbrain structure, plays a key role in regulating hippocampal ripple activity and memory consolidation. We performed *in vivo* simultaneous recordings in the median raphe nucleus and hippocampus, and found that when a group of raphe neurons were active, ripples were absent. To determine a causal relationship, we employed an optogenetic approach and found that photostimulation of raphe neurons strongly suppressed hippocampal ripple activity. Conversely, photoinhibition of raphe neurons increased hippocampal ripple activity. We then examined whether such manipulation of raphe neurons would interfere with memory consolidation using a fear conditioning procedure, and found that photostimulation during subsequent sleep/ rest significantly reduced freezing behavior when mice were tested 24 hours later. Our results demonstrate an unprecedented role of the median raphe nucleus in regulating hippocampal ripple activity and memory consolidation processes. We anticipate that our study is a beginning to understand how subcortical and cortical regions interact to regulate memory processes and to provide insights for the treatment of affective/anxiety disorders arising from dysregulated memory processes.

Disclosures: D.V. Wang: None. **H. Yau:** None. **C.J. Broker:** None. **J. Tsou:** None. **A. Bonci:** None. **S. Ikemoto:** None.

Poster

559. Memory Consolidation and Reconsolidation: Neural Mechanisms

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 559.18/UU43

Topic: F.02. Animal Cognition and Behavior

Title: Rapid encoding of novel spatial information requires LTD

Authors: *D. M. ASHBY[1], Y. WANG[1,2]
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Abstract: The exploration and encoding of a novel environment is a fundamental learning process that occurs on a short time scale, and is a useful model for studying how the hippocampus encodes and represents complex and arbitrary associations, as is required for episodic memory. Novelty exploration has been demonstrated to promote LTD induction in area CA1 of the hippocampus, but it is unclear what role this LTD plays as the novel space is represented and encoded in the hippocampus. Here we show that novelty exploration produces AMPAR endocytosis-dependent LTD in CA1 in the absence of a paired LTD induction protocol, and that blockade of AMPAR endocytosis/LTD produces a prolonged exploration period in a novel environment. Male Sprague-dawley rats were chronically implanted with recording arrays in CA1, while afferent pathways were stimulated with bipolar stimulation electrodes. After recovery from surgery, baseline fEPSP responses were measured in a familiar environment, after which rats were allowed to explore a highly novel environment. A decrease in evoked potentials consistent with LTD was observed upon novelty exploration, which was blocked by IV administration of an interference peptide (Tat-GluA2-3Y) that blocks regulated AMPA receptor endocytosis. To assess the effects of this LTD on the acquisition of novel spatial information, the GluA2-3Y peptide was administered prior to a spatial recognition memory test in which rats are allowed to spontaneously explore both a familiar and novel spatial location. While vehicle administered rats showed a transient preference for the novel location, inhibition of AMPA receptor endocytosis produced a prolonged exploratory preference for the novel spatial location, indicating that behavioral habituation to the novel location was impaired when LTD processes were blocked. These results demonstrate a behaviorally relevant role for a rapid LTD-like process in the acquisition of novel spatial information, with important implications for the mechanisms of information processing in the hippocampus.

Disclosures: D.M. Ashby: None. **Y. Wang:** None.

Poster

559. Memory Consolidation and Reconsolidation: Neural Mechanisms

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Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant RO1AG037984

NIH Grant RO1AG036800

Evelyn F. McKnight Brain Research Foundation

Title: Inflammation as a potential mediator of decreased NMDA receptor function and the onset of age-related cognitive decline: A test for the effectiveness of anti-inflammatory drugs

Authors: *A. KUMAR, A. RANI, T. C. FOSTER
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Abstract: A redox mediated decrease in N-methyl-D-aspartate (NMDA) receptor function in the hippocampus is associated with the emergence of impaired episodic spatial memory. However, the source of reactive oxygen species that contributes to impaired NMDA receptor function is unclear. Inflammation provides one potential source for reactive oxygen species suggesting a possible mechanism through which inflammation could compromises neurons leading to the onset and progression of cognitive decline. Therefore, the current studies tested the hypothesis that treatments to reduce inflammation would enhance NMDA receptor function in older animals. For these studies, young (5-6 mo, n = 20) and aged (18-20 mo, n = 22) male F344 rats were first tested on a one day version of the water maze which is sensitive to spatial episodic memory. Young rats and aged rats that exhibited impaired learning were included for treatment. Animals were treated with the nonsteroidal anti-inflammatory drug, indomethacin (Indo, 2.5 mg/kg; young-Indo = 11; aged-Indo = 7), delivered in a frozen treat (orally, twice per day, 3 weeks) and compared to vehicle controls (young-Veh = 9; aged-Veh = 9). Following 3 weeks of treatment with indomethacin, subsequent re-testing on the water maze indicated superior learning associated with indomethacin ($p<0.001$) treatment and post hoc test indicated a drug effect in young and aged rats ($p<0.05$). Hippocampal slices were prepared and input-output curves of CA3-CA1 NMDA receptor-mediated synaptic responses were generated. Examination of synaptic NMDA receptor input-output curves indicated a tendency ($p=0.06$) for an interaction of treatment and stimulation intensity mainly due to an increased response for Indo treated animals. Indeed, no aged difference was observed for Indo groups and a tendency ($p=0.09$) for an interaction of age and stimulation intensity was observed only for the vehicle treated animals due to a reduced response in aged animals. NMDA receptors are critical for long-term potentiation (LTP). LTP was induced by theta burst stimulation consisting of 4 sets of 5 bursts of 4 pulses at 100 Hz with 200 ms intervals between bursts and the 4 sets separated by a 10-second interval. An ANOVA indicated an interaction of age and treatment ($p<0.05$) due to increased LTP for

aged-Indo relative to aged-Veh ($p<0.05$). The results suggest that inflammation may act through NMDA receptors to contribute to the onset and progression of age-related cognitive decline.

Disclosures: **A. Kumar:** None. **A. Rani:** None. **T.C. Foster:** None.

Poster

559. Memory Consolidation and Reconsolidation: Neural Mechanisms

Location: Halls A-C

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Program#/Poster#: 559.20/UU45

Topic: F.02. Animal Cognition and Behavior

Support: NIH grant NS061103

Title: Coordinated increase of excitatory and Purkinje cell somatic synapses on eyeblink projection neurons of the anterior interpositus after eyeblink conditioning

Authors: *J. GONZALEZ-JOEKES, B. G. SCHREURS
WVU, Morgantown, WV

Abstract: The effect of eyeblink conditioning on excitatory and inhibitory somatic synapses on eyeblink projection neurons of the anterior interpositus nucleus (AIN) was examined. Rabbits were divided into three groups that received different behavioral procedures. The experimental group received paired delay training with a tone conditioned stimulus and an airpuff unconditioned stimulus. There were two control groups, one received unpaired stimulus presentations, and the other did not receive any stimuli. After behavioral procedures, eyeblink projection neurons of the AIN were identified using a retrograde transneuronal tracer. A coordinated increase in the number of excitatory and inhibitory Purkinje cell somatic synapses was observed in subjects that acquired eyeblink conditioned responses. These results support a parallel and correlated mechanism of cerebellar learning mediated by excitatory (presumably from mossy fibers) and inhibitory Purkinje cells. In contrast, control subjects that received unpaired stimulus presentations showed an increase in the number of inhibitory somatic synapses from local interneurons, suggesting a possible role of feedback inhibition that may also explain the retardation effect in learning observed after unpaired stimulus preexposure. Another important finding was that the somatic surface area of eyeblink projection neurons was highly correlated with the number of somatic synapses; suggesting that synaptic remodeling is a bilateral process that entails proportional structural alterations on the postsynaptic neuron.

Disclosures: J. Gonzalez-Joekes: None. **B.G. Schreurs:** None.

Poster

559. Memory Consolidation and Reconsolidation: Neural Mechanisms

Location: Halls A-C

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Program#/Poster#: 559.21/UU46

Topic: F.02. Animal Cognition and Behavior

Support: the RIKEN Brain Science Institute

Howard Hughes Medical Institute

Title: The role of dopamine 1 and 5 receptors in hippocampal dependent learning and memory

Authors: *J. SARINANA[1], T. KITAMURA[1], P. KÜNZLER[2], L. SULTZMAN[3], S. TONEGAWA[3]
[1]RIKEN–MIT Ctr. for Neural Circuit Genet., Massachusetts Inst. of Technology, The Picower Inst. for Learning and Me, CAMBRIDGE, MA; [2]Inno-Motion Ltd., Zürich, Switzerland; [3]MIT, Cambridge, MA

Abstract: Mounting evidence supports the role of midbrain dopaminergic neurons in aversive Pavlovian conditioning. Activation of the hippocampal dopamine 1-class receptors (D1R and D5R) are implicated in Pavlovian conditioning, such as contextual fear conditioning (CFC). However, the specific role of the D1R versus D5R in hippocampal dependent CFC has not been investigated. Generation of D1R- and D5R-specific *in situ* hybridization probes showed that D1R and D5R mRNA expression was greatest in the dentate gyrus (DG) of the hippocampus. To identify the role of each receptor in CFC we generated spatially restricted KO mice that lack either the D1R or D5R in DG granule cells. DG D1R KOs displayed significant fear memory deficits while DG D5R KOs did not. Furthermore, D1R KOs but not D5R KOs, exhibited generalized fear between two similar but different contexts. Home cage c-Fos expression was relatively low in the DG of control mice, but significantly increased when mice were exposed to the training context or when they were fear-conditioned. In contrast, c-Fos expression was elevated in the DG of D1R KOs, in the home cage as compared to the ¬control mice, which did not further increase when the KOs were exposed to the training context or when they were fear-conditioned. DG D5R KOs did not exhibit any differences in c-Fos expression under these conditions, compared to the control mice. Overall our results suggest that DG D1Rs, but not D5Rs, contribute to the formation of distinction contextual representations of novel

environments by keeping DG baseline activity low in familiar contexts (i.e., the home cage environment) and promoting DG activity upon an exposure to novel contexts.

Disclosures: J. Sarinana: None. **T. Kitamura:** None. **P. Künzler:** None. **L. Sultzman:** None. **S. Tonegawa:** None.

Poster

559. Memory Consolidation and Reconsolidation: Neural Mechanisms

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Topic: F.02. Animal Cognition and Behavior

Support: NSERC Grant 400176

Title: Muscarinic receptor-mediated destabilization of object memories is blocked by proteasome inhibition

Authors: *M. L. STIVER, D. L. JACKLIN, N. VICIC, J. CARLIN, M. O'HARA, B. D. WINTERS
Dept. of Psychology and Collaborative Neurosci. Program, Univ. of Guelph, Guelph, ON, Canada

Abstract: Consolidated memories can become destabilized and susceptible to updating and modification upon retrieval, requiring a post-reactivation reconsolidation process in order to persist in long-term memory. Destabilization is most reliably prompted when novel information is present during memory reactivation. We hypothesized that the neurotransmitter acetylcholine (ACh) plays an important role in novelty-induced memory destabilization due to its established involvement in cognitive functions related to new learning. Accordingly, we investigated the effects of cholinergic manipulations in rats using an object recognition paradigm that requires reactivation novelty to destabilize object memories. The muscarinic receptor antagonist scopolamine, delivered pre-reactivation either systemically (0.3 mg/kg) or infused directly into the perirhinal cortex (PRh)(10 μg/μl), a brain region strongly implicated in object memory, blocked this novelty-induced memory destabilization. Conversely, systemic injection (0.1 mg/kg oxotremorine) or intra-PRh microinfusion (0.25 μg/μl carbachol) of muscarinic receptor agonists mimicked the destabilizing effect of novel information presented during reactivation. Furthermore, preliminary data suggest that the destabilization seen with muscarinic receptor agonism requires proteasome-regulated protein degradation within the PRh. The destabilizing

effects of carbachol delivered directly into the PRh were blocked with pre-reactivation microinfusion of the proteasome inhibitor β-lactone (32 ng/μl). The bidirectional cholinergic effects reported here suggest a crucial influence of ACh on memory destabilization and the updating functions of reconsolidation. This is a hitherto unappreciated mnemonic role for ACh with implications for its potential involvement in cognitive flexibility and the dynamic process of long-term memory storage. A connection between muscarinic receptor activation and proteasome activity provides a basis for investigation of the intracellular pathways involved in ACh-mediated object memory destabilization. This research has the potential to expand our understanding of how memories are returned to a labile state in order to be modified.

Disclosures: **M.L. Stiver:** None. **D.L. Jacklin:** None. **N. Vicic:** None. **J. Carlin:** None. **M. O'Hara:** None. **B.D. Winters:** None.

Poster

560. Hippocampal and Cortical Circuits II

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Topic: F.02. Animal Cognition and Behavior

Support: McKnight Brain Research Foundation

CIHR SIB171357

NIH Grant AG012609

Title: Aging is associated with altered intrinsic neural dynamics in the basolateral complex of the amygdala

Authors: *R. D. SAMSON[1,2], A. W. LESTER[1,2], P. LIPA[1,2], C. A. BARNES[1,2,3]
[1]Evelyn F. McKnight Brain Inst., [2]ARL Div. of Neural Systems, Memory & Aging, [3]Departments of Psychology, Neurol. and Neurosci., Univ. of Arizona, Tucson, AZ

Abstract: Emotion regulation is thought to be preserved, if not improved with aging. That is, older adults show a "positivity effect", as shown by a greater attention to and memory for positive items or events as compared to young adults. Currently, two competing theories argue that the positivity effect is as result of either a decline in amygdala function, or decreased prefrontal cortical control over the amygdala (Nashiro et al., 2012). To further our understanding of how aging impacts the function of the amygdala, we recorded from the amygdala of 19 rats

(10 young and 9 old) and have analyzed both intrinsic neuronal properties of its cells as well as their short time-scale interactions. In line with the idea that amygdala networks are altered with aging, we found neurons of this structure to be disinhibited in aged rats. Indeed, we found that the average firing frequency of neurons of the basolateral complex of the amygdala (BLA) in aged rats (4.6 Hz) to be greater than that of young rats (3.7 Hz). In contrast, neurons recorded in the cortical area ventral to the BLA did not differ across age. The age difference in firing frequency of BLA cells could in part be explained by the finding that a larger proportion of neurons fired at less than 2 Hz in young rats, whereas a greater proportion of cells fired above 5 Hz in aged rats. To further investigate the cellular properties of BLA neurons, spike waveform characteristics (peak-to- trough width, half-amplitude width) and spike dynamics (autocorrelation functions) were assessed; only the peak-to-trough width was different in aged rats and was found to be shorter (average over all BLA neurons). To further dissect whether the effects of aging are heterogeneous across different cell types, cross-correlation analysis will be performed to identify excitatory and inhibitory neuron types based on their short-latency interactions. Thus far, our results suggest that amygdala networks in rats do change with aging, and may actually be overactive. It remains to be determined, however, whether this change is detrimental to amygdala function or serves as a compensatory mechanism to maintain activity in downstream targets in aged rats.

Disclosures: R.D. Samson: None. **A.W. Lester:** None. **P. Lipa:** None. **C.A. Barnes:** None.

Poster

560. Hippocampal and Cortical Circuits II

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Topic: F.02. Animal Cognition and Behavior

Support: McKnight Brain Research Foundation

NIH Grant AG012609

Title: Behavior of normal aged rats mimics the pattern of task performance of rats with hippocampal lesions on a W-track continuous spatial alternation task

Authors: *A. R. UPRETY[1,2], A. I. ESPINOZA[1,2], A. RICHARDS[1,2], A. C. SMITH[1], C. A. BARNES[1,2,3]

[1]Evelyn F. McKnight Brain Inst., [2]ARL Div. of Neural Systems, Memory & Aging, [3]Departments of Psychology, Neurol. and Neurosci., Univ. Arizona, Tucson, AZ

Abstract: The aged population and life expectancy has been steadily increasing as significant progress has been made in medical treatment, making it vital to understand the physiological and cognitive changes that may occur during the aging process. The hippocampus is clearly an important contributor to the formation and storage of episodic memories that are known to be affected during aging in humans and in animal models of normal aging. Critical to uncovering the neurobiological mechanisms that underlie memory deficits in aging is the application of cognitive tests that have been validated through the use of lesion methodologies. A particularly interesting apparatus in this regard is the W-track continuous spatial alternation task developed by Kim and Frank (2009) in which hippocampal lesions were shown to disrupt animal performance. Among the error types that can be detected in the task include outbound, inbound or exaggerated side-to-side errors, as well as an overall learning rate of the task. Compared to non-lesion sham controls, hippocampal lesioned animals make many more errors, and are slower to improve on the task. We compared the performance of old rats on the W-track continuous spatial alternation task to young controls using a state-space algorithm to allow accurate characterization of learning performance (Smith et al., 2004). While both young and old rats take longer to learn the outbound compared to the inbound routes on the track, the old rats tend to learn the task rules for outbound and inbound choices more slowly than do the younger animals, and make several times more side-to-side errors than the younger animals. The pattern of results from older animals is reminiscent of the pattern of performance in hippocampus-lesioned rats, although less severe. These data suggest that this task will be sensitive to age-related neurobiological changes within the hippocampus and may provide a means to better understand defects in communication between the hippocampus and prefrontal cortical circuits.

Disclosures: A.R. Uprety: None. **A.I. Espinoza:** None. **A. Richards:** None. **A.C. Smith:** None. **C.A. Barnes:** None.

Poster

560. Hippocampal and Cortical Circuits II

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Topic: F.02. Animal Cognition and Behavior

Support: McKnight Brain Research Foundation

NIH Grant AG012609

Title: Age-related changes in high-frequency local field activity in the rodent hippocampus during ripple and inter-ripple periods

Authors: *J.-P. WIEGAND[1,2], D. T. GRAY[1,2], L. A. SCHIMANSKI[1,2], P. LIPA[1,2], C. A. BARNES[1,2,3,4], S. L. COWEN[1,2,3]
[1]Evelyn F. McKnight Brain Inst., [2]ARL Div. of Neural Systems, Memory & Aging, [3]Dept. of Psychology, [4]Departments of Neurol. and Neurosci., Univ. of Arizona, Tucson, AZ

Abstract: Ripples are brief (50-150 ms), high-frequency (80-200 Hz) oscillations in the local field potential in the hippocampus (Buzsaki et al., 1992). Given the hypothesized association between ripples, memory consolidation, and homeostatic plasticity, we explored whether there might be age-associated changes in ripple characteristics that contribute to age-related memory loss. To investigate this, local field potentials were recorded from CA1 during rest sessions before and after rats performed a place-dependent eyeblink conditioning task. High-frequency (80-500 Hz) oscillatory activity in the hippocampus of old (n=6) and young (n=6) male F344 rats during ripple events (50-150 ms in duration) and during inter-ripple periods was recorded. Two features of these local field potentials were found to differ between the young and old animals. Specifically, during hippocampal ripple periods the mean frequency in old rats was 6 Hz lower than that observed in young rats (174 Hz old, 180 Hz young, unpaired t-test, $p<0.05$). Additionally, during the inter-ripple periods, old rats showed greater local field power in high frequency bands (150 to 500 Hz; unpaired t-test, $p<0.05$). The increased power during inter-ripple periods in old rats reduced the signal-to-noise ratio between ripple and inter-ripple intervals compared to the young animals. The combination of reduced frequency during the ripple periods and the reduction in the contrast between ripple and inter-ripple periods in old animals could conceivably result in impaired spike timing and altered ripple-associated functions such as the transfer of information from the hippocampus to extra-hippocampal targets during memory consolidation.

Disclosures: J. Wiegand: None. **D.T. Gray:** None. **L.A. Schimanski:** None. **P. Lipa:** None. **C.A. Barnes:** None. **S.L. Cowen:** None.

Poster

560. Hippocampal and Cortical Circuits II

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Support: McKnight Brain Research Foundation

NIH Grant AG012609

Title: Age-associated changes in spike-timing of hippocampal principal cells and interneurons during ripple oscillations

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Abstract: Ripples are brief (50-150 msec), high-frequency (80-200 Hz) local-field oscillations generated in the hippocampus and associated with memory consolidation and homeostatic plasticity. We explored the hypothesis that normal aging alters the timing of the spiking response of individual neurons during the ripple oscillation. To investigate this hypothesis, we measured single-unit and high-frequency local-field activity in CA1 in aged (n=6) and young (n=6) rats during rest sessions that preceded and followed performance on a place-dependent eyeblink conditioning task. We observed that neurons with excitatory firing responses during the ripple oscillation exhibited notably similar mean firing responses in aged and young rats; however, putative interneurons that exhibited suppressed activity during ripple oscillations remained suppressed 40 msec longer in aged animals. Furthermore, although the mean firing response of principal cells during ripple oscillations was similar in aged and young animals, principal cells in aged rats responded to a narrower range of phases of the ripple oscillation (span of 21 degrees) relative to principal cells in young animals (span of 28 degrees). Taken together, these results suggest that aging results in alterations in the timing of spiking activity relative to local-field oscillations with the specific form of the alteration depending on the cell type. Such changes could disrupt ripple-associated processes in aged animals such as memory consolidation.

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Poster

560. Hippocampal and Cortical Circuits II

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

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Topic: F.02. Animal Cognition and Behavior

Support: McKnight Brain Research Foundation

Title: Non-linear optical imaging: A powerful new technique for acquiring high-resolution brain images and possible application for identifying cell types and neuronal activity

Authors: *M. A. MILLER[1], S. MEHRAVAR[2], D. T. GRAY[1,3], A. A. KOSHY[1,4,5], C. M. CABRAL[4], M. K. CHAWLA[1,3], K. Q. KIEU[2], C. A. BARNES[1,3,4,6,7], S. L. COWEN[1,3,6], N. N. PEYGHAMBARIAN[2]
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Abstract: Current imaging techniques for whole-brain visualization such as confocal or electron microscopy are costly, time consuming and labor intensive. Furthermore, using such techniques for the identification of cell types and markers of neuronal activity typically require thin (~40 uM) slices of brain tissue in conjunction with slow and costly techniques such as immunohistochemistry. Recent advances in non-linear optics by the College of Optical Sciences at the University of Arizona have allowed harmonic frequency and resonance changes by Raman amplification to be used to image thick (1 mm) brain slices (cortex and hippocampus) with sub-micron resolution. Ongoing analyses of these images suggest that the technique may also produce non-linear optical signatures corresponding to specific cell types (e.g., interneurons or glial cells). To investigate this possibility, we are combining this technique with standard immunohistochemistry and signal co-registration analysis to identify recently-activated neurons using the immediate early gene *Arc*. In addition, we are using other markers such as parvalbumin, GFAP and tyrosine hydroxylase to determine whether this imaging technique produces signatures that correspond to specific cell-types. If effective, this imaging method could open up the possibility of rapidly identifying patterns of neuronal activation and cell type distributions in entire brains (rodent and primate) at sub-cellular resolution.

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Poster

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Topic: F.02. Animal Cognition and Behavior

Support: McKnight Brain Research Foundation

UA BIO5 Institute

Title: Novel method for behavior-driven molecular and structural investigation in rodent whole brain

Authors: *M. K. CHAWLA[1,2], D. T. GRAY[1,2], A. E. COMRIE[1,2], B. K. BAGGETT[3], U. UTZINGER[3], C. A. BARNES[1,2,4]
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Abstract: Methods for identifying the regional distribution of neuronal activity within the brain during specific behaviors are labor intensive, time consuming and suffer from the necessity to prepare thin (20 micron) sections when *in situ* hybridization methods are employed. To circumvent these limitations, we are currently developing a novel method that allows behavior-induced activity markers to be imaged in intact brain tissue. This involves combining a recently developed whole brain clarification method (CLARITY; Chung et al., 2013) that provides the capacity to image deep into intact brain tissue, with a gene expression, cellular activity marker method (catFISH; Guzowski et al., 1999) that labels only those cells active in a given behavioral experience. Prior to using spatial exploration behavior we used a rat that was given maximal electro-convulsive shock treatment (that enables rapid transcription of immediate early genes) to maximize *Arc* expression. Current experiments are being done using exploratory behavior. Brain was quick frozen in isopentane that was cooled in a dry-ice ethanol slurry. The frozen brain was then placed in a 50 ml centrifuge tube containing hydrogel solution for post-fixation which allows cross linkage with formaldehyde in the presence of hydrogel monomers, covalently linking tissue elements to monomers that are then polymerized into a hydrogel mesh via thermal initiation. An electric field (25 volts) was applied across the sample in ionic detergent in an electrophoretic chamber which actively transports micelles through the tissue, which removes brain lipids, leaving the fine structure and cross linked biomolecules in place. The cleared brain tissue (~2 mm slab) was then processed for *in situ* hybridization using full length *Arc* digoxigenin tagged *cRNA* probe (Chawla et al., 2005) followed by CY3 TSA amplification. Tissue was counterstained with DAPI and submerged in 85% glycerol for imaging. Images were collected using an advanced intravital multi-photon microscope and a 3-D rendering of the collected images was performed. Cell nuclei with *Arc* transcription foci and cytoplasmic *Arc* were clearly visible up to ~300 μm deep in the tissue. These results provide evidence for the first time that we can combine *Arc in situ* hybridization with CLARITY methods in a slab of cleared brain. Future experiments will be carried out to increase signal penetration, imaging depth, and

eventually be applied in whole brains of animals that have undergone exploratory behaviors to visualize the activity of entire circuits.

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Poster

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Title: Selective changes in inhibitory networks of the medial temporal lobe correlate with behavioral and electrophysiological deficits in aged rhesus macaques

Authors: *D. T. GRAY[1,2], A. THOME[1,2], C. A. ERICKSON[1,2,4], P. LIPA[1,2], C. L. TAKAMATSU[1,2], A. E. COMRIE[1,2], C. A. BARNES[1,2,3]
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Abstract: Hippocampal neurons have been shown to encode features of episodic memory in multiple animal models. These representations rely on complex neural codes, and basic electrophysiological characteristics of neurons in the hippocampus have been suggested to underlie the ability of these networks to encode and retrieve information. In rodent models, activity of neurons in specific subregions of the hippocampus increases with age, and this hyperexcitability correlates with performance deficits in various medial temporal lobe-dependent behaviors. While the origins of this increased neural output remain poorly understood, several studies suggest that inhibitory circuits in select hippocampal regions change with age. No study to date has examined the relationship between the behavioral, electrophysiological, and molecular components of these deficits in the same animals. Furthermore, it is unknown whether similar age-related alterations occur in non-human primates. To address these questions, ensemble single unit electrophysiological recordings and counts of parvalbumin- and

somatostatin-expressing GABAergic interneurons were obtained from various subregions of the temporal lobe in three middle aged and two aged rhesus macaques which were behaviorally characterized in a delayed nonmatching-to-sample task. Age-related behavioral deficits were significantly correlated with both neural hyperexcitability and decreases in the number of somatostatin-containing interneurons in CA3, but not CA1 or the perirhinal cortex. Together, these findings are consistent with data suggesting that age-related declines in episodic memory are due, at least in part, to network dysfunction in specific regions of the medial temporal lobe which arise from losses in a specific class of interneuron. Furthermore, the selective impairment of CA3 suggests that this region may be a hub of neural dysfunction in the natural aging process that gives rise to behavioral deficits observed near the end of the lifespan of primates.

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Poster

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state of Arizona and ADHS

Title: PACAP expression is downregulated in aged nonhuman primates

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Abstract: Pituitary adenylate cyclase activating polypeptide (PACAP) is considered to be a potent neurotrophic and neuroprotective peptide. PACAP exists in two forms. The 38-amino acid form (PACAP-38) is the major form in the brain and peripheral organs such as pancreas and

respiratory tract. The shorter 27-amino acids form corresponds to the N-terminal of PACAP-38 (PACAP-27) that contains the biological active region and is preserved during evolution. Both forms of PACAP bind to and activate G protein-coupled receptors (PAC1, VPAC1, and VPAC2). We characterized changes in PACAP in human Alzheimer's disease cortex and in AD transgenic mice and found significant decreases in PACAP protein and mRNA levels (Han et al., 2014, Neurobiol Aging). As aging has been identified as the most significant risk factor for AD, it is essential to understand how PACAP changes during the aging process. Because postmortem brain tissue from young humans is rarely available, in this pilot study we examined PACAP expression in four nonhuman primates (*Macaca mulatta*) from mature adult to aged (12 to 30 years, equivalent to 36 to 90 human years). For the immunohistochemistry, the tissues were mounted on glass slides and treated with 3% H2O2 in methanol. After blocking with goat serum, the primary PACAP antibody was added to incubate in a humid chamber overnight at 4ºC. The slides were washed and incubated with a secondary antibody for 1 hour at room temperature, followed by DAB chromogen imaging. We used the size exclusion method in Image J software to select neuronal populations, measure the immunodensity and quantify the PACAP (+) cells. We analyzed the neurons from the visual cortex, the parietal cortex, the post-cingulate gyrus, the hippocampal formation, and the temporal cortex. PACAP expression was high in the temporal cortex particularly in younger monkeys, very low in parietal cortex and not detectable in hippocampus or postcingulate gyrus. While larger numbers of animals will be needed to confirm our preliminary results, it appears that PACAP expression in temporal cortex is inversely related to aging in nonhuman primates.

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Poster

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Support: NIH MH51570

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ONR M00014-10-1-0936

Title: Unique contributions of medial and lateral entorhinal cortices to episodic memory in a context-guided object-association task

Authors: *C. S. KEENE, J. H. BLADON, S. MCKENZIE, H. EICHENBAUM
Boston Univ., Boston, MA

Abstract: The entorhinal cortex is critically positioned to impact memory and the hippocampus. Specifically, evidence suggesting roles in spatial navigation and processing of objects in space suggest entorhinal cortex may be particularly situated to integrate items and space to impact episodic memory. However, it remains to be seen how medial entorhinal cortex (MEC) and lateral entorhinal cortex (LEC) contribute to learning and memory in the presence of task-relevant objects and contexts. To address this, single unit recordings from MEC or LEC were collected from rats trained in a task designed to distinguish neural activity associated with object, place, and spatial-contextual stimuli. In this task, rats were required to select one of two objects within each of two distinctive spatial contexts differing in multiple features. Specifically, object X was rewarded when it appeared within either of two positions within spatial context A, whereas object Y was rewarded when it appeared within either of two positions within spatial context B. In addition, rats foraged in an open field environment following testing in the context-guided object-association task in order to identify spatial properties of the cells (e.g., grid cells). Single unit and ensemble analysis of these recordings indicate a striking degree of overlap in function between MEC and LEC, while also highlighting complementary, but unique, contributions to episodic memory. In particular, preliminary analysis of ensemble recordings in LEC and MEC indicate that LEC population representations more strongly distinguish the item-place combinations within contexts, whereas MEC population representations more strongly distinguish item-position combinations within contexts. One interpretation of this finding is that LEC may be specialized for pattern separation of item-place information within a context, while MEC may be specialized for pattern separation across contexts. Additional analyses are underway to determine how these representations develop while learning novel object-context associations. Research supported by NIMH MH51570, MH094263 and ONR M00014-10-1-0936.

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Poster

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Topic: F.02. Animal Cognition and Behavior

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Title: Investigating the role of the medial entorhinal cortex in the hippocampal encoding of time and space for memory function

Authors: *N. T. ROBINSON[1], J. W. RUECKEMANN[1], J. B. PRIESTLEY[1], A. D. GARCIA[1], V. A. SMEGLIN[1], A. CHUONG[2], E. BOYDEN[2], H. B. EICHENBAUM[1]
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Abstract: The hippocampus and entorhinal cortex generate the neural activity patterns that enable the formation of spaitotemporally organized episodic memories. It is thought that the lateral entorhinal cortex provides object/event information that is integrated onto a spatiotemporal contextual framework within the hippocampus. The mechanisms that underlie the creation of hippocampal spatiotemporal firing fields and their characteristics remain largely unknown. We report that transient inactivation of the medial entorhinal cortex (MEC) during a mnemonic temporal delay or spatial experience disrupts the existing spatiotemporal firing fields. The red-shifted inhibitory opsin JAWS was expressed in MEC through adeno-associated viral injection. Rats were implanted stereotaxically with bilateral 3-fiber optic arrays in MEC and recording tetrodes were lowered into CA1. Animals performed an object-delay-response association task in which they ran on a treadmill during the delay period. On each trial, one of two distinct objects was encountered before each 8s treadmill run, after which the animal was required to match the object identity to either digging or withholding digging in order to receive reward. During the treadmill run, many CA1 neurons fired at successive brief moments, the sequence of "time cell" firings filled the entire delay, and time cell sequences differed depending on the object that began the trial. Transient MEC inactivation during the delay disrupted temporal firing fields in CA1 both during and following the inactivation period. Neurons with firing fields prior to the inactivation timing remained relatively stable. The disruption of time cell sequences was accompanied by a behavioral deficit implicating MEC activity and hippocampal time cell sequences in effective task performance. Inactivation of MEC during track running had a variety of time locked affects, with place fields being disrupted, remapping or created. Our results support a crucial role for MEC in the stable expression of spatiotemporal coding that supports memory function.

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Poster

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Support: NIH MH094263

MHMH51570

DMS-1042134

Title: Single cell and ensemble odor-place representations in the dentate gyrus and CA1 of the hippocampus

Authors: *L. M. RANGEL[1], P. D. RIVIÈRE[2], C. H. BUDLONG[3], I. HEIMBUCH[2], B. S. PORTER[4], H. EICHENBAUM[3]
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Abstract: The traditional hypothesized role of the dentate gyrus (DG) of the hippocampus is to provide distinct representations of events at the population level for similar experiences, thus providing an orthogonalizing code within the hippocampal circuit. We examined how the DG and CA1 subregions of the hippocampus represent multiple features of repeated experiences in its single cell and ensemble activity during an odor-place conditional learning paradigm. We found that in addition to exhibiting activity selective for distinct spatial locations irrespective of changing task conditions (place cells), DG and CA1 cells were also selective for individual odor cues that informed subsequent behavior in the task. The firing rates of these cells were often additionally modulated by odor-position conjunctions, or the combined presentation of a given odor cue in a specific location. We examined the extent to which DG and CA1 activity contained information for each of these parameters. Both DG and CA1 ensembles demonstrated decreases in the similarity of their firing rates across trial types in which there were different task conditions, such as changes in the spatial contexts in which odor cues were presented. The degree to which different task conditions elicit changes in DG versus CA1 ensembles was evaluated. Lastly, during cue presentation we observed large increases in the power of beta (15-30Hz) frequency oscillations during cue presentation. We then assessed differences in how beta coherent cells in DG and CA1 represent multi-dimensional task features compared to non-beta coherent cells. This study highlights the potential importance of dentate gyrus activity in the formation of odor-place associations.

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Poster

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Title: Bidirectional hippocampal-prefrontal interactions support context guided memory

Authors: *R. J. PLACE[1], A. FAROVIK[2], H. EICHENBAUM[3]
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Abstract: The medial prefrontal cortex (mPFC) and hippocampus (HPC) are both necessary for contextual-guided memory retrieval. Here we pursed predictions of a model of the flow of information between the mPFC and HPC consistent with known anatomical connectivity. According to this model, ventral CA1 (vHPC) may send meaningfully distinct contextual information directly to mPFC (Komorowski et al., 2013). Subsequently, mPFC may employ this contextual information to generate decision rules and apply them to bias object representations in perirhinal and lateral entorhinal cortex, and thence to the hippocampus, thereby guiding the retrieval of the contextually appropriate object associations in dorsal CA1 (dHPC) (Navawongse et al., 2013). To test this model, we recorded local field potentials (LFPs) from dHPC, vHPC, and mPFC simultaneously in rats as they learned to use different spatial contexts to guide the selection of conflicting object-reward associations. On each trial, rats first explored one of the contexts, then were allowed to sample two objects and select the one associated with reward in that context. Cross-correlations of systematically time-shifted theta-filtered amplitude-envelopes (Adhikari et al., 2010) were used to characterize the directionality of mPFC-HPC information flow during the separate context exploration and object sampling periods. During context exploration, dHPC theta leads that in vHPC, suggesting that vHPC integrates specific spatial information from dHPC. Then, vHPC theta leads that in mPFC, consistent with the prediction that vHPC sends contextual information to mPFC. During subsequent object sampling, the direction of information flow is reversed, with mPFC theta leading dHPC, consistent with the prediction that mPFC biases HPC representations in order to retrieve specific task-relevant

experiences that guide decision making. These results suggest bidirectional dynamics within a circuit of mPFC-HPC interactions that support context guided memory.

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Poster

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Keck Nakfi

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Title: Name-calling in the hippocampus (and beyond): Coming to terms with neuron types and properties

Authors: *D. J. HAMILTON, D. W. WHEELER, C. WHITE, C. L. REES, A. O. KOMENDANTOV, S. VENKADESH, G. A. ASCOLI
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Abstract: “What's in a name? That which we call a [neuron] by any other name would [fire] as [frequently].” Widely spread naming inconsistencies in neuroscience pose a vexing obstacle to effective communication within and across areas of expertise. Hippocampome.org is a web-accessible neuroinformatics resource that organizes existing data about essential properties of all known neuron types in the rodent hippocampal formation. In this context, a neuron type is identified by its (putative) neurotransmitter and the presence of axons and dendrites across the distinct layers of dentate gyrus, CA3, CA2, CA1, subiculum, and entorhinal cortex. Each type is further characterized by any available information on biomarker expression and electrophysiological features. Hippocampome.org links the evidence supporting assignment of a property to a type to relevant literature sources with direct pointers to text quotes or figures. Mining this knowledge from peer-reviewed reports reveals the troubling extent of terminological ambiguity and lack of definitions. Examples span simple cases of using multiple synonyms and

acronyms for the same molecular biomarkers (or other property) to more complex cases of neuronal naming. New publications use different terms without mapping them to previous terms. As a result, neurons of the same type are often assigned different names while neurons of different types are bestowed the same name. Furthermore, non-unique properties are frequently used as names, and several neuron types are not named at all. One of the benefits of Hippocampome.org is to recognize and clarify this nomenclature confusion by mapping all encountered synonyms and homonyms. Moreover, all key properties are defined in the Neuron Term Portal, a curated catalog of human- and machine-readable definitions also encompassing other brain regions (e.g. neocortex). Based on these resources, we developed a robust approach to provide each neuron type with an informative name and unique identifier.

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Poster

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Title: Firing pattern classification of hippocampal neurons

Authors: *A. O. KOMENDANTOV, D. W. WHEELER, C. L. REES, C. WHITE, D. J. HAMILTON, S. VENKADESH, G. A. ASCOLI
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Abstract: Collecting knowledge on the structural and physiological properties of hippocampal neurons is important for understanding the computational function of the hippocampus. The Hippocampome.org knowledge base identifies more than 100 neuron types in the rodent hippocampal formation (dentate gyrus, CA3, CA2, CA1, subiculum, and entorhinal cortex) based

on the locations of their axons and dendrites, putative excitatory/inhibitory outputs, and molecular marker expressions. For every hippocampal neuron type, electrophysiological data are also collected, quantified, and analyzed from available published experiments, including firing responses to depolarizing current injections. We have developed and implemented protocols to classify firing responses based on the analysis of transient and steady-state activity (e.g. spiking, bursting, stuttering, and silence) as well as their regularity/irregularity. Leveraging this automated classification approach, we characterized the set of all known firing responses exhibited by each neuron type, which defines its firing pattern phenotype. These results guide, constrain, and validate the design of simple neuronal models with different dynamics, which are suitable for large-scale network simulations. At the same time, this work reveals several statistical associations of firing responses, firing pattern phenotypes, and other electrophysiological properties with morphological features and molecular marker expression.

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Poster

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Title: Using Hippocampome.org to unravel the circuitry of the hippocampal connectome

Authors: *C. L. REES, D. W. WHEELER, C. WHITE, A. O. KOMENDANTOV, D. J. HAMILTON, S. VENKADESH, G. A. ASCOLI
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Abstract: Hippocampome.org is a knowledge base of neuron types in the hippocampal formation (dentate gyrus, CA3, CA2, CA1, subiculum, and entorhinal cortex), with evidence culled from the information-rich rodent literature. We collate morphological, molecular expression, and electrophysiological data to describe a set of more than 100 neuron types.

Amassing this information into a centralized resource yields numerous benefits, including a platform for data sharing, the opportunity to establish community-wide interpretation and nomenclature standards, and, notably, comprehensive knowledge of type-based circuitry. Information on network connectivity is compiled in two stages: by (1) literature mining for experimentally-verified data on synapses (or lack thereof) between types and (2) determining the binary (and later, weighted) potential for synapsing that arises from a spatial overlap of axons and dendrites of any two types. Our analyses of these connectomic data employ graph theory metrics, including degree distribution, betweenness centrality, rich club coefficient, and motif composition, to unveil the fundamental architectural principles of the network. Furthermore, using measures like absorption and driftness, we investigate the relationship between network topology and the dynamic behaviors of information flow, which may foster intuition on hippocampal operation. Consequently, by gathering data and performing analyses at the mesoscopic level of neuron types, we circumvent the time and data requirements of projects aimed at mapping the circuitry of every individual neuron. This strategy provides immediate traction toward the ultimate goal of obtaining and comprehending the full hippocampal connectome.

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Poster

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Title: Parameter optimization in a CA3 network model by evolutionary algorithms

Authors: *S. VENKADESH, A. O. KOMENDANTOV, D. W. WHEELER, C. L. REES, C. WHITE, D. J. HAMILTON, G. A. ASCOLI
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Abstract: Modeling biologically realistic large-scale spiking neural networks (SNN) is essential for understanding cognition and behavior. Izhikevich neurons are widely used in large-scale SNN simulations, due to their computational efficiency and ability to reproduce the variety of spiking patterns observed in real neurons. However, the parameters of a large-scale network of Izhikevich neurons create a massive search space, making it computationally challenging to constrain the model based on experimental evidence. We aim to build a real-scale model of the rodent hippocampus using information available from Hippocampome.org. To help constrain the model, we leverage the optimization capabilities of evolutionary algorithms. As an initial proof of principle, we designed a simple CA3 network model with pyramidal cells and two interneuron types (one perisomatic and one dendrite-targeting). First, we identify the ranges of Izhikevich model parameters that reproduce the firing patterns experimentally exhibited by these three neuron types. The quantitative features used to compare simulated and observed firing patterns include average inter-spike interval, spike frequency adaptation, and latency to first spike. Next, we optimize the network parameters, such as connection probabilities and strengths, to obtain desired network activity and target firing rate for each neuron type. While this pilot phase in its limited scope runs on a CPU architecture, our implementation is compatible with Graphical Processing Unit (GPU) based systems, thus allowing for scalability.

Disclosures: S. Venkadesh: None. **A.O. Komendantov:** None. **D.W. Wheeler:** None. **C.L. Rees:** None. **C. White:** None. **D.J. Hamilton:** None. **G.A. Ascoli:** None.

Poster

560. Hippocampal and Cortical Circuits II

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Keck Nakfi

ONR MURI N00014-10-1-0198

Title: Hippocampome.org: a knowledge base of neuron type properties for the rodent hippocampus

Authors: *D. W. WHEELER[1], C. WHITE[1], A. O. KOMENDANTOV[1], F. FAGHIHI[1], C. L. REES[1], D. J. HAMILTON[1], S. VENKADESH[1], B. HOLMES[2], G. A. ASCOLI[1]
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Abstract: Hippocampome.org is an open-access knowledge base of neuron types in the rodent hippocampal formation. Our extensive literature mining and curation protocols have identified over 100 neuron types to date based on their main neurotransmitter and neurite morphology. We focus on the presence or absence of axons and dendrites in twenty-six neuroanatomical parcels corresponding to widely agreed upon subregions (dentate gyrus, CA3, CA2, CA1, subiculum, and entorhinal cortex) and their respective layers (e.g. CA1 oriens, radiatum, etc.). We integrate these morphological characteristics with information on electrophysiological features and molecular marker expression. Each property is directly linked to the specific supporting evidence in the relevant publication. In addition, we assign each neuron type a unique identifier to unambiguously connect all references to their appropriate content. This collation of knowledge into a centralized online repository enables (1) data mining for novel correlations among types and properties, (2) inferring potential connectivity between neuron types, (3) characterizing neuronal dynamics for use in computational models, (4) analytics via user-friendly browsing and searching capabilities, and (5) inter-relating newly acquired neuronal information with the existing content of the knowledge base.

Disclosures: D.W. Wheeler: None. **C. White:** None. **A.O. Komendantov:** None. **F. Faghihi:** None. **C.L. Rees:** None. **D.J. Hamilton:** None. **S. Venkadesh:** None. **G.A. Ascoli:** None. **B. Holmes:** None.

Poster

560. Hippocampal and Cortical Circuits II

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Topic: F.02. Animal Cognition and Behavior

Support: Canadian Foundation for Innovation

NSERC

Krembil Foundation

Title: Memory-guided visual search reveals behavioral and autonomic markers of aging and Alzheimer's disease

Authors: *M. DRAGAN[1,4,2], T. K. LEONARD[4,3], A. M. LOZANO[5,6], M. MCANDREWS[5,6], K. NG[6], J. D. RYAN[5,7], D. F. TANG-WAI[5,6], J. WYNN[7,5], K. L. HOFFMAN[3,4,2]
[1]York Univ., Mississauga, ON, Canada; [2]Biol., [3]Psychology, York Univ., Toronto, ON, Canada; [4]Ctr. for Vision Res., Toronto, ON, Canada; [5]Univ. of Toronto, Toronto, ON, Canada; [6]Toronto Western Hosp., Toronto, ON, Canada; [7]Rotman Res. Institute, Baycrest., Toronto, ON, Canada

Abstract: Prodromal and typical Alzheimer's Disease (AD) is characterized by episodic memory deficits that are thought to correspond to disease progression in the hippocampal formation. AD therapies targeting hippocampal function may be best evaluated through the use of specific and selective hippocampal tasks. We used a hippocampal-dependent episodic target-in-scene detection task (Chau et al., 2011) to determine if task performance shows age-related decline, and if performance of the AD group is further impaired relative to an age-matched cohort. AD patients (N=7) were selected based on their participation in an ongoing clinical trial, using deep-brain stimulation targeting the fornix. To enroll in the clinical trial, patients must have scored between 12-24 on the Alzheimer's Disease Assessment Scale -cognitive test 11 (ADAS-cog11), and either 0.5 or 1 on the Clinical Dementia Rating. Testing described here was conducted before the treatment conditions were applied. Older adults (N=20) were divided into healthy (N=10; Montreal Cognitive Assessment: MoCA score >=26) and at-risk for developing mild cognitive impairment (N=7; MoCA score <=24); three participants fell in between these cutoffs. Younger Adults (N=17) were university students tested on the same task. Participants were asked to find items ('targets') that appeared/disappeared in alternation within flickering images of natural scenes. Previously, we observed that faster search times on repeated trials was due to explicit recall of the targets-in-scenes (Chau et al., 2011). . In this study, older adults from healthy, at-risk, and AD groups had longer search times and required more fixations compared to younger adults on repeated trials. Individuals with AD were significantly worse on both measures relative to any other population ($p<0.001$). Autonomic engagement was evaluated by measuring pupillary responses, revealing increasingly slower responses in aged and AD populations. Furthermore, memory effects on pupillary responses were only seen in younger adults and healthy older adults, not in the at-risk or AD groups. Our results suggest that this nonverbal memory-guided visual search task can be used to detect age-related impairments, to differentiate age-related impairments from those associated with AD, and to act as a possible early diagnostic aid, specifically by discriminating the pupillary responses of unimpaired groups from those at-risk for or affected by Alzheimer's Disease.

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Poster

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CFI

Ontario MRI ERA

Title: Sharp wave ripples in the primate hippocampus during visual exploration

Authors: *T. K. LEONARD[1], E. N. ESKANDAR[5], J. L. GERRARD[6], D. KAPING[7], J. M. MIKKILA[2], S. PATEL[8], T. WOMELSDORF[3], K. L. HOFFMAN[4]
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Abstract: Hippocampal neurons replay activity from previous experiences during sharp-wave ripples (SWRs). In rodents, SWRs have been associated with learning during quiescent and waking states. In many of these studies, analysis of waking activity is restricted to periods of immobility, but SWR-associated replay also occurs during exploration and it appears to play a

specific role in rapid learning through experience. Exploration in the task environments is operationalized as walking speed, leaving open the question of whether ambulatory movement is necessary and sufficient for generating eSWRs. In primates, SWRs have only been described during inactive states such as sleep, quiescent wakefulness, and under anesthesia ; their prevalence in waking exploration is unknown. Finally, the detection of SWRs during exploration is complicated by non-SWR activity that occurs in overlapping 'epsilon' oscillatory bands ('high gamma', 'high-frequency oscillations' or HFOs). Here, we describe SWRs in the macaque during visual exploratory behaviors that were found to be distinct from high gamma events or HFOs. During visual exploration eSWR events do occur in non-human primates. Furthermore, for visual search, eSWRs occurred more often during spatially constrained sequences of saccades than during periods of spatially diffuseness . These results suggest that in primates eSWRs may similarly contribute to learning from visual experiences.

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Poster

560. Hippocampal and Cortical Circuits II

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Topic: F.02. Animal Cognition and Behavior

Support: Alzheimer's Association

Alzheimer's Society of Canada

Krembil Foundation

NSERC DG

NSERC CREATE VSA

Title: State- and frequency-dependent changes in medial temporal lobe activity after Deep Brain Stimulation in the macaque

Authors: *A. GÓMEZ PALACIO SCHJETNAN, T. K. LEONARD, K. HOFFMAN
Dept. of Psychology, Ctr. for Vision Res., York Univ., Toronto, ON, Canada

Abstract: Deep brain stimulation (DBS) has been applied to various brain structures and pathways in an attempt to selectively alter neural activity associated with disease states. The optimal frequency of stimulation for specific targeted regions and the effectiveness of a given stimulation protocol across differing ongoing brain states is less well understood, but has widespread implications for DBS-treatment strategies. To determine the influence of different stimulation protocols, and their possible dependence on brain state, we tested frequency- and behavioral state-dependence of neural activity in the medial temporal lobe in response to stimulation of the anterior portions of the extended circuit, including the fornix and connected structures of the basal forebrain and anterior thalamus. We delivered unilateral, bipolar stimulation trains of 10 bursts, where each burst contained 8 biphasic pulses of 100μs duration at a rate of 500 Hz. Each train of 10 bursts was delivered at intervals corresponding to 1.25, 2.5, 4, 9, and 11 Hz. During stimulation experiments, a chronically-implanted multi-electrode/tetrode array sampled local field potentials from different sites in the medial temporal lobe of a rhesus macaque, while the animal was: 1) performing a goal-direct memory task 2) during rest periods where the animal sat in the darkened booth and occasionally slept. Our preliminary results show an interaction between inter-burst frequency and behavioral state. The higher frequencies, i.e. shorter inter-burst intervals, evoked stronger theta/alpha-band power than lower-frequency bursts. This effect occurred in resting states rather than during attentive, goal-seeking states. Our preliminary results suggest that 1) changes in stimulation patterning - here, burst frequency - lead to different responses in targeted pathways, and 2) behavioral state during stimulation can determine the effects of stimulation, presumably as a consequence of underlying brain states. These state-dependent modifications support further investigation of closed-loop, state-contingent stimulation including stimulation during sleep. Supported by: Alzheimer's Association, Alzheimer's Society of Canada, Krembil Foundation, NSERC DG, NSERC CREATE VSA (TKL).

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Poster

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Canadian Foundation for Innovation

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Alzheimer Society

Title: Theta-gamma cross-frequency coupling in the macaque hippocampus during learning and recollection

Authors: *J. M. MIKKILA[1,2,3], T. K. LEONARD[1,2,3], R. MONTEFUSCO-SIEGMUND[1,2], K. L. HOFFMAN[1,2,4]
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Abstract: One of the best-characterized examples of cross-frequency rhythmic interactions in the brain is the modulation of gamma oscillations by the theta rhythm in the hippocampus [1]. The functional correlates of this phenomenon, however, are only beginning to be understood. Hippocampal theta-gamma cross-frequency coupling (CFC) has been implicated in object-location associative learning in rats [2] and working memory in humans [3]. Here we ask whether or not theta-gamma coupling in primate hippocampus changes as a function of associative learning and recall. We conducted multi-electrode recordings in the hippocampus of two macaques as they performed a goal-directed visual search task to locate an object embedded in a natural scene [4]. Animals saw each scene twice, providing an opportunity to locate the object faster on the second trial, indicating memory for the location of the object in the scene. After identifying theta bouts (4-12 Hz) in the local field potentials, we calculated the modulation index [2] between theta phase and higher-frequency amplitudes. We observed bouts of theta (4-12 Hz) oscillatory activity during trial and after in the inter-trial interval. Identified bouts during search lasted on average 1.74 seconds (SD +/- 1.52 s) ; when average search time per trial was 15.53 seconds. The strength of the phase-amplitude coupling between theta and gamma (30-90 Hz) oscillations and the power of the coupled frequencies differed by memory condition. Repeated trials in which the object location was forgotten, and novel trials that lead to subsequent forgetting show stronger coupling than their 'remembered-object' counterparts. In line with previous reports [5], there was more theta power in novel trials that were subsequently remembered. This demonstrates that cross-frequency effects may not be directly related to the strength of the constituent oscillations. Prima facie, these results may be counterintuitive: stronger coupling is associated with worse performance; however, other tasks describing CFC effects include well-learned examples whereby early in learning the coupling is weakest. Further

examination of various behavioral correlates and of extended learning are required to better understand the significance of the cross-frequency effects we observed. [1] Lisman J & Jensen O. (2013). Neuron 77: 1002-16. [2] Tort A, Komorowski RW, Mannsf J, Kopell N, Eichenbaume H. (2009). PNAS 106: 20942-7 [3] Axmacher N, Henseler M, Jensen O, Weinreich I, Elger CE, Fell J. (2010). PNAS 107: 3228-33. [4] Chau, V.L., Murphy, E.F., Rosenbaum, R.S., Ryan, J.D. & Hoffman, K.L. (2011). Front. Behav. Neurosci. 5 [5] Jutras M & Buffalo E. (2014) NeuroImage 85: 694–701

Disclosures: J.M. Mikkila: None. **T.K. Leonard:** None. **R. Montefusco-Siegmund:** None. **K.L. Hoffman:** None.

Poster

561. Invertebrate Learning and Memory II

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Topic: F.02. Animal Cognition and Behavior

Support: R15MH090998-01

Title: Characterization of the rapid transcriptional response to long-term sensitization training in *Aplysia* californica

Authors: S. HERDEGEN[1], G. HOLMES[1], *R. CALIN-JAGEMAN[2], I. E. CALIN-JAGEMAN[1]
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Abstract: We used a custom-designed microarray and qPCR to characterize the rapid transcriptional response to long-term sensitization training in the marine mollusk *Aplysia californica*. *Aplysia* were exposed to repeated noxious shocks to one side of the body (4 ten-second shocks at 90mA, 30 min ISI), a procedure known to induce a transcription-dependent and long-lasting increase in reflex responsiveness that is restricted to the side of training. One hour after training, pleural ganglia from the trained and untrained sides of the body were harvested; these ganglia contain the sensory nociceptors which help mediate the expression of long-term sensitization memory. Microarray analysis on 8 animals suggests that long-term sensitization training strongly and rapidly regulates at least 102 transcripts. We used qPCR to test a subset of these transcripts and found that 86% were confirmed in the same samples, and 83% of these were again confirmed in an independent sample (n = 9 animals). Thus, our new microarray design shows very strong predictive and convergent validity for analyzing the transcriptional

correlates of memory in *Aplysia*. Fully validated transcripts include some previously identified as regulated in this paradigm (apC/EBP and apEgr) but also include novel findings. Specifically, we show that long-term sensitization training rapidly up-regulates the expression of transcripts which seem to encode a C/EBP gamma, a glycine transporter, and a vacuolar protein-sorting-associated protein.

Disclosures: S. Herdegen: None. **R. Calin-Jageman:** None. **G. Holmes:** None. **I.E. Calin-Jageman:** None.

Poster

561. Invertebrate Learning and Memory II

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Topic: F.02. Animal Cognition and Behavior

Support: NIMH R01 MH 041083

NIMH F31 MH 100889-01A1

Title: Distinct growth factor families engage spatially and temporally unique molecular cascades during two-trial long-term memory formation in *Aplysia*

Authors: *A. M. KOPEC, T. J. CAREW
Ctr. for Neural Sci., New York Univ., New York, NY

Abstract: In adult animals growth factors (GFs) signaling via TrkB and TGFβr-II receptors are required for long-term memory (LTM). However, the unique contribution of each GF family to memory formation is unknown. *Aplysia* californica is a powerful model system for examining molecular cascades during LTM formation. In *Aplysia*, repeated sensitizing stimuli (e.g. tail shocks) induce MAPK activation (P-MAPK) and gene expression in tail sensory neurons. Both P-MAPK and gene expression are required for LTM formation and are downstream of TrkB and TGFβr-II signaling. Thus, assaying P-MAPK and gene expression as a proxy for LTM formation provides the opportunity to assess the regulation of signaling pathways common to both GF families. We previously analyzed trial-specific GF regulation of P-MAPK in two-trial LTM analog training using tail nerve shocks (TNSs; Philips et al 2007; 2013). We found that Trial 1 induces synaptic TrkB signaling for an early phase of P-MAPK, and Trial 2 induces somatic TGFβr-II signaling for a late phase of P-MAPK (Kopec & Carew, 2013a). Here we examined the

GF-dependency of genes regulated during learning in *Aplysia*: ApC/EBP, ApUCH, and ApKHC1. We found that both Trial 1 and Trial 2 induce ApC/EBP expression (during the training interval and after training, respectively), while ApUCH and ApKHC1 were not modulated. Next, TNSs were given in the presence of either a TrkB-Fc or a TGFβr-II-Fc chimera, which sequesters endogenous TrkB or TGFβr-II ligands, respectively. We found that Trial 1 TrkB signaling is required for Trial 1-dependent ApC/EBP expression, and Trial 2 TGFβr-II signaling is required for Trial 2-dependent ApC/EBP expression. Furthermore, blocking Trial 1 TrkB signaling disrupts Trial 2-dependent ApC/EBP expression, suggesting that Trial 2 TGFβr-II signaling interacts with Trial 1 TrkB signaling to prolong ApC/EBP expression. Taken with our earlier data, these results show that both TrkB and TGFβr-II signaling are engaged during two-trial LTM to regulate MAPK activation and transcription, but with unique spatial, temporal, and mechanistic profiles. These data are consistent with the view (Kopec and Carew, 2013b) that signaling by individual GFs should be considered as a component of a complex and interactive molecular network involving multiple GF families.

Disclosures: A.M. Kopec: None. **T.J. Carew:** None.

Poster

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Program#/Poster#: 561.03/UU71

Topic: F.02. Animal Cognition and Behavior

Title: Taste avoidance conditioning with electrical shock as unconditional stimulus in Lymnaea stagnalis

Authors: *M. SAKAKIBARA, S. TAKIGAMI
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Abstract: New taste avoidance conditioning paradigm has developed with an electrical shock as an unconditional stimuli (US) paired with sucrose application as a conditional stimuli (CS). Our previous study demonstrated that Lymnaea stagnalis can be conditioned with 20 paired presentations of sucrose as CS and gentle tactile stimulus as US. In this study we examined whether snails are capable to be conditioned with 100 mM sucrose application as CS paired with brief electrical stimulus (1000 V, 80 micro-A, 0.2 s) application in place of mechanical tapping to animal's head as US. Snails were evaluated with the feeding score, i.e., the number of mouth openings per minute in response to sole CS application examined at 10 min and/or 24 h

following the conditioning paradigm. We defined that a 10 min post-test characterized as a short term memory (STM) whereas a 24 h post-test characterized as a long term memory (LTM). In this study we evaluated the number of CS-US pairings from 5 to 20 whether snails acquired STM/LTM. As a result animals showed significant reduced feeding scores to sucrose application after 15 paired presentations of CS-US pairings leading to both STM and LTM, furthermore their memory persisted for at last one week. We next made intracellular recordings from right pedal dorsal 11 (RPeD11) neuron, which is known to process invasive stimulus such as mechanical tactile and/or chemical stimulus to control the whole-body-withdrawal response which is the only available escape behavior to this animal. Excitatory post synaptic potential was recorded in response to US application thus the electrical voltage stimulus was recognized as the invasive stimulus as same as the mechanical tactile stimulus we previously employed as US. With this new methodology we do not require the skill for the experimenter to give a tactile stimulus.

Disclosures: M. Sakakibara: None. **S. Takigami:** None.

Poster

561. Invertebrate Learning and Memory II

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Title: Nitric oxide synthase is involved in maintenance but not in induction of activity-dependent LTP in the vertical lobe of the octopus

Authors: *A. TURCHETTI-MAIA[1,2], T. SHOMRAT[1,3], B. HOCHNER[1,4]
[1]Dept. Neurobiology, Silberman Inst. of Life Sciences, The Hebrew Univ., Jerusalem, Israel; [2]The Edmond and Lily Safra Ctr. for Brain Sci., Jerusalem, Israel; [3]The Ruppin Academic

Center, Sch. of Marine Sci., Michmoret, Israel; [4]Interdisciplinary Ctr. for Neural Computation, Jerusalem, Israel

Abstract: The vertical lobe (VL) of Octopus vulgaris, an area known to be involved in learning and memory, shows a robust activity-dependent LTP. This LTP, which occurs at the 'fan-out' glutamatergic synaptic input to the VL, seems to be non-NMDA dependent and presynaptically expressed; therefore it seems that the associative mechanism that mediates the Hebbian property of octopus LTP has not converged to vertebrate like mechanisms. Nitric oxide (NO), produced by nitric oxide synthase (NOS), has been associated with a variety of learning modalities in vertebrates and invertebrates. Here we examined the effect of NOS inhibitors on plasticity in slices of octopus VL. We observed that NOS inhibitors showed an inhibitory effect on LTP expression without affecting LTP induction by high frequency (50Hz) stimulation, as full LTP could be induced in the presence of NOS inhibitors (revealed after drugs washout). Once NOS inhibitors were applied to already induced LTP, they inhibited the synaptic field potential (fPSP), but did not depotentiate it. It is likely that NOS inhibitors negate the presynaptic effect of NO, as they both reduced the fPSP amplitude and reversed the effect of LTP on twin pulse facilitation to pre-tetanization level. In contrast, slowly developed LTP, induced by 0.1Hz stimulation, was attenuated by NOS inhibitors possibly because of an indirect effect via blockage of NO induced synaptic facilitation. We are checking the possibility that the tonic activation of NOS is mediated by PKC or PKD, because phorbol ester (5μM PDBu) activates full LTP even when synaptic transmission is blocked in zero Ca++ EGTA solution and/or AMPA-like glutamatergic transmission is also blocked. These results suggest that LTP expression occurs through synaptic facilitation induced by elevation of NO tonic concentration, which is likely to be generated by activity dependent NOS long-term activation. We conclude that molluscan NO/NOS system is conserved in the octopus VL, though the mechanism of involvement is adapted to mediate LTP maintenance rather than the conservative mechanism of LTP induction.

Disclosures: **A. Turchetti-Maia:** None. **T. Shomrat:** None. **B. Hochner:** None.

Poster

561. Invertebrate Learning and Memory II

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Topic: F.02. Animal Cognition and Behavior

Support: NSERC

Title: The role of lrn-2/scd-2 in sensory integration and learning in Caenorhabditis elegans

Authors: *G. S. WOLFE[1], D. VAN DER KOOY[2]
[1]Inst. of Med. Sci., [2]Mol. Genet., Univ. of Toronto, Toronto, ON, Canada

Abstract: The cellular and molecular mechanisms of learning and memory can be studied in detail by taking advantage of the nematode, Caenorhabditis elegans. With a fully mapped connectome and genetic tractability, *C. elegans* has a wide variety of behaviours controlled by comparatively few neurons. We have characterized an EMS derived mutant strain, lrn-2, which shows deficits in both sensory integration and a wide variety of associative learning paradigms. Having deficits in these two established processes suggests that this gene is either a key component of separate sensory integration and associative learning pathways, or it is evidence that sensory integration and learning are the same psychological process. Snip-SNP mapping and genome sequencing suggested that the locus of the lrn-2 mutation was the gene scd-2, which is homologous to human anaplastic lymphoma kinase. This was supported by lrn-2 and scd-2 failing to complement each other after a cross, and by a wild type scd-2 containing fosmid successfully rescuing associative learning in a lrn-2 background. The primary test of associative learning used in this project involved learning about pathogenic bacteria. The N2 control strain learns to associate the pathogenicity of PA14, a strain of P. aeruginosa, with the odor of the bacteria after 4 hour exposure. This results in N2 worms leaving the PA14, and migrating to a safe E. coli lawn on the other side of the plate. The lrn-2 mutants do not learn this association and remain on the pathogenic bacteria. The sensory integration deficit is demonstrated by the mutant's inability to integrate two cues by crossing an aversive copper barrier to reach an attractive diacetyl odorant. Both learning and sensory integration involve processing of multiple sensory cues leading to altered behavioral output. While sensory integration does not require these changes to persist, learned behaviors do, as memories. Since lrn-2/scd-2 plays a role in both sensory integration and associative learning, they may be part of the same psychological process. The gene fsn-1 has been shown to have an upstream suppressive effect on scd-2 sensory integration; however, we have found that in an associative learning assay, fsn-1 may have act differently, downstream of scd-2. Furthermore, preliminary tests suggest that there is no measurable learned memory in the sensory integration assay, supporting the idea that they may be separate processes with some shared machinery. In order to further investigate the relationship between these processes, cell specific rescues of scd-2 in a mutant background will be performed to find where it must be expressed for both sensory integration as well as learning and memory.

Disclosures: G.S. Wolfe: None. **D. van der Kooy:** None.

Poster

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Topic: F.02. Animal Cognition and Behavior

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Title: BK SLO-1 channel mediates specific aspects of locomotion, posture, and habituation learning on and off alcohol in *C. elegans*

Authors: C. H. C. LIN[1], S. KHAN[1], D. HSIAO[1], A. HUNG[1], S. SA[1], B. MENON[1], F. PSOLTANI[1], D. CHAIM[1], *C. H. RANKIN[2]
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Abstract: Alcohol intoxication impairs locomotion, posture, and learning, but the underlying mechanism is not well understood. We use the model system Caenorhabditis elegans to investigate the mechanisms by which alcohol affects behavior. Previous research on *C. elegans* showed that a calcium-activated, voltage-gated potassium (BK) channel (SLO-1) plays a critical role in alcohol-induced behavior (Davies et al, 2003). In this study we investigated the role BK channels in different aspects of locomotion, posture, and learning using a high throughput tracking system "Multi-Worm Tracker". To investigate the role of SLO-1 on locomotion and body posture, we tracked worms on alcohol (400mM ethanol (v/v) plates; ~ 0.08-0.1% BAC) or off alcohol (0mM) for 60mins. Exposure to ethanol led to a depression is body curve (posture) in wild-type worms compared to unexposed worms; mutants missing a functional slo-1 gene had the same body curve (posture) on and off alcohol. Worms with a mutation missing the SLO-1 calcium-sensing region showed less body curve depression on alcohol compared to wildtype on alcohol. This suggests alcohol's depressive effect on body curve/posture requires a functional SLO-1 channel and missing the calcium-sensing region reduces the effect. To study the role of BK channel in learning under normal and intoxicated conditions, we focused on a non-associative type of learning, habituation. We induced habituation by giving repeated mechanical stimuli at 10s, 15s, 30s, or 60s interstimulus- intervals (ISI); habituation was analyzed as decrements in response probability, magnitude or speed. Unexposed slo-1 mutants habituated slower than wild-type worms for response speed, suggesting a role of SLO-1 in slowing down speed responses to repeated stimuli. We also found that SLO-1 became increasingly more important role as stimulus ISIs increased specifically in habituation of response probability. Wildtype worms on alcohol habituated faster in response probability (unrelated to fatigue), and this alcohol habituation phenotype was not present in slo-1 mutants. Our genetic rescue studies suggest that SLO-1 in neurons was responsible for the alcohol habituation phenotype. Interestingly, rescues only in muscles showed an opposite phenotype to alcohol habituation.

Disclosures: **C.H.C. Lin:** None. **C.H. Rankin:** None. **S. Khan:** None. **D. Hsiao:** None. **A. Hung:** None. **B. Menon:** None. **F. Psoltani:** None. **S. Sa:** None. **D. Chaim:** None.

Poster

561. Invertebrate Learning and Memory II

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 561.07/UU75

Topic: F.02. Animal Cognition and Behavior

Title: A single gustatory sensory neuron of caenorhabditis elegans generates memory-dependent behaviors in nacl chemotaxis

Authors: *L. WANG[1], M. TOMIOKA[2], H. KUNITOMO[1], Y. IINO[1,2]
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Abstract: Animals show various behaviors in response to environmental conditions, which are often plastic according to previous experiences. *C. elegans*, sensing chemicals with a limited number of sensory neurons, is an ideal model for analyzing the role of each neuron in innate and learned behaviors. ASE neurons (ASE-Left neuron and ASE-Right neuron) play major roles for salt chemotaxis but have lateralized functions: ASER neuron is stimulated by decreases in NaCl concentration and ASEL neuron is stimulated by increases in NaCl concentration. Until now, our lab has focused on the roles of ASER and its signaling pathways in salt chemotaxis and other behaviors, but whether and how ASEL regulates the behaviors in salt chemotaxis remains unknown. In this study, chemotaxis assays and optogenetics combined with behavioral quantification with worm tracking system were used to investigate the ASEL's roles in worm behaviors. By using transgenic worms in which channelrhodopsin (ChR2) was expressed in the ASEL neuron, the neuron was activated by blue-light illumination and the behavioral response was recorded. The result indicated that worms showed behavioral responses to ASEL activation after cultivation with NaCl, but no or small responses after cultivation in NO NaCl conditions. After cultivation in the presence of NaCl, the worms' turning rate decreased during ASEL stimulation and increased immediately after termination of ASEL stimulation. The behavior of ASER-ablated mutants was tested in chemotaxis assays. The chemotaxis index was almost zero for worms that had been cultured in NO NaCl conditions, which was consistent with the result of optogenetics and was possibly because activation of ASEL does not generate behavioral responses. On the other hand, when worms were cultured in the presence of NaCl, worms migrated to higher concentrations, which was also identified with above optogenetics' result and

our lab's former data, and it was possibly explained by the behavior that worms cultivated with NaCl went forward when NaCl concentration increased, but turned when NaCl concentration decreased, both contributing to migration to higher concentrations.

Disclosures: L. Wang: None. **M. Tomioka:** None. **H. Kunitomo:** None. **Y. Iino:** None.

Poster

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Topic: F.02. Animal Cognition and Behavior

Support: NIH Cognitive Aging RO1 AG034446

Keck Foundation

Title: Cellular and molecular mechanisms of short-term associative memory in *C. elegans*

Authors: *A. SYLVAIN, G. STEIN, M. RAHIMI, C. T. MURPHY
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Abstract: An important challenge is to understand how associations are formed and maintained in order to alter behavioral responses. Acquiring an in-depth understanding of memory formation on both the molecular and circuit level is important not only to gain an understanding of normal brain function, but can also lead to an understanding of disease states such as age-related cognitive decline and Alzheimer's disease. The simplicity of the *C. elegans* nervous system makes it a practical model organism to study memory encoding. Our lab has developed a short-term associative memory (STAM) assay in *C. elegans*, in which worms learn to associate food with an odor and can remember this association for over one hour. Here we use this assay to identify regulators of *C. elegans* short-term memory processes, by testing genetic mutants and assessing deficits in associative memory formation. We find that, as in higher organisms, *C. elegans* STAM requires calcium and cAMP signaling and is dependent on translation, but independent of transcription and the transcription factor CREB. Using GCaMP imaging, we found that STAM conditioning induces increased odor-evoked Ca^{2+} responses in the sensory neuron AWC^{on}, and that the number of animals responsive to the odor decreases with increased time post-conditioning. Our tests suggest that several neurons post-synaptic to the sensory neuron AWC^{on} are not required for normal STAM. Our data confirm that STAM in *C. elegans* is

a distinct behavioral paradigm controlled by a small neural circuit that requires canonical memory pathways. Study of short-term associative memory in *C. elegans* could lead to the rapid discovery of novel conserved short-term memory regulators.

Disclosures: **A. Sylvain:** None. **G. Stein:** None. **M. Rahimi:** None. **C.T. Murphy:** None.

Poster

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Topic: F.02. Animal Cognition and Behavior

Support: NIH R01 AG034446

Glenn Foundation

Title: Transcriptional profiling of dissociated adult *C. elegans* neurons reveals short-term memory components of Insulin/FOXO signaling

Authors: *R. AREY, R. KALETSKY, A. WILLIAMS, V. LAKHINA, J. LANDIS, C. MURPHY
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Abstract: The ability to form associative memories is one of the most important functions of an organism's nervous system. However, the molecular mechanisms underlying learning and associative memory formation have yet to be fully elucidated. Identifying molecules involved in these processes is important not only to gain an understanding of normal brain function, but can also lead to an understanding of disease states such as age-related cognitive decline and Alzheimer's disease. We have developed a novel positive olfactory assay to study learning and memory in the nematode *C. elegans* (Kauffman et al., 2010), providing the opportunity to identify molecules that are most essential for learning and memory. We previously found that worms with a mutant form of the insulin/IGF-1 receptor daf-2, which have an extended lifespan, have increased short-term and long term memory (Kauffman et al., 2010). The molecular components that contribute to daf-2's threefold increased short-term memory are unknown, except that it is dependent on the FOXO transcription factor daf-16. We recently developed a technique for lysis of adult worms followed by Fluorescence-activated cell sorting (FACS) to isolate individual tissues, such as neurons. Using this technique in daf-2 and daf-2;daf-16

mutants, we have identified Insulin/IGF-1-like signaling (IIS) neuronal target genes. We have used RNAi to test whether the IIS-regulated neuronal genes enable daf-2's enhanced short-term associative memory (STAM). We have found that some of these neuron-specific IIS targets are required for daf-2's extended STAM. Ongoing studies are examining the roles of these IIS targets in wild-type short term memory and how daf-2 affects the expression of these genes. These studies will allow for the identification of genes that were not previously known to play a role in memory function, and may uncover novel genes involved in associative memory formation in higher organisms.

Disclosures: R. Arey: None. **R. Kaletsky:** None. **A. Williams:** None. **V. Lakhina:** None. **J. Landis:** None. **C. Murphy:** None.

Poster

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Topic: F.02. Animal Cognition and Behavior

Support: BBSRC BB/KO18515/1

Title: Interneuronal mechanism for Tinbergen's hierarchical model of behavioral choice

Authors: M. CROSSLEY, Z. PIRGER, S. NASKAR, Z. LASZLO, G. KEMENES, M. O'SHEA, *P. R. BENJAMIN, I. KEMENES
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Abstract: Recent studies of behavioral choice support the notion that the decision to carry out one behavior rather than another depends on the reconfiguration of shared interneuronal networks. We investigated another decision-making strategy, derived from the classical ethological literature which proposes that behavioral choice depends on competition between autonomous networks. According to this model, behavioral choice depends on inhibitory interactions between incompatible hierarchically-organized behaviors. We provide evidence for this by investigating the interneuronal mechanisms mediating behavioral choice between two autonomous circuits that underlie whole-body withdrawal and feeding in the pond snail Lymnaea,. Whole-body withdrawal is a defensive reflex that is initiated by tactile contact with predators. As predicted by the hierarchical model, tactile stimuli that evoke whole-body withdrawal responses also inhibit on-going feeding, even in the presence of feeding stimulus. By

recording neurons from the feeding and withdrawal networks we found no direct synaptic connections between the interneuronal and motoneuronal elements that generate the two behaviors. Instead, we discovered that behavioral choice depends on the interaction between two unique types of interneurons with asymmetrical synaptic connectivity that allows withdrawal to override feeding. One type of interneuron, the PlB (Pleuro-Buccal), is an extrinsic modulatory neuron of the feeding network that completely inhibits feeding when excited by touch-induced monosynaptic input from the second type of interneuron, PeD12 (Pedal-Dorsal 12). PeD12 plays a critical role in behavioral choice by providing a synaptic pathway joining the two behavioral networks that underlies the competitive dominance of whole-body withdrawal over feeding.

Disclosures: **M. Crossley:** None. **Z. Pirger:** None. **S. Naskar:** None. **Z. Laszlo:** None. **G. Kemenes:** None. **M. O'Shea:** None. **P.R. Benjamin:** None. **I. Kemenes:** None.

Poster

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Program#/Poster#: 561.11/UU79

Topic: F.02. Animal Cognition and Behavior

Support: BBSRC BB/KO18515/1

Title: Memory lapses are a general feature of consolidation after both reward and aversive classical conditioning

Authors: F. LORENZETTI, M. CROSSLEY, S. NASKAR, M. O'SHEA, P. R. BENJAMIN, *I. KEMENES
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Abstract: Memory consolidation is generally conceived as a process whereby new information sequentially moves to successively longer-term stores. In invertebrates and vertebrates, including humans, there are short periods of memory lapses during consolidation. Formerly these have been seen simply as moments of vulnerability in memory formation. Our recent work on the snail Lymnaea however suggests that they are adaptive, allowing consolidation to be regulated so that acquisition and storage are effectively modified by new information after initial learning. Recently, we found that one-trial appetitive classical conditioning using sucrose as the unconditioned stimulus (US) and amyl acetate (AA) as the conditioned stimulus (CS), was accompanied by memory lapses at 30 min and 2h after acquisition. Now we show that lapses at

similar time points also occur following either reward training with a novel appetitive stimulus (pairing AA with serine) or aversive conditioning using the same CS paired with quinine. This suggests that memory lapses could be a general feature of consolidation. The vulnerability of memory consolidation to disturbances during lapses was tested by application of either aversive or appetitive stimuli following classical reward conditioning (pairing amyl acetate with sucrose). Both quinine and serine were found to disrupt memory formation, but only when applied as a disturbance during periods of lapses. Interestingly, memory formation following appetitive classical conditioning can be disrupted by the presentation of an alternative appetitive stimulus at a lapse point. Lapses in memory formation have now been demonstrated at similar time points after conditioning with the aversive US quinine and the appetitive US serine, as well as sucrose. Thus, these lapses in memory formation appear to be a general feature of consolidation after classical conditioning. There is also generalization among the stimuli that can act as a disturbance when applied at a lapse point, as quinine, serine, and mechanical stimulation all successfully disrupt memory formation.

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Poster

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Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant R01 NS019895

Title: Doxorubicin attenuates serotonin-induced long-term synaptic facilitation by phosphorylation of p38 MAPK in *Aplysia*

Authors: ***R.-Y. LIU**, Y. ZHANG, B. L. COUGHLIN, L. J. CLEARY, J. H. BYRNE
Univ. Texas Med. Sch. Houston, HOUSTON, TX

Abstract: A substantial proportion of cancer patients report significant impairments in cognitive function after chemotherapy, often referred to as "chemobrain." Although the major chemotherapeutic effects of doxorubicin (DOX) are due to oxidative damage of cellular membranes and interference with DNA replication and RNA synthesis, DOX also affects multiple isoforms of MAPK, such as extracellular signal-regulated kinase (ERK) and p38

mitogen-activated protein kinase (p38 MAPK), two kinases important for long-term memory (LTM) formation. Consequently, we examined the hypothesis that a component of chemobrain may be due to altered kinase signaling in memory circuits. In the adjacent poster, Zhang et al. demonstrated that DOX can activate both ERK and p38 MAPK in *Aplysia* sensory neurons. In this study, we evaluated the impact of DOX on long-term enhanced excitability (LTEE) and long-term synaptic facilitation (LTF), both of which are cellular correlates of LTM. In isolated *Aplysia* sensory neurons, DOX alone produced a significant increase in cell excitability 24 h after treatment ($p < 0.05$), as did 5-HT ($p < 0.05$). The two effects were not summative, however, as 5-HT-induced LTEE appeared to be attenuated by DOX, although this effect was not statistically significant. In sensorimotor neuron co-cultures, DOX alone appeared to produce a small synaptic facilitation, but this effect was not statistically significant. 5-HT-induced LTF was significantly reduced by DOX ($p < 0.05$). To determine whether activation of p38 MAPK was responsible for the impairment of 5-HT-induced LTF by DOX, we assessed LTF in the presence of SB203580 (SB), a specific inhibitor of p38 MAPK. Interestingly, the increase in magnitude of EPSPs produced in the 5-HT + DOX + SB group was significantly greater than in the 5-HT + DOX group ($p < 0.001$), suggesting that inhibition of p38 MAPK rescued the deficit in LTF induced by DOX. Moreover, no significant difference was observed in LTF between the 5-HT and 5-HT + DOX + SB groups, suggesting that the rescue is complete. Therefore, our results suggest that the deleterious effects of DOX on memory are due, at least in part, to activation of the p38 MAPK pathway.

Disclosures: **R. Liu:** None. **Y. Zhang:** None. **B.L. Coughlin:** None. **L.J. Cleary:** None. **J.H. Byrne:** None.

Poster

561. Invertebrate Learning and Memory II

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Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant R01 NS019895

Title: Doxorubicin enhances phosphorylation of ERK and p38 MAPK in *Aplysia* sensory

Authors: ***Y. ZHANG**, R.-Y. LIU, L. J. CLEARY, J. H. BYRNE
Neurobio. & Anat., Univ. Texas, HOUSTON, TX

Abstract: Doxorubicin (DOX) is an anthracycline widely used for cancer chemotherapy. Its antitumor actions include oxidative damage to cellular membranes and interference with DNA replication and RNA synthesis. However, DOX also affects the extracellular signal-regulated kinase (ERK) and p38 mitogen-activated protein kinase (p38 MAPK) isoforms of MAPK (Poizat et al., 2005; Small et al., 2003). Because these two MAPK isoforms play antagonistic roles in long-term memory (LTM) formation, their activation by DOX in neurons, if present, could have secondary effects on cognitive functions such as learning and memory. Here, we used cultured sensory neurons from *Aplysia* to examine the effects of DOX on levels of phosphorylated ERK (pERK) and p38 MAPK (phospho-p38 MAPK) as well as its effects on MAPK phosphatase MKP1. As a first step, immunofluorescence techniques were used to measure pERK levels at different concentrations and at different exposure times. A 30-min treatment with 10 μM DOX led to a 113 ± 29% ($n = 5$, $t_4 = 4.23$, $p < 0.05$) increase in pERK compared to controls. Treatments with lower concentrations (2.5 μM and 5 μM) were without effect. However, treatment with 2.5 μM DOX for a longer period (2 h) led to a 41 ± 14% increase in pERK ($n = 4$, $t_3 = 4.63$, $p < 0.05$). These results indicate that DOX is an effective activator of the ERK cascade in neurons and the magnitude of the effects are time and concentration dependent. Immunofluorescence techniques were also used to examine whether DOX affects levels of phospho-p38 MAPK after 2 h treatment with 2.5 μM DOX, a treatment duration and concentration used in the physiological studies in the accompanying abstract by Liu et al. DOX treatment led to a 73 ± 12% increase in phospho-p38 MAPK compared to controls ($n = 4$, $t_3 = 3.813$, $p < 0.05$). These results indicate that in SNs, DOX is an effective activator of both p38 MAPK and ERK. In breast epithelial and breast carcinoma cells, DOX increases pERK by decreasing the expression of MAPK phosphatase MKP1. To determine protein levels of MKP1 in neurons after DOX treatment, SNs were exposed to 10 μM DOX for 30 min or 2.5 μM DOX for 2 h, treatments that significantly enhanced ERK phosphorylation (see above). The 30 min treatment with 10 μM DOX produced a 9 ± 7% ($n = 4$) increase in MKP1, but this change was not statistically significant ($t_3 = 1.316$, $p = 0.3$), whereas the 2 h treatment of 2.5 μM DOX induced a modest (-13 ± 9%) but significant decrease in MKP1 levels ($n = 8$, $t_7 = 2.658$, $p < 0.05$). Therefore, down regulation of MKP1 expression may contribute, at least in part, to the prolonged (2 h) DOX-induced activation of ERK. The effects of DOX on excitability and synaptic plasticity are described in a separate abstract (Liu et al, 2014).

Disclosures: **Y. Zhang:** None. **R. Liu:** None. **L.J. Cleary:** None. **J.H. Byrne:** None.

Poster

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Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant RO1 MH-55880

Title: Evidence for attention in a non-visual invertebrate, *Aplysia*

Authors: K. PEARCE, *T. W. ABRAMS
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Abstract: Attention is typically associated with animals with highly evolved visual systems, such as primates and owls. Attention-like processes with visual cues have also been demonstrated in fruit flies. Wan et el. (2012, Nat Neurosci 15:1144-52) suggested that a molecular switch that shifts afferent synapses between active and silent states may function in an attention-like process in *Aplysia*. We now asked whether this more primitive invertebrate, which lacks an image-forming visual system, exhibits attention. Attention is a limited resource and is degraded by multiple competing stimuli. Distraction is only possible if a subject attends to a stimulus. We tested whether *Aplysia* could be distracted from a stimulus that had gained importance through training with a fear conditioning paradigm adapted from Walters et al. (1981, Science 211:504-6), in which a neutral chemosensory CS is paired repeatedly with tail shock. For fear conditioning, animals were exposed to a dilute carrot juice CS for 90 sec; a tail shock was delivered during the final 15 sec of CS exposure. Sensitized control animals received the same CS and tail shock during training, but with the two stimuli separated by 15 min. After a series of a series of 6 training trials over 2 days, siphon withdrawal responses were tested on day 3, in the presence or absence of the CS. Fear conditioned animals showed a much more dramatically enhanced withdrawal response when tested in the presence of the CS, than did sensitized animals. Interestingly, fear conditioned animals tested in the absence of the CS showed <50% of the response as sensitized animals, suggesting that they had less general, non-specific fear. To test for attention to the CS, animals were tested with a very weak vibration stimulus as a distractor presented 5 sec before the CS. The distractor had no effect on the responses of sensitized animals tested with or without the CS. In contrast, when tested with the vibration distractor in the presence of the CS, fear conditioned animals showed little or no enhanced response to the test stimulus compared with pretests responses. We obtained similar effects of a distractor on responses to a fear conditioning CS that had been paired with tail shock when weak vibration was the CS, and either carrot juice or light flashes served as the distractor. Thus, the effect of the distractor was not due to inhibition of the behavioral response, as the same vibration distractor from the first protocol evoked an enhanced defensive response when used as the fear conditioning CS. In summary, these results suggest that *Aplysia* can only enhance their defensive responses when exposed to a fear conditioned CS if they are “paying attention” to this stimulus.

Disclosures: K. Pearce: None. **T.W. Abrams:** None.

Poster

561. Invertebrate Learning and Memory II

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Topic: F.02. Animal Cognition and Behavior

Support: NIH

NSF

Title: Genomic dissection of the *Aplysia* californica siphon-withdrawal reflex circuit: One neuron at a time

Authors: *C. BOSTWICK[1], Q. YANG[3], T. P. MOROZ[1], A. B. KOHN[2], R. D. HAWKINS[3,4], L. L. MOROZ[1]
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Abstract: Analyzing single neurons is critical as studies have indicated that there is high variability in gene expression even within supposedly homogeneous cell populations. Here, we examined the transcriptome of single neurons that control the siphon-withdrawal reflex in the marine sea hare, *Aplysia californica*. The siphon and gill withdrawal reflex of *A. californica* has been studied for decades as a model circuit for understanding the molecular processes underpinning learning and memory that occurs when the animal undergoes sensitization training or classical conditioning. Three cell types were considered: the LE sensory neurons, LFs motor neurons, and L29 interneurons, which modulate the circuit. Unbiased transcriptome profiling of these single neurons (RNA-Seq) allowed us to explore the diversity of molecular components present in the circuit. It appears that each neuron has a unique expression profile with several hundred to thousands of differentially expressed transcripts. Interestingly, we discovered the CCAAT-enhancer-binding protein (ApC/EBP) was enriched in L29 interneurons compared to LE and LFs neurons. We also found expression of both isoforms of protein kinase C (PKC) and HCN channels to be relatively higher in LFs motor neurons. Also of interest is the set of novel uncharacterized neuropeptide candidates found to be differentially expressed in each of the neuronal types investigated. We suggest that small signal peptide molecules can act as both anterograde and retrograde messengers within the circuit. Comprehensive neuron-specific expression profiles could provide unique molecular resources and novel molecular targets which

might play essential roles in synaptic transmission and neural plasticity within this important model learning and memory circuit.

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Poster

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Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant GM097502

Title: Hyperpolarization-activated, cyclic nucleotide-gated cation (HCN) channels contribute to classical conditioning of the *Aplysia* siphon-withdrawal reflex

Authors: *R. D. HAWKINS[1,2], P. KUZYK[3], I. ANTONOV[1], Q. YANG[1], C. BOSTWICK[3,4], A. KOHN[3], L. L. MOROZ[3,4]
[1]Neurosci., Columbia Univ., New York, NY; [2]New York State Psychiatric Inst., New York, NY; [3]Whitney Lab., Univ. of Florida, St. Augustine, FL; [4]Neurosci., Univ. of Florida, Gainesville, FL

Abstract: Hyperpolarization-activated, cyclic nucleotide-gated cation (HCN) channels are critical regulators of neuronal excitability and have been implicated in different forms of neuroplasticity. Here, we examined their distribution, properties, and possible involvement in simple forms of learning in *Aplysia*. First, we cloned and characterized the expression (RNA-seq) and distribution (*in situ* hybridization) of HCN channel in the CNS of *Aplysia* californica. acHCN shares many canonical regions with other HCN channels. It is predominantly expressed in motor neurons of *Aplysia*, although we also observed moderate expression in other neuronal types. To characterize the biophysical and pharmacological properties of the channel we expressed acHCN construct in Xenopus laevis oocytes. The expressed channel exhibits all the major properties of HCN channels, including activation by both hyperpolarization and cyclic nucleotides, permeability to potassium and sodium ions, and inhibition by Cs+ and ZD7288. ZD7288 also suppressed spiking in buccal and pedal motoneurons, suggesting a role of HCN in *Aplysia* feeding and locomotor circuits respectively. Next, we used ZD7288 to test whether HCN channels are involved in two simple forms of learning in the semi-intact siphon-withdrawal preparation. We first gave a train of 4 shocks to the tail, which produces behavioral sensitization

of the siphon withdrawal reflex that lasts approximately 1 hr. However, bathing the abdominal ganglion in ZD7288 (100 μM) had no significant effect on the sensitization. We then examined classical conditioning. We compared changes in the withdrawal reflex in groups that received either paired or unpaired training with a siphon tap CS and a tail shock US while the abdominal ganglion was bathed in either normal seawater (control) or ZD7288. In the control group, paired training produced an increase in the response to the CS compared to either the pretest or unpaired training, demonstrating classical conditioning. ZD7288 (100 μM) significantly reduced the conditioning. As controls, it did not have significant effects on the amplitude of the initial response to either the CS or US. These results suggest that HCN channels contribute to associative learning (conditioning) but not nonassociative learning (sensitization) of the withdrawal reflex. Furthermore, the pattern of results with ZD7288 during conditioning (a partial blockade that becomes larger with additional training) is similar to the pattern with injection of the NO scavenger oxymyoglobin into the motor neuron (Antonov et al, 2007), suggesting that NO may act through cGMP to activate HCN channels in the motor neuron during conditioning.

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Poster

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Topic: F.02. Animal Cognition and Behavior

Support: NIH grant NS083690

Title: *Aplysia* neurotrophin and its Trk receptor play an essential role during the transition from short-term to intermediate-term facilitation produced by 5HT at *Aplysia* sensory-motor neuron synapses

Authors: *I. JIN[1], S. KASSABOV[1], H. UDO[2], R. NICHOLLS[1], E. R. KANDEL[1], R. D. HAWKINS[1]
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Abstract: We have previously found that spontaneous neurotransmitter release from the presynaptic neuron during short-term facilitation (STF) acts as an anterograde signal to recruit postsynaptic mechanisms of intermediate-term facilitation (ITF), an early step in a cascade

leading to long-term facilitation (LTF) and synaptic growth at *Aplysia* sensory-motor neuron synapses in culture (Jin et al., 2012a,b). In addition, we found that an endogenous neurotrophin (ApNT) and its receptor (ApTrk) are important for LTF (Kassabov et al., 2013). We report here that presynaptic ApNT/ApTrk signaling also plays a major role in the transition from STF to ITF by enhancing spontaneous transmitter release. 2 X 5 min or a 10 minute bath application of 5HT produces ITF and an increase in mEPSC (mini) frequency, with little change in amplitude. 10 min application of mature ApNT also increased mini frequency, and blocking ApTrk by bath application of Fc fragments reduced the increase produced by 10 min 5HT. These results suggest that ApNT/ApTrk signaling is involved in the enhancement of spontaneous release produced by 5HT during the induction of ITF. Injection of siRNAi against ApNT into the SN reduced the increase in mini frequency produced by 5HT, implicating a presynaptic role for ApNT. Injection of ApTrk antisense into the SN similarly reduced the increase in mini frequency produced by 5HT. Conversely, activation of presynaptic ApTrk receptors with a hybrid system (Knight et al., 2000) increased mini frequency. These results suggest an autocrine feedback, in which presynaptic ApTrk is also involved. 10 min activation of heterologous octopamine (OA) receptors which are linked to the production of cAMP and activation of PKA in the SN also produces an increase in mini frequency, and that increase was reduced by injection of ApTrk antisense in the SN, suggesting that ApTrk is downstream of PKA. Conversely, injection of a peptide inhibitor of PKA into the SN blocked the increase in mini frequency produced by 5-HT, OA, or mature ApNT, suggesting that PKA is downstream of ApNT. Collectively, these results suggest that activation of presynaptic 5HT (or OA) receptors stimulates cAMP, PKA, and the release of ApNT, which binds to presynaptic ApTrk autoreceptors and acts back through PKA to enhance spontaneous release of glutamate from the SN. Thus, ApNT has a critical role as a presynaptic autocrine signal involved in the enhancement of spontaneous release and subsequent recruitment of postsynaptic mechanisms of ITF and LTF. In addition, presynaptic ApNT, ApTrk, and PKA apparently form a self-amplifying feedback loop that could increase activation of PKA and recruit presynaptic mechanisms of LTF.

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Poster

562. Decision-Making: Neuropharmacology

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Title: High-yawning rats are more anxious than low-yawning in elevated plus-maze and in the light-dark box: Effects of diazepam

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Abstract: High-yawning (HY) subline of Sprague-Dawley rats none only yawned more than the low-yawning (LY), but they also show other changes more ambulation and less fecal boluses in the open-field test. In a novel environment HY rats showed more grooming bouts and time spent in grooming behavior suggesting that HY rats are more anxious than LY subline. The aim of this study is to study diazepam in the HY and LY rats exposed to behavioral standardized test to measure anxiety, the elevated plus-maze (EPM) and in the light-dark box (LDB). All subjects were maintained under standard conditions with a light-dark cycle 12/12 with lights on at 0700 with free access to rodent pellets and water. At 100 days old, male subjects were tested in the EPM and in the LDB. All experiments did between 1100 to 1300h. We also analyzed the effects the diazepam a well-known benzodiazepine. We used 0.25, 0.50 and 1 mg/kg doses administered by intraperitoneal (i.p.) injections. The behavior in the EPM and LDB was measured after 15 min of injection. The behavioral display were recorded using video camera by 5 min in each test and analyzed off-line by a blind observer using The Observer XT software. Data analyzed through ANOVA followed by Tukey test. The results showed that male HY rats are more anxious because they showed less entries and less the time spent in the open arms of the EPM respect to that showed by LY sublines ($P<0.05$). Similar results were obtained in the LDB with less entrances in the illuminated compartment and the time spent on it by the HY rats ($P<0.05$). Importantly, HY subline showed in both tests vicarious trial and error (VTE) behavior. However, both sublines are sensible to the action of diazepam being higher the effects with the 1 mg/kg dose ($P<0.05$). In conclusion, HY rats are more anxious in the EPM and LDB tests. Importantly, HY subjects showed more VTE behavior suggesting obsessive-compulsive disorder (OCD). Importantly, the benzodiazepine reduced VTE display and increased the time spent in the open arms in the EPM and light compartment in the LDB. So, HY subline is an adequate model for perseverance and OCD.

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Poster

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Title: A DA-SP-ENK interface promotes the translocation of the δ-opioid receptor on cholinergic interneurons in the striatum

Authors: *E. M. HEATH[1], B. CHIENG[1], V. LAURENT[1], J. BERTRAN-GONZALEZ[3], M. CHRISTIE[2], B. BALLEINE[1]
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Abstract: The ∂-opioid receptor (DOR) is a heavily expressed in the striatum, particularly on cholinergic interneurons (CINs). Under baseline conditions these neurons contain large amounts of receptor sequestered in intracellular pools. During Pavlovian conditioning, DOR in the nucleus accumbens shell (NACsh) of DOR-eGFP mice translocates from the cytoplasm to the somatic membrane of CINs. The molecular mechanism that triggers this event is unknown. This study investigates the role of dopamine (DA) and substance P (SP) in this insertion. SP (300nm) was administered unilaterally to the striatum of DOR-eGFP mice *in vivo* and to slices *in vitro* for 30 minutes. In a separate experiment, the D1-receptor agonist chloro-APB (100μM) was infused into the striatum of DOR-eGFP mice either alone or with the D2-receptor agonist quinpirole (10μM) for 45 minutes. DOR-eGFP distribution was visualised with immunofluorescence and its levels at the membrane of CINs quantified with ImageJ. *In vivo,* SP produced a significant increase in the membrane levels of DOR-eGFP in both the dorsal medial striatum (DMS) and NACsh. This was blocked by administration of n-acetyltryptophan (NAT) (1μM), an NK1R antagonist. *In vitro*, SP treatment significantly increased the levels of membrane DOR-eGFP in the nucleus accumbens core (NACco) and the NACsh. Infusion of chloro-APB into the striatum significantly lowered levels of DOR-eGFP at the somatic membrane of CINs whereas co-treatment with quinpirole significantly increased membrane levels of DOR-eGFP. In conclusion, SP signalling triggers the translocation of DOR on CINs in the striatum. Concurrent D1- and D2-receptor activation also triggers the translocation of DOR, possibly by stimulating the release of SP from D1-positive striatonigral medium spiny neurons (MSNs) while simultaneously inhibiting the release of enkephalin from D2-positive striatopallidal MSNs. These data suggest that a DA-SP-ENK interface may drive DOR translocation in the striatum.

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Poster

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Medical Research Council

Title: Pharmacological fingerprints of contextual uncertainty

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Abstract: Adapting to environmental dynamics requires us to distinguish truly significant changes from chance fluctuations. This presents the brain with several forms of uncertainty and prediction errors (Yu & Dayan, 2005): 1) expected uncertainty (EU) arises from a combination of ignorance and known but irreducible variability in a given environment; 2) this determines the surprise that arises when prevailing expectations are dashed; 3) unexpected uncertainty (UU) occurs when one encounters a new environment governed by novel rules. Theoretical, and indirect behavioural and neuroimaging evidence (Iglesias et al, 2013; Payzan-LeNestour et al., 2013; Galea et al., 2012) has proposed distinct neuropharmacological underpinnings for these types of uncertainty, with EU linked to acetylcholine (ACh), UU to norepinephrine (NE), and aspects of surprise to dopamine (DA). Here we manipulated these neuromodulators pharmacologically to assess their causal impact on these forms of uncertainty. In a double-blind between-subjects design (n=128), human subjects received biperiden (ACh muscarinic antagonist), prazosin (NE antagonist), haloperidol (DA antagonist) or placebo before performing a probabilistic choice reaction time (RT) task, in which the underlying probabilistic rule controlling the trial-by-trial occurrence of one of four imperative stimuli switched every 50 trials. We modelled individual learning and contextual uncertainty within a Bayesian framework

(Mathys et al., 2011; Yu & Dayan, 2005). After estimating the parameters of subject-specific choice trajectories, we established the relative contributions of EU, UU and surprise on observed RTs. Our preliminary results indicate dissociable roles for ACh, NE and DA in signalling contextual uncertainty, which were not due to group differences in baseline impulsivity, risk-taking or distractibility. Subjects with reduced ACh signalling were slower to learn novel contextual rules. Ongoing analyses are probing the exact nature of the changes elicited by NE and DA signal suppression, but indicate separable impairments in contextual learning and processing of surprise. Collectively, our current results suggest dissociable neuromodulatory fingerprints for the control of our adaptive responses to dynamic and unexpected changes in the environment.

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Poster

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NIMH MH094966

Title: Animal models of effort-related decision making: Reversal of the effects of tetrabenazine with the MAO-B inhibitor deprenyl (selegiline)

Authors: *H. M. CONTRERAS[1], M. A. ROWLAND[1], S. E. YOHN[1], M. W. JONES[1], M. CORREA[2,1], J. D. SALAMONE[1]
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Abstract: Considerable evidence indicates that nucleus accumbens dopamine (DA) is a critical component of the neural circuitry that regulates behavioral activation and effort-related processes. Motivated behaviors often are characterized by a high degree of behavioral activation and work output, and organisms frequently make effort-related decisions based upon cost/benefit analyses. Moreover, people with depression and other disorders often show effort-related motivational symptoms, such as anergia, psychomotor retardation, and fatigue. It has been

suggested that tasks measuring effort-related choice behavior could be used as animal models of these motivational symptoms, and in the present studies the effort-related effects of the vesicular monoamine transport (VMAT-2) inhibitor tetrabenazine (TBZ) were investigated. TBZ produces depressive symptoms in humans, and because of its selective inhibition of VMAT-2, it preferentially depletes DA. Effort-based decision making is studied with tasks offering choices between high effort options leading to highly valued reinforcers vs. low effort/low reward options. Several recent studies have shown that TBZ can alter effort-related choice in rats, and bias animals towards low effort alternatives. These effects are seen at doses that reduce extracellular DA in nucleus accumbens, and alter expression of DARPP-32 in accumbens medium spiny neurons in a pattern indicative of reduced transmission at both D1 and D2 DA receptors. In addition, these behavioral effects can be reversed by the adenosine A2A antagonist MSX-3 and the catecholamine uptake inhibitor/antidepressant bupropion. The present studies characterized the ability of the monoamine oxidase -B (MAO-B) inhibitor deprenyl (selegiline) to reverse the effort-related effects of TBZ. Originally developed as an antiparkinsonian drug, deprenyl has been shown to have antidepressant effects in humans, and to induce antidepressant-like effects in traditional rodent models of depression. Rats were assessed using a concurrent fixed-ratio 5/chow feeding choice task. Tetrabenazine shifted response choice in rats, producing a decrease in lever pressing and a concomitant increase in chow intake. Co-administration of the MAO-B inhibitor deprenyl (2.5 mg/kg) reversed the effects of 0.75 mg/kg TBZ, increasing lever pressing and decreasing chow intake in TBZ-treated rats. Future research will characterize the effects of MAO-A selective drugs for their effects on effort-related choice. These studies have implications for the potential use of MAO inhibitors as treatments for the motivational symptoms of depression and related disorders.

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Poster

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Title: Orally ingested curcumin attenuates shifts in effort-related choice behavior induced by the vmat-2 inhibitor tetrabenazine: Implications for depression

Authors: *S. E. YOHN[1], A. MISTRY[1], S. COLLINS[1], L. XIE[2], A. MANCHANDA[3], B. BOLLING[2], R. BOGNER[3], M. CORREA[4,1], J. D. SALAMONE[1]
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Abstract: Nucleus accumbens (NAc) dopamine (DA) is involved in behavioral activation and effort-related motivational processes. Behavioral paradigms have been developed to assess effort-related choice in rodents, including maze procedures and operant tasks. Administration of the vesicular monoamine transport-2 (VMAT-2) inhibitor tetrabenazine (TBZ) produces an alteration of response allocation in the FR5/chow procedure, biasing animals toward the lower effort alternative (i.e. consumption of freely available chow rather than lever pressing for a preferred food). The effects of TBZ are consistent with those of accumbens DA depletions and DA D1 or D2 family antagonists. It has been suggested that tasks assessing effort-related choice in rodents can model motivational symptoms such as psychomotor retardation, anergia, and fatigue, which can be seen in depression and other disorders. Previous studies have shown the effort-related effects of TBZ can be attenuated through co-administration of the monoamine-oxidase (MAO)-B inhibitor, deprenyl, as well as the antidepressant bupropion. Curcumin, a naturally occurring compound found in turmeric, has proven to be successful in traditional rodent models of depression. Curcumin acts as an MAO-A/B inhibitor, but it has low bioavailability due to poor absorption, rapid metabolism, and fast elimination. Prior studies have enhanced bioavailability through addition of excipients, and have generally employed gavage feeding. Although highly effective, this method can cause esophageal injury as well as restraint-associated distress, particularly with repeated use. Moreover, it does not mimic human ingestion. The current studies involved two experiments. The first experiment assessed the ability of rats to ingest food pellets formulated with curcumin and the excipient neusilin, while the second was undertaken to determine if ingested curcumin could reverse the effort-related effects of TBZ. Two different formulations of curcumin were tested to observe consumption; the crystalline (CRYS) and coground (CGR) formulations differed in the way that they were milled. Rats readily consumed CRYS curcumin pellets, but did not consume the CGR pellets. Thus, CRYS pellets were administered in the FR5/chow procedure at two different time points prior to testing. When administered three hours before testing, ingestion of 160 mg/kg CRYS curcumin significantly reversed the effort-related effects of TBZ. These studies demonstrate that orally ingested curcumin in rats can exert effort-related motivational effects. These results have implications for the clinical use of curcumin to treat motivational symptoms in humans.

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Poster

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UCONN SURF Grant

Title: Animal models of effort-related decision making: Reversal of the effects of the VMAT-2 inhibitor tetrabenazine with drugs acting on dopamine transmission

Authors: *J. D. SALAMONE[1], S. E. YOHN[1], H. M. CONTRERAS[1], E. J. NUNES[1,2], P. A. RANDALL[1,3], M. A. ROWLAND[1], S. COLLINS[1], M. W. JONES[1], Y. BAQI[4,5], C. E. MÜLLER[5], M. CORREA[1,6]
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Abstract: Motivated behaviors can be characterized by a high degree of behavioral activation and work output, and animals often make effort-related decisions based upon cost/benefit analyses. Moreover, people with depression and other disorders show effort-related motivational symptoms, such as anergia, psychomotor retardation, lassitude and fatigue. Nucleus accumbens dopamine (DA) is a critical component of the neural circuitry that regulates behavioral activation and effort-related processes. Effort-based decision making is studied with tasks offering choices between high effort options leading to highly valued reinforcers vs. low effort/low reward options, and it has been suggested that such tasks could be used as animal models of motivational symptoms. In the present studies the effort-related effects of the vesicular monoamine transport (VMAT-2) inhibitor tetrabenazine (TBZ) were investigated. TBZ inhibits

VMAT-2 and thereby blocks vesicular storage, preferentially depleting DA in the striatal complex. TBZ also produces depressive symptoms in humans. Recent studies have shown that TBZ alters effort-based choice in rats, and biases animals towards low effort alternatives across multiple behavioral tasks (operant lever pressing and T-maze choice tasks). These effects are seen at doses that reduce extracellular and tissue levels of DA in nucleus accumbens, and alter expression of DARPP-32 in accumbens medium spiny neurons in a manner consistent with reduced D1 and D2 receptor transmission. Because tasks involving effort-related choice may be useful for drug development, additional studies investigated the ability of various drugs that are putative or established antidepressants to reverse the effects of TBZ. The effort-related behavioral effects of TBZ were reversed by the adenosine A2A antagonist MSX-3, the MAO-B inhibitor deprenyl (selegiline), and the catecholamine uptake inhibitor/antidepressant bupropion. The 5-HT uptake inhibitor fluoxetine and the norepinephrine (NE) uptake inhibitor desipramine failed to reverse the effects of TBZ, and higher doses of fluoxetine and desipramine in combination with TBZ led to further behavioral impairments. This pattern of effects indicates that drugs acting on DA transmission are relatively effective at reversing the effort-related effects of TBZ. Moreover, adenosine A2A antagonism may be effective because of interactions between adenosine A2A and dopamine D2 receptors in nucleus accumbens. These results are consistent with the hypothesis that drugs acting directly or indirectly to enhance DA transmission may be effective at treating effort-related psychiatric symptoms in humans.

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Poster

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UCONN SURF Grant

Title: Animal models of effort-related decision making: The antidepressant fluoxetine potentiates effort-related effects of the dopamine depleting agent tetrabenazine

Authors: *M. A. ROWLAND[1], H. M. CONTERAS[1], M. W. JONES[1], S. E. YOHN[1], M. CORREA[2,1], J. D. SALAMONE[1]
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Abstract: Impairments in behavioral activation and effort-related motivational function are often seen in depression and other disorders. Symptoms such as psychomotor retardation, anergia, apathy, avolition, and fatigue are common in psychiatric patients, and are highly resistant to treatment. Dopamine (DA), particularly in the nucleus accumbens, is involved in effort-related functions. DA antagonism and accumbens DA depletions make animals more sensitive to work-related response costs, and bias them towards low effort choices in decision making tasks. Thus, tasks that assess effort-related decision making can be useful for the development of animal models of motivational dysfunction. In the present studies, the vesicular monoamine transporter (VMAT-2) inhibitor tetrabenazine (TBZ) was used to induce effort-related dysfunctions in rats. TBZ induces depressive symptoms in humans, and low doses preferentially deplete DA. Several recent studies have shown that TBZ can alter effort-related choice in rats, and bias animals towards low effort alternatives. These effects are seen at doses that reduce both extracellular and tissue levels of DA in nucleus accumbens. In addition, these behavioral effects can be reversed by the adenosine A2A antagonist MSX-3, the catecholamine uptake inhibitor/antidepressant bupropion, and the MAO-B inhibitor deprenyl. The present studies characterized the ability of the selective serotonin reuptake inhibitor (SSRI) fluoxetine to reverse the effort-related effects of TBZ. SSRIs inhibit the uptake of serotonin into nerve terminals; fluoxetine (Prozac) is a commonly prescribed antidepressant that selectively inhibits uptake at the 5-HT transporter. Rats were assessed using a concurrent fixed-ratio 5/chow feeding choice task. TBZ (0.75 mg/kg) shifted response choice in rats, decreasing lever pressing while increasing intake of the freely available chow. When administered without TBZ, 10.0 mg/kg of fluoxetine decreased lever pressing in rats, but did not increase chow consumption as is seen in the TBZ condition. Co-administration of fluoxetine (2.5-10.0 mg/kg) with TBZ failed to reverse the effects of TBZ. In fact, co-administration of fluoxetine with TBZ produced further effort-related impairments, decreasing lever pressing at all doses of fluoxetine tested. These effects are consistent with human clinical studies showing that SSRIs are not highly effective at treating motivational dysfunction in depressed patients, and can induce symptoms such as fatigue and apathy. These studies have implications for the potential use of SSRIs as treatments for the motivational symptoms of depression and related disorders.

Disclosures: M.A. Rowland: None. **H.M. Conteras:** None. **M.W. Jones:** None. **S.E. Yohn:** None. **M. Correa:** None. **J.D. Salamone:** None.

Poster

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Title: Dopamine depletion reduces preference for physical effort in animals with low but not high experience of exercise: Shift to sedentary sources of reinforcement

Authors: *L. LOPEZ-CRUZ[1], N. SAN MIGUEL[1], P. BAYARRI[1], J. MEDRANO[1], C. CARRATALÁ[1], L. MONFERRER[1], J. D. SALAMONE[2], M. CORREA[1,2]
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Abstract: Organisms frequently make cost/benefit analyses in which they weigh the value of rewards vs. the costs involved in procuring them. These decision-making processes include assessments of effort-related costs and other factors, and can involve cognitive as well as physical effort. The mesolimbic dopamine (DA) system plays a critical role in behavioral activation, exertion of effort, and effort-based decision-making, and DA antagonism and depletion in this system has been shown to induce anergia in effort-based decision tasks. Exercise has been demonstrated to have protective effects in animal models of pathologies characterized by motor disturbances such as Parkinson's disease, which involves DA loss in the nigrostriatal system. However, the beneficial effects of physical activity on symptoms such as mental fatigue or anergia, present in many psychiatric and neurological pathologies, also need to be explored. To assess DAergic involvement in the activational component of motivation and in effort based decision-making when multiple reinforcers are available, mice received injections of tetrabenazine (TBZ), a VMAT-2 inhibitor that produces a reversible DA depletion. These animals were tested with a mouse T-maze task developed for the assessment of preference between physical activity (wheel running) in one arm vs. sedentary reinforcers such as a freely available sucrose pellets in another arm, as well as a non-social (neutral) odor in the third arm. Additionally, to study the protective effects of physical exercise, different groups of animals were exposed to different regiments of exercise (i.e. forced versus voluntary by environmental enrichment). Under standard conditions, mice spent more time running and less consuming sucrose or sniffing. TBZ produced a shift in the relative preference; it reduced the choice of the

reinforcer that involved vigorous activity, but increased consumption of a reinforcer that required little effort (sucrose). None of these doses of TBZ reduced RW performance or sucrose consumption when animals could not choose between them. Finally, mice that were extensively exposed to exercise (forced or by environmental enrichment) did not show TBZ-induced shifts in preference towards low-effort reinforcers such as sucrose or olfactory stimuli. These results suggest that exercise could act as a preventive therapy for the anergia-inducing effects of DA depletion. Thus, DA depletion produced effects indicative of anergia (lack of energy), but did not impair the primary rewarding effects of sucrose.

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Poster

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Title: Low dopamine D2/D3 receptor availability is associated with steep discounting of delayed rewards in methamphetamine dependence

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Abstract: Background: Steep temporal discounting, or a tendency to undervalue delayed outcomes, is commonly exhibited by individuals with psychiatric disorders that are associated with aberrant dopaminergic neurotransmission. Studies of substance use disorders have indicated

that steeper temporal discounting is predictive of a lower probability of achieving protracted abstinence, and that interventions that reduce temporal discounting can improve treatment response. Dopamine signaling influences intertemporal choice, and indirect evidence suggests that low dopamine D_2-type receptor availability in the striatum may contribute to a propensity to sacrifice long-term goals for short-term gain; however, this possibility has not been tested directly. Methods: We investigated whether striatal D_2/D_3 receptor availability is negatively associated with preference for smaller, more immediate rewards over larger, delayed alternatives among research participants who met DSM-IV criteria for methamphetamine (MA) dependence. Fifty-eight adults (n = 27 MA-dependent, n = 31 control) completed the Kirby Monetary Choice Questionnaire, and underwent positron emission tomography scanning with [^{18}F]fallypride. Results: MA users displayed steeper temporal discounting and lower striatal D_2/D_3 receptor availability than controls. Discount rate was significantly negatively correlated with striatal D_2/D_3 receptor availability among MA users (r = -.342, p = 0.041) and in the combined sample (r = -.246, p = 0.032), but not among controls (r = -.121, p = 0.258). Conclusions: These results provide the first direct evidence of a link between deficient D_2/D_3 receptor availability and steep temporal discounting. This finding fits with reports that low striatal D_2/D_3 receptor availability is associated with a higher risk of relapse among stimulant users, and may help to explain why some individuals chose to continue using drugs despite knowledge of their future negative consequences.

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Poster

562. Decision-Making: Neuropharmacology

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 562.10/VV3

Topic: F.03. Motivation and Emotion

Support: CNPq !C Researcher Scholarship

CAPES 059573/2010

Title: The effects of cocaine on oral sucrose self-administration by 6-hydroxydopamine lesioned male and female rats

Authors: *H. M. BARROS[1], K. M. BISOGNIN[2], L. S. UMPIERREZ[2], T. B. LOS SANTOS[2], L. FREESE[2], M. F. SOUZA[2]
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Abstract: The attention deficit hyperactivity disorder (ADHD) is one of the most common psychiatric disorders of childhood and it can persist into adulthood. In adults, the most common comorbidity of ADHD is Substance Use Disorder. Cocaine abuse and dependence are more precocious in ADHD patients. Because females are more vulnerable to the effects of cocaine, our objective was to evaluate the self-administration of cocaine in male and female rats in a model to ADHD. Male (M) and female (F) Wistar rats, PN 4, were randomized to intrathecal 6-OHDA (100µg free base) or SHAM 10µL (0.1% ascorbic acid). When 30 days old the rats were placed daily in operant conditioning sessions (3 hours, FR1) for oral sucrose self-administration added or not with cocaine (0, 0.2, 0.3, 0.4 mg/ml), for 28 days. Horizontal activity was estimated on PN 21 and post cocaine 0,2 mg, 0,3 mg and 0,4 mg. For analysis of reinforcement and of locomotion a Two Way ANOVA was used, considering lesion and sex as factors. Tukey test was used for post hoc comparisons when appropriate. As expected, females showed more active lever presses than males and lesioned animals present less sucrose self-administration. When considering cumulative responses in the active lever, the lesioned animals presented less lever presses than the non-lesioned animals for oral sucrose self-administration when given cocaine (F/SHAM - 1415.23 ± 32.86; M/SHAM -1180.84 ± 32.86; F/6OHDA- 917.33 ± 28.17; M/6OHDA - 538.12 ± 28.12; $p < 0.001$). The mean number of daily reinforcements showed that cocaine decreased sucrose self-administration, dose-dependently, in males. Higher dose-dependent effect was seen in M/6OHDA. F/SHAM showed more sucrose consumption in the presence of cocaine and in F/6OHDA cocaine did not retain its dose-dependent effect towards sucrose self-administration. These results show that 6-OHDA lesion decrease sucre self-administration, as expected and that cocaine behaviuoral effects on sucrose reinforcement is sensitized in 6-OHDA lesioned males. Cocaine effects on sucrose self-administration are less intense in females, irrespective of previous dopaminergic lesion or not. Further studies should elucidate the participation of the pre-synaptic dopaminergic neurons in sucrose consumption in female rats. **Support:** CNPq 1C Researcher Grant; CAPES 059573/2010

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Poster

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Program#/Poster#: 562.11/VV4

Topic: F.03. Motivation and Emotion

Support: MRC Grant GO300155

NIHR BRC PhD Studentship

Title: The effects of acute fluoxetine administration on functional abnormalities during temporal discounting in children with ADHD

Authors: *C. O. CARLISI, L. J. NORMAN, K. CHANTILUKE, A. CHRISTAKOU, M. J. BRAMMER, V. GIAMPIETRO, K. RUBIA
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Abstract: The use of temporal foresight is critical in successful decision making. Temporal discounting is the rate at which a subjective value of reward decreases with increasing time delay and is a validated measure of temporal foresight and impulsivity. Patients with Attention Deficit Hyperactivity Disorder (ADHD) are impulsive and exhibit behavioural and neural deficits in temporal discounting and decision making because of this phenotype. The role of the selective serotonin reuptake inhibitor (SSRI) fluoxetine in ADHD is relatively unexplored, but evidence supports an involvement of serotonin in impulsivity, temporal discounting and in ADHD. Therefore, we wanted to examine whether acute fluoxetine administration would ameliorate atypical behavioural and brain response in ADHD on a temporal discounting task. 12 adolescent boys with ADHD and 20 controls (15.09+/-1.76y) completed a temporal discounting task which required participants to choose between receiving a certain amount of hypothetical money now or receiving £100 in either one week, month or year in an fMRI scanner. ADHD patients were scanned twice in a placebo-controlled randomised design under either Fluoxetine or placebo. Controls were scanned once, off medication. Activations to delayed vs immediate reward choices were modelled, with the modulatory effects of medication on the ADHD group's activations to delayed choices as the primary effect of interest. Repeated measures whole brain analysis for delayed vs immediate choices ($p<0.005$) revealed a significant medication effect in the right inferior frontal/superior temporal cortex, which was enhanced in activation with fluoxetine relative to placebo. Placebo, on the other hand, enhanced activation in the left cerebellum and left precentral gyrus relative to fluoxetine. Comparisons between healthy controls and patients under either drug condition to test for normalisation effects will be presented at the conference. Inferior frontal dysfunction is consistently implicated in ADHD during several cognitive functions including temporal foresight (Rubia et al., 2009, Chantiluke et al., 2014). Our recent meta-analysis showed that psychostimulants most consistently upregulate activation in this region during neurocognitive tasks in ADHD (Rubia et al., 2014). The present findings show that serotonin agonists may have a similar effect of upregulating the inferior frontal cortex in ADHD in the context of an impulsiveness measure of temporal discounting.

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Poster

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Program#/Poster#: 562.12/VV5

Topic: F.03. Motivation and Emotion

Support: National Center for Responsible Gaming

NIH NS074014

Title: Effects of mixed-function serotonergic compounds in a novel rodent cost/benefit decision-making task

Authors: *A. L. PERSONS, S. E. TEDFORD, T. NAPIER
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Abstract: Preclinical and clinical studies suggest that drug and behavioral addictions (e.g., gambling) have overlapping features, and may involve the serotonergic (5-HT) system. Our preclinical studies indicate that mixed-function compounds that act on multiple 5-HT receptors can attenuate the behavioral and neurobiological effects of methamphetamine. Mirtazapine (an FDA-approved atypical antidepressant that acts on 5-HT2A/2C, 5-HT3 and adrenergic α2 receptors) decreases methamphetamine-induced behaviors such as drug-seeking (Graves & Napier. Biol. Psychiatry. 69:275,2011) and conditioned place preference (Herrold et al. Drug Alcohol Depend. 99:231,2009, Voigt et al. Behav. Brain Res. 225:91,2011; Voigt & Napier. Front. Behav. Neurosci. 5:92,2011). Additionally, we revealed that mirtazapine can reduce preference for larger, albeit costlier, reinforcement options and promote more advantageous decision-making in a cost/benefit decision-making task (Persons et al. Program No. 635.12. Neuroscience Meeting Planner. San Diego, CA: Society for Neuroscience. Online). Here we extend this work to determine if other mixed-function serotonergic compounds can alter decision-making. We implemented a novel rat model of cost/benefit decision-making that uses intracranial self-stimulation (ICSS) as the positive reinforcer in a lever-pressing operant task. Two options of cost/benefit decision-making included the choice between (i) the unpredictable occurrence of a high effort/large reinforcer (HE/LR) option, and (ii) the predictable occurrence of a low effort/small reinforcer (LE/SR) option. In this paradigm, the response cost placed on

obtaining the large reinforcer is the exertion of greater physical effort (i.e., increased lever pressing) necessary to obtain the reward. Increasing the effort required for the large reinforcer reduces the rats' tendency to select the HE/LR lever, and their preference switches to the LE/SR lever. The task allows the rats to demonstrate their well-documented tendency to prefer some level of risk when effort is relatively low. Results to date show the following: (i) Pretreatment with mianserin (5mg/kg) had no effect on preference for the HE/LR lever compared to baseline preference (76% vs. 62%). (ii) Pretreatment with ketanserin (5mg/kg) decreased preference for the HE/LR lever by ~30% (79% vs. 49%), suggesting that ketanserin was able to reduce gambling-like behavior. (iii) Treatment with vehicle for either test compound had no effect on baseline preference for the HE/LR lever. Further assessments of low-dose (1mg/kg) mianserin and ketanserin are ongoing.

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Poster

562. Decision-Making: Neuropharmacology

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Topic: F.03. Motivation and Emotion

Title: Nicotinic receptors in the ventral tegmental area mediates uncertainty-driven exploration in mice

Authors: J. NAUDÉ[1], S. TOLU[1], M. DONGELMANS[1], N. TORQUET[1], U. MASKOS[2], *P. FAURE[3]
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Abstract: Smoking alters evaluative and decision processes: smokers are more impulsive, less sensitive to adverse consequences and exhibit less exploratory behaviors. Exploration is an important adaptive process that may help discovering more advantageous alternatives. In particular, exploration can be directed towards uncertain options, as reducing uncertainty has an informational value. However, the neural basis of uncertainty-driven exploration remains unclear and how nicotine can interact with these processes is unknown. In studies with non human animals, food commonly serves as the reward, often requiring food deprivation which can affect response to uncertainty itself. Using a behavioral paradigm that can be considered as a gambling task, where animals make a continuous series of choices between probabilistic brain stimulation

rewards (ICSS), we deciphered the role of nicotinic control of dopamine activity in uncertainty-driven exploration. Acute nicotine injection (n=10) increases exploration of uncertain options compared to saline control (n = 10). Compared to wild-type mice (n = 12), mice lacking nAChR β2-subunit (n=11) have an altered balance between exploitation of known rewards and exploration of uncertain options. Targeted re-expression of functional β2-containing nAChRs in the VTA (n = 11) restored this balance. Hence, our findings show that cholinergic modulation of the VTA is a neural basis for uncertainty-driven exploration. We also demonstrate a decrease in exploration following chronic nicotine exposure (n = 10), which modifies the dopaminergic system by acting on nicotinic acetylcholine receptors (nAChRs). Thus, by acting on VTA nAChRs, chronic nicotine not only exerts its reinforcing properties, but also favors the tendency to focus on known rewards, encompassing smoking itself and decisions unrelated to drugs.

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Poster

563. Motivation and Emotions: Neurocircuitry

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Title: The forebrain circuitry underlying fear behavior: Connections between the mediodorsal thalamus, frontal cortices and basolateral amygdala in mice

Authors: *F. MATYAS[1], J. LEE[2], H.-S. SHIN[2], L. ACSÁDY[1]
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Abstract: A large forebrain circuit, including the thalamus, amygdala and frontal cortical regions, is responsible for the establishment and extinction of fear-related memories. Understanding interactions among these three regions is critical to decipher the basic mechanisms of fear. With the advancement of molecular technology and optogenetics, mouse has become the main species to study fear-related behaviours. However, the basic connectivity pattern of the forebrain circuits involved in processing fear has not been described in this species. In this study we mapped - using single and double, anterograde and retrograde tracings - the connectivity between three key nodes of the circuit, the basolateral nucleus of the amygdala (BLA), the mediodorsal nucleus of the thalamus (MD) and the medial prefrontal cortex, which were shown to have closed triangular connectivity in rats. In contrast to rat, we found no evidence for this closed loop in mouse. There was no major input from BLA to MD and little overlap between medial prefrontal regions connected with both BLA and MD. The common nodes in the frontal cortex, which displayed reciprocal connection with both BLA and MD were the agranular insular cortex and the border zone of cingulate/M2 cortex. In addition BLA can indirectly affect MD via the orbital cortex. We attribute the difference between our results and earlier rat studies to methodological problems rather than to genuine species difference. Our data demonstrate that BLA and MD communicate via cortical sectors whose roles in fear-related behavior have not been extensively studied. In general, our study provides the morphological framework for the studies of murine fear related behaviors.

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Poster

563. Motivation and Emotions: Neurocircuitry

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Topic: F.03. Motivation and Emotion

Support: FAPESP Processo 11/08575-7

FAPESP Processo 13/20602-5

Title: The role of amygdala in human aggression disorders

Authors: ***F. V. GOUVEIA**[1], C. C. OLIVEIRA[1], L. T. C. SANTOS[1], M. D. J. SENO[1], E. T. FONOFF[2], E. ALHO[2], S. P. RIGONATTI[2], J. P. OTOCH[3], M. J. TEIXEIRA[2], *. C. R. MARTINEZ[1]

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Abstract: Aggression is a heterogeneous behavior that has multiple ways and it is related to violence, impulsiveness, irritability and hostility. Drug treatment with antipsychotic and anticonvulsant drugs are most commonly used for aggressive disorders. However, there is a small population of patients that displays extremely high levels of aggression, has drug-refractory symptoms and remain under maximum restrain measures. Some hormones, like testosterone and cortisol are implicated in the modulation of aggression. The amygdala is an important structure in the neurocircuitry of aggressive behavior and previous studies using lesions of the amygdala showed improvement in patient condition. Nevertheless, those studies had many limitations as follow-up, criteria for selecting patients and the surgery techniques were obsolete. As an attempt to reintegrate extremely aggressive patients into society, a judicial law proposed amygdala lesion in three adult men. In order of that and to have a better understanding of the neurobiology of human aggression, we analyzed the levels of different hormones - thyroid-stimulating hormone (TSH), T4, T3, Cortisol, Luteinizing Hormone (LH), Estradiol, Prolactin, Progesterone, testosterone, and sex hormone-binding globulin (SHBG) - before, 1 and 6 months after surgery. Our results showed an average lesion of 59.68% of the amygdala nuclei. There was a positive correlation between the ratio testosterone/cortisol and aggressive behavior ($r2=0.13$, $F(1,7)=1.04$, $p>0.05$). There were no statistical differences in all other hormones studied TSH ($F(4,10)=0.83$, $p>0.05$), T4 ($F(4,10)=0.14$, $p>0.05$), T3 ($F(4,10)=0.22$, $p>0.05$), LH ($F(4,10)=0.14$, $p>0.05$), FSH ($F(4,10)=0.07$, $p>0.05$), Estradiol ($F(4,10)=1.10$, $p>0.05$), Prolactin ($F(4,10)=0.13$, $p>0.05$), Progesterone ($F(4,10)=0.68$, $p>0.05$), SHBG ($F(4,10)=1.23$, $p>0.05$). The Overt Aggression Scale showed a significant reduction in aggression in one month in comparison with before and 6 months after the procedure ($F(4,10)=5.29$, $p<0.05$). Our results showed that partial amygdala lesion reduced aggression after surgery but this is not a long lasting effect and this decreased was accompanied by a reduction in the ratio testosterone/cortisol with no alteration in other hormones.

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Poster

563. Motivation and Emotions: Neurocircuitry

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Topic: F.03. Motivation and Emotion

Support: ZIA MH002928-01

Title: Amygdala lesions compromise reinforcement learning to impact behavioral flexibility

Authors: *D. R. LUCAS, III, V. D. COSTA, O. DAL MONTE, P. H. RUDEBECK, E. A. MURRAY, B. B. AVERBECK
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Abstract: The primate amygdala is implicated in reward-guided behavior, including the ability to flexibly alter stimulus-reward associations. Despite this understanding, lesions of the amygdala are not reported to cause deficits in reversal learning. Past studies often assessed the performance of monkeys when they first experienced reversals as opposed to when performance had asymptoted, and exclusively used deterministic rewards. As such, it is not clear how the amygdala contributes to reversal learning when behavioral strategies are stable and feedback is stochastic. To address these issues, we examined the role of the amygdala in reversal learning when rhesus macaques were trained to expect a reversal in the stimulus-reward contingencies of the task. The performance of macaques with bilateral excitotoxic amygdala lesions (n = 4) and intact controls (n = 3) was assessed on two different versions of a two-arm bandit reversal-learning task. In the first version, macaques initially learned to choose between two novel, deterministically rewarded options and task progression was tied to reaching a criterion level of performance after each reversal. In each daily session, macaques completed a total of 8 serial reversals. In a second version of the task the animals learned to choose between two choice options, but the reversal of stimulus-reward contingencies did not depend on the monkeys' performance. Instead, reversals randomly occurred between trials 30 and 50 in a fixed block of 80 trials. In each daily session, macaques completed up to 36 blocks, with choices rewarded deterministically or probabilistically. To analyze choices across tasks we developed a formal, Bayesian model of reversal behavior and used it to guide fitting of reinforcement learning models. Results indicated that amygdala lesions compromise the ability to reverse stimulus-reward associations by disrupting reinforcement learning. In both tasks, across the acquisition and reversal phases, macaques with amygdala lesions exhibited less consistent choice behavior, as indicated by a smaller inverse temperature parameter compared to controls. Monkeys with amygdala lesions also showed heightened sensitivity to negative vs. positive feedback, compared to controls. These effects were not due to adoption of a win-stay/lose-shift strategy. Thus, monkeys with amygdala lesions were slower to reverse their choice behavior-especially when feedback was stochastic. In summary, amygdala lesions cause noisy choice behavior and heightened sensitivity to negative feedback that enhances or impairs behavioral flexibility based on unexpected or expected choice uncertainty.

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Poster

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Topic: F.03. Motivation and Emotion

Support: Helen Hay Whitney Foundation

Title: The control of social and self-directed behaviors

Authors: ***W. HONG**, D.-W. KIM, D. J. ANDERSON
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Abstract: Animals exhibit a broad range of innate behaviors that are essential for their survival and reproduction. These include responses to predators or prey, social behaviors among conspecifics such as mating, aggressive and communicative behaviors, as well as self-directed behaviors such as grooming and feeding. The regulation and maintenance of a proper balance between social and self-directed behaviors is of importance in social species such as humans. Abnormalities in this balance are associated with several psychiatric disorders such as autism. Several subcortical limbic nuclei in the hypothalamus and amygdala have been implicated in playing an important role in regulating social behaviors. In this poster, I will present our work on investigating the circuits that control the choice between innate social and self-directed behaviors in these subcortical limbic circuits. A better understanding of how social vs. self directed behaviors are regulated may provide insights into human psychiatric disorders involving social behaviors.

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Poster

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Title: Ambiguous social threat perception and the role of the basolateral amygdala - Evidence from fMRI

Authors: *R. HORTENSIUS[1], D. TERBURG[2,3], B. MORGAN[4], D. J. STEIN[3], J. VAN HONK[2,3,5], B. DE GELDER[6,1,7]
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Abstract: Resolving ambiguity of social threat is important for adaptive reactions to face and body expressions. The amygdalae (AMG) play an important role in the processing of social signals but it has so far been difficult in human studies to investigate the contribution from the different amygdalae nuclei. Previously, we have shown that bilateral damage of the basolateral amygdala (BLA) in subjects with Urbach-Wiethe disease (UWD), a genetic disorder that results in bilateral calcification of the AMG, show a deficit in ignoring bodily threat signals that render the facial expression ambiguous. The current study investigated the neural signature of this face-body incongruence effect. Six subjects with UWD and twelve matched controls viewed facial and bodily expressions presented in isolation (happy, fear, and control), and in congruent and incongruent face-body compounds with their attention always directed at the facial expression. Given the importance of the BLA and the frontal cortex in regulation of the central-medial amygdalae (CMA) and subsequent attention and reactions to ambiguous social emotional cues,

differences in activation between UWD and control subject were predicted to emerge in frontal regions and in the CMA when viewing incongruent face-body compounds. Results show that UWD subjects compared to the control group show an increase in activity in the lingual gyrus and inferior occipital gyrus, but a decrease in activity in the inferior parietal lobule and precuneus, when observing emotional facial and bodily expressions. Importantly, UWD but not control subjects show an increase in activity in the CMA and superficial AMG for incongruent compared to congruent compounds. Furthermore, a decrease in activity in the inferior frontal gyrus for incongruent relative to congruent compounds was found in UWD subjects compared to the control subjects. These results suggest that in the absence of a functional BLA processing of ambiguous social threat is sustained by increased CMA activation together with decreased activation in the inferior frontal gyrus. The present study extends previous findings on the role of the AMG in ambiguous threat processing and throws new light on the role of its subnuclei.

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Poster

563. Motivation and Emotions: Neurocircuitry

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Title: Long range amygdalar inputs to cortico-PAG neurons in infralimbic cortex

Authors: M. K. KAUSHIK[1], A. N. FERREIRA[2], H. YOUSUF[1], M. T. DINH[2], *P. L. SHEETS[1]
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Abstract: Layer 5 (L5) pyramidal neurons are the primary subcerebral output pathway of the infralimbic cortex (IL) which is a cortical area that plays an important role in the extinction of fear memories. There is strong reciprocal circuitry between the IL and amygdala, a structure pivotal in fear and emotion processing. However, specific targeting of amygdalar inputs to

defined populations of L5 IL neurons is unknown but is important for understanding the dynamics of neural circuitry that regulates fear behavior. Our lab has identified a large subset of L5 pyramidal neurons in IL that send projections to the ventral periaqueductal grey (vPAG), a midbrain structure important for autonomic components of fear behavior. We have also identified a non-overlapping subset of L5 IL neurons that project to the amygdala. Using the mouse as a model system and applying anatomical labeling strategies, whole-cell recordings, and adenoviral optogenetics, we aimed to elucidate the organization and strength of amygdalar input to L5 cortico-vPAG (CvP) and L5 corticoamygdalar (CA) neurons in IL. CvP and CA neurons were identified via *in vivo* injection of fluorescent retrograde tracers into the vPAG and amygdala, respectively. Concurrently, adeno-associated virus (AAV1.CAG-ChR2-Venus.W.SV40) was injected into the amygdala for anterograde infection of channelrhodopsin-2 (ChR2) into amygdalar axons in IL. While performing whole-cell recordings of retrogradely labeled CvP and CA neurons in IL of coronal brain slices, we optogenetically stimulated AAV-ChR2 infected amygdalar axons to test for long-range input from the amygdala. We detected excitatory monosynaptic amygdalar inputs in both L5 CvP and L5 CA neurons following optogenetic stimulation. Recorded inputs were stronger in L5 CvP and L5 CA neurons compared to labeled L2 CA neurons in IL. Additionally, activation of amygdalar axons caused robust disynaptic inhibition in both L5 CvP and L5 CA neurons. Our current experiments are focused on determining preferential connectivity of amygdalar inputs to CvP and CA neurons. These results suggest that amygdala inputs can modulate or shape IL output to subcortical structures important in fear.

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Poster

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Topic: F.03. Motivation and Emotion

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NIH Grant RO1MH085082

Title: Dissection of neural circuitry underlying trauma-induced enhancements in fear and aggression

Authors: *M. ZELIKOWSKY, D. J. ANDERSON
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Abstract: Fear organizes and orchestrates a host of species-specific defense reactions that enable an animal to adaptively respond to threat. However, fear can be maladaptive when elicited in non-threatening situations or in excess, and the persistence of such inappropriate fears is thought to contribute to the formation of anxiety disorders and phobias. One poignant example of this is post-traumatic stress disorder (PTSD), wherein following an extremely traumatic event, animals display a host of maladaptive behaviors. Thus far, scientific research has largely focused on PTSD symptoms such as the propensity to acquire new fears, exaggerated fear responses to mild stressors, increased substance abuse, depression, and anxiety, while relatively fewer studies have examined the effects of trauma on social behaviors such as sex and aggression. Moreover, most rodent models of PTSD that have examined aggression report that trauma produces an overall reduction in aggression. Thus, current rodent PTSD models fail to capture the increases in aggressiveness, violence, and anger that often characterize humans with PTSD. Using mice, we adapted a model of PTSD in which mice received a single traumatic event comprised of multiple unsignaled, unpredictable footshocks in a novel environment. We found that following trauma, mice showed enhanced fear learning, increased aggression and alterations in adaptive mating behavior. Importantly, we found that these disruptions in adaptive behavior persisted despite extensive extinction of the context in which the trauma occurred. Lastly, using behavioral, pharmacogenetic, optogenetic and pharmacological techniques, we investigated and characterized the neural circuitry involved in trauma-enhanced fear and aggression.

Disclosures: M. Zelikowsky: None. **D.J. Anderson:** None.

Poster

563. Motivation and Emotions: Neurocircuitry

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Topic: F.03. Motivation and Emotion

Support: NIH Grant R01MH085082

Klarman Foundation

Hilda and Preston Davis Foundation

NARSAD Young Investigator Award

Title: Dissecting a central amygdala neural circuitry for feeding behavior

Authors: *H. CAI[1], D. J. ANDERSON[2]
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Abstract: Central amygdala (CEA) has long been indicated to play a role in regulating feeding behavior. However, due to its cellular complexity, previous lesion studies have yielded conflicting results, reporting positive, negative or no effect of CEA neurons in regulating feeding and body weight. Thus a clear view of the role of CEA in regulating feeding behavior has yet to emerge. Using novel genetic methods, we have identified a specific subpopulation of neurons in CEA. Here we show that optogenetic manipulations of the neural activity of these neurons directly regulate feeding behaviors. We further dissected the underlying neural circuitry through which these neurons regulate feeding behavior. An understanding of this neural pathway will yield new insight into the neural circuitry mechanism of eating disorders.

Disclosures: H. Cai: None. **D.J. Anderson:** None.

Poster

563. Motivation and Emotions: Neurocircuitry

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 563.09/VV15

Topic: F.03. Motivation and Emotion

Title: Rapid control over food intake by accumbal to hypothalamic projection neurons

Authors: *E. C. O'CONNOR, S. LEFORT, V. PASCOLI, C. LUSCHER
Dept. of Basic Neurosciences, Univ. of Geneva, Geneva, Switzerland

Abstract: When feeding, animals must be able to rapidly switch away from food intake in response to sensory and external stimuli, like taste or predator threat. Projections from the nucleus accumbens (NAc) to the lateral hypothalamus (LH) are particularly important for conveying feeding relevant information. However, the identity of cells involved in this circuit and how they control moment-to-moment food intake is not known. Here we characterize the functional anatomy of NAc to LH projections in feeding. First, we establish a free-feeding paradigm in mice whereby analysis of licking microstructure provides precise temporal

information on food intake. Experiments using pharmacological and optogenetic inhibition of NAc neurons *in vivo* confirms the NAc as a key regulator of feeding. Next, we use a combination of neural tracing, *ex vivo* electrophysiology and optogenetic assisted circuit mapping to identify a specific population NAc neurons that project to the LH. Cell type and projection specific optogenetics in freely behaving mice is used to demonstrate causality between the activity of these NAc to LH projection neurons and rapid control over food intake. In summary, we find that a specific population of NAc neurons that project to LH control moment-to-moment food intake, providing a neural basis for the rapid switching of feeding behavior in response to sensory and external stimuli.

Disclosures: E.C. O'Connor: None. **S. Lefort:** None. **V. Pascoli:** None. **C. Luscher:** None.

Poster

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Support: 5R01MH070053-10

1F32HD055198-01

HHMI

Title: Control of stress-induced persistent anxiety by an extra-amygdala septohypothalamic circuit

Authors: *T. E. ANTHONY[1], N. DEE[2], A. BERNARD[2], W. LERCHNER[1,3], N. HEINTZ[4], D. J. ANDERSON[1]
[1]Biol., Caltech, Pasadena, CA; [2]Allen Inst. for Brain Sci., Seattle, WA; [3]NIH, Bethesda, MD; [4]The Rockefeller Univ., New York, NY

Abstract: Traumatic stress induces persistent increases in anxiety, but the neural substrates underlying this effect are poorly defined. Classical ablation experiments have suggested that the septum suppresses emotional responses to stress, but its circuitry has not previously been dissected using modern genetic tools. We have used optogenetic approaches to investigate the function of a subpopulation of lateral septal (LS) neurons marked by expression of the type 2 CRF receptor (Crfr2). Crfr2+ cells include GABAergic projection neurons that connect with the

anterior hypothalamus. Surprisingly, we find that these LS outputs enhance stress-induced behavioral measures of anxiety. Moreover, transient activation of Crfr2+ neurons promotes, while inhibition suppresses, persistent anxious behaviors. LS Crfr2+ outputs also positively regulate circulating corticosteroid levels. These data identify a subset of LS projection neurons that promote, rather than suppress, stress-induced behavioral and endocrinological dimensions of persistent anxiety states, and provide a cellular point-of-entry to LS circuitry.

Disclosures: **T.E. Anthony:** None. **N. Dee:** None. **A. Bernard:** None. **W. Lerchner:** None. **N. Heintz:** None. **D.J. Anderson:** None.

Poster

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Location: Halls A-C

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Program#/Poster#: 563.11/VV17

Topic: F.03. Motivation and Emotion

Title: *In vivo* calcium imaging of nucleus accumbens neurons in freely feeding mice

Authors: *S. LEFORT, E. C. O'CONNOR, C. LÜSCHER
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Abstract: Calcium imaging techniques in awake animals have been extensively used in the past decade to monitor neuronal activity within superficial cortical areas. However, imaging of subcortical brain structures has been limited due to optical limitations. Recent developments in microscopy, and more specifically in microendoscopy, have extended the possibilities for deep brain imaging of neuronal activity in freely behaving animals. Here we took advantage of different Cre mouse lines, combined with the improved genetically encoded calcium indicator GCaMP6s and deep brain calcium imaging techniques, to study neuronal activity in the nucleus accumbens (NAc) of mice engaged in a simple free-feeding task. After infecting GCaMP6s in specific NAc cell types and implanting a lens targeting the NAc, mice were first habituated to the weight of the microendoscope prior to being introduced to a feeding chamber. Imaging of NAc neurons expressing GCAMP6s was then performed while animals were given free access to a palatable fat solution. Analysis of licking microstructure provided precise temporal information on food intake that could be used to correlate with recorded calcium activity in specific NAc neuron populations. These data support the use of microendoscopy to study population activity of identified neurons in deep brain structures of freely behaving mice.

Disclosures: **S. Lefort:** None. **E.C. O'Connor:** None. **C. Lüscher:** None.

Poster

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Support: Indiana CTSI Research Enhancement Grant

Navari Family Foundation Grant

Title: Modulation of the amygdala-dorsal periaqueductal grey pathway by long-range inputs from the infralimbic cortex and ventral periaqueductal grey

Authors: *A. FERREIRA[1], P. L. SHEETS[2]
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Abstract: A major focus of fear research involves understanding communication between the medial prefrontal cortex (mPFC) and the amygdala. However, another critical structure in fear is the midbrain periaqueductal grey (PAG). The dorsal and ventral columns of the PAG contribute to opposing behaviors related to fear expression and fear extinction, respectively. How specific input-output pathways of the PAG connect to the mPFC-amygdala network is poorly understood. Here, we used anatomical labeling techniques, acute brain slice recordings, and adenoviral optogenetics (AAV1.CAG-ChR2-Venus.W.SV40) to investigate long-range connectivity of the PAG and infralimbic cortex (IL) in the amygdala. Retrograde tracer injection into the dorsal PAG (dPAG) labeled neurons in the central amygdala (CeA) showing a direct pathway from the amygdala to the PAG. Following injection of AAV-ChR2 into the ventral PAG (vPAG) and layer 5 of infralimbic cortex (IL), optogenetically activated inputs were detected in the intercalated nucleus of the amygdala (ITC) and the CeA, respectively. Optogenetic activation of vPAG-ITC axons resulted in disynaptic activation and inhibition of CeA-dPAG neurons. Therefore, there is an indirect vPAG to dPAG pathway that is routed through amygdala circuits. Preliminary data also suggests that descending IL input excites CeA-dPAG neurons. Our results suggest that the amygdala serves as an important node for vPAG and IL input to indirectly influence dPAG activity. Further dissection of this circuit will provide insight into the mechanism of fear extinction as it relates to the mPFC-PAG-amygdala network.

Disclosures: **A. Ferreira:** None. **P.L. Sheets:** None.

Poster

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U.S. Department of Veterans Affairs I01BX007080

NIH Grant RO1-NS44373

Stress and Motivated Behavior Institute

Title: Characterization of amino acid neurotransmitter release in the prelimbic cortex of anxiety vulnerable rats in response to basolateral amygdala stimulation

Authors: *J. E. CATUZZI[1,2], D. M. GREGOR[2], K. C. H. PANG[1,2,3], K. D. BECK[1,2,3]
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Abstract: Avoidance is a symptom common to all anxiety disorders and can be modeled in rodents using an active lever-press avoidance paradigm. During this task, an animal learns to actively avoid shock by pressing a lever. Extinction-resistance of this avoidant behavior is believed to signify the pathological avoidance characteristic of anxiety disorders. The Wistar Kyoto (WKY) rat serves as a model of anxiety vulnerability as this strain rapidly acquires and is resistant to extinguish this avoidant behavior. Interestingly, previous research from our laboratory has shown WKY rats lack NMDA-dependent synaptic plasticity in two brain regions known to mediate avoidant behavior: the amygdala and prefrontal cortex. Specifically, WKY rats lack long-term potentiation (LTP) within the basolateral amygdala (BLA) to prelimbic cortex (PL) projection using stimulation parameters known to induce NMDA-dependent LTP in outbred Sprague Dawley (SD) rats. In an attempt to determine the mechanism by which WKY rats lack LTP in the BLA to PL projection, we sought to characterize amino acid neurotransmitter release in the PL in response to BLA stimulation in WKY and outbred control SD rats under urethane anesthesia. We focused our assessment on glutamate, the endogenous ligand of the NMDA

receptor, and the NMDA receptor co-agonists d-serine and glycine. In this study, a microdialysis probe was implanted into the PL, and a stimulating electrode was implanted into the BLA. Dialysate samples were collected in 20-minute intervals and amino acid neurotransmitters were assessed using high-performance liquid chromatography (HPLC). Glycine release was significantly lower in WKY rats compared to SD rats. No effect of stimulation was observed on neurotransmitter release in WKY or SD rats. The reduction in extracellular glycine observed in the PL of WKY rats may represent a mechanism by which this strain fails to maintain LTP within the BLA to PL projection. Moreover, the reduction in extracellular glycine may underlie the avoidance susceptibility expressed by this strain.

Disclosures: J.E. Catuzzi: None. **D.M. Gregor:** None. **K.C.H. Pang:** None. **K.D. Beck:** None.

Poster

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Topic: F.03. Motivation and Emotion

Support: F30MH100835

R01MH090264

Title: Excitation/inhibition balance in the nucleus accumbens microcircuit underlies stress resilience or susceptibility

Authors: *M. HESHMATI[1], S. A. GOLDEN[1], H. ALEYASIN[1], D. J. CHRISTOFFEL[1], M. L. PFAU[1], I. MAZE[3], P. H. GOFF[2], G. E. HODES[1], L. A. KHIBNIK[1], N. REBUSI[1], J. L. ABLES[3], S. J. RUSSO[1]
[1]Neurosci., [2]Icahn Sch. of Med. at Mount Sinai, New York, NY; [3]The Rockefeller Univ., New York, NY

Abstract: Dysregulation of the mesolimbic reward circuit is a cardinal feature of mood and stress disorders. We are investigating shifts in excitation/inhibition balance on nucleus accumbens (NAc) medium spiny neurons (MSNs) following repeated social defeat stress, a mouse model of depression. Previously, we observed an increase in excitatory synapses in the NAc of mice that are susceptible to stress. Now, we show a concurrent decrease in spontaneous inhibitory postsynaptic currents restricted to the indirect pathway in susceptible mice, with an

overall elevation of excitation to inhibition ratio in dopamine receptor D2-expressing MSNs compared to control and stress resilient mice. On the other hand, we observe increased inhibition of direct pathway MSNs in resilient mice after stress. Susceptible mice have an overall decrease in vesicular GABA transporter (vGAT) protein, which is not due to decreased inhibition by parvalbumin-positive interneurons but may be caused by changes to other interneuron subtypes within the NAc. We are currently using Cre-dependent miRNA-mediated knockdown of neuroligin-2 *in vivo* to manipulate inhibitory synapses within the NAc microcircuit and test their contribution to stress susceptibility or resilience in a cell-type specific manner.

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Poster

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Topic: F.03. Motivation and Emotion

Support: MRC Career Development Award to HFC

Title: Normalising high trait anxiety in monkeys; the role of hippocampal-medial prefrontal circuitry

Authors: J. ZEREDO[1,3], Y. SHIBA[1,2], *A. ROBERTS[1,2], H. F. CLARKE[1,2]
[1]Physiology, Develop. and Neurosci., [2]Behavioural and Clin. Neurosci. Inst., Univ. of Cambridge, Cambridge, United Kingdom; [3]Univ. of Brasilia, Brasilia, Brazil

Abstract: Depression and anxiety are highly prevalent, debilitating conditions, which are only partially alleviated by current treatments. There are many theories of depression and anxiety, and two of the most prominent implicate a smaller, overactive, anterior hippocampus (antHipp) with reduced glutamate (Yüksel and Öngür 2010, Biol Psychiatry,68(9):785-94), and altered activity in medial prefrontal cortex (PFC) including Brodmanns areas 32 and 25 (Hamilton et al. 2011, Mol Psychiatry, 16, 763-772). The antHipp sends direct projections to the medial PFC yet there has been no attempt to integrate these hypotheses and investigate how the antHIpp might modulate the medial prefrontal cortex in the regulation of anxiety related behaviour. To test the hypothesis that increasing glutamate within the antHipp alleviates anxiety (both autonomic and

behavioural indices) via the mPFC, high trait anxious marmosets received bilateral intracerebral cannulae targeting the antHipp, area 32 and area 25, and had telemetry probes inserted into their descending aorta for remote measurement of cardiovascular activity. Marmosets were then trained in a context of unpredictable threat (pseudoconditioning, 5 days of unpaired CS and aversive US; 12 each), a known trigger for anxiety. The anxiety elicited by this context results in an increased response to a novel neutral CS when presented in the same context, despite the absence of the aversive US. Marmosets show a strong anxiety response: decreased heart rate and very low vigilant scanning, possibly indicative of disengagement from the environment. Compared to saline, these effects were alleviated peripheral anxiolytic treatment (0.25mg/kg diazepam), and also by antHipp activation (100pg/µl LY341495 & 10ng/µl CGP52432 infused at 0.5µl /min). However, the anxiolytic effects of antHipp activation were blocked by concomitant inactivation (0.1mM muscimol & 1.0mM baclofen; 0.5 µl infused at 0.25µl/min) of area 25, but not area 32. Thus, the antHipp acts to modulate anxiety related behaviour via its connectivity with distinct regions of the medial PFC. In particular, the anxiolytic effects of antHipp activation are dependent upon an intact area 25 and not area 32.

Disclosures: J. Zeredo: None. **H.F. Clarke:** None. **A. Roberts:** None. **Y. Shiba:** None.

Poster

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Topic: F.03. Motivation and Emotion

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NIDA R21DA035144

McDonnell Center for Systems Neuroscience

Title: Activation of noradrenergic locus coeruleus neurons promotes anxiety-like and aversive behaviors

Authors: *J. G. MCCALL[1], R. AL-HASANI[1], E. R. SIUDA[1], D. Y. HONG[1], C. P. FORD[2], M. R. BRUCHAS[1]
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Abstract: The locus coeruleus (LC) and its projections are the primary source of norepinephrine for the mammalian forebrain. The LC is a target for corticotropin-releasing factor (CRF), opioid neuropeptide containing neurons, and is highly enriched with all four opioid receptor types. LC tonic firing increases during stress and this increase is thought to be controlled by opioid and CRF activity. Therefore, we hypothesized that specific modulation of LC neuronal firing could lead to anxiety-like and aversive behaviors. We used optogenetic stimulation to specifically increase LC-NE neuronal activity. Consistent with previous reports, we demonstrate increased LC firing rate in response to stress *in vivo*. We investigated the role of this increase in LC-NE activity in negative affective behaviors. We report consistent firing of LC-NE neurons following repeated 5hz light stimulation *in vitro* and *in vivo*. We examined the effect of increasing tonic LC-NE firing on anxiety- and aversion-like behaviors using both conditioned place aversion and a real-time aversion paradigm. We found that increasing tonic firing of LC-NE neurons, consistent with CRF release in the LC, induces a subsequent aversion to the stimulated context. This same stimulation is aversive in real-time and scales linearly with frequency, with animals choosing to avoid increased activation of these neurons. Increased LC-NE firing also results in decreased open arm time in the elevated zero maze, and time in the center of the open field test, both assays for anxiety-like behavior. Furthermore, the same stimulation that drives aversion and anxiety-like behavior is sufficient to reinstate a cocaine place preference. We next used viral tracing to determine key sites of afferent innervation to the LC. These studies identified the central amygdala (CeA) as a structure with robust projections to the LC. We then investigated the CRF-containing CeA inputs into the LC that regulate its firing in these behaviors. We found that stimulation of these CRF terminals in the LC increases the firing rate of a subset of LC neurons and is sufficient to recapitulate the behaviors seen by directly increasing the firing rate of LC neurons. These data suggest that increasing the firing rate of LC-NE neurons is sufficient to produce negative affective behaviors including aversion and anxiety. Together these results suggest that noradrenergic locus coeruleus tone is important for anxiety and aversive behavioral states as well as reinstatement to drug seeking.

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Poster

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Support: CNPq

CAPES

FAPERJ

Title: Is it safe? Brain areas involved in the the processing of safety signals

Authors: *F. S. ERTHAL[1], I. MOCAIBER[2], L. OLIVEIRA[3], M. PEREIRA[3], V. ROCHA-REGO[1], I. FIGUEIRA[1], E. VOLCHAN[1]
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Abstract: For survival it is crucial to continuously evaluate the presence or absence of risk in the environment, identifying signals of danger or security. An important internal factor that modulates safety detection mechanisms is a trait disposition favoring positive affect. Positive affect trait was shown to enhance the engagement in safety contexts, capturing contextual cues of security and transforming them more efficiently into implicit modulations of the balance between the circuits underpinning predispositions to deal with threat and safe situations. Interestingly, trauma-exposed victims with post traumatic stress disorder, compared to trauma-exposed controls, fail to learn safety signals besides presenting low levels of positive affect. Further, patients with post traumatic stress disorder present volumetric reduction in the pregenual anterior cingulate cortex, a region which is associated with positive affect and may be involved in the processing of safety signals. The aim of this study was to search for putative brain areas tuned to safety cues processing in healthy subjects . We focused in the pregenual anterior cingulate, specifically the region which showed reduced volume in patients with post traumatic stress disorder.. Highly unpleasant pictures were shown in two contexts in which a prior description presented them as taken from movie scenes (fictitious/safe) or real scenes (control). In whole brain analysis, the comparison between unpleasant pictures in safe vs control contexts revealed activations in the pregenual anterior cingulate cortex and in the anterior insula ($p<0.001$ uncorrected). Beta values (task versus baseline), extracted from an unbiased region-of-interest in the pregenual anterior cingulate cortex (coordinates taken from the very same region which is reduced in volume in post traumatic stress disorder), were significantly higher in the “safe” compared to the “control” context (T-test, $p<0,05$). Additionally, we found a significant correlation between an index of engagement of the pregenual anterior cingulate cortex (beta of the safe context minus beta of the control context) and the scores on positive affect trait. These results suggest that the anterior insula and the pregenual anterior cingulate cortex are involved in the processing of safety signals. Involvement of the latter seems more conspicuous in individuals with higher positive affect. A rupture in the normal functioning of the pregenual anterior cingulate cortex in traumatized victims with PTSD may account for the failure in the processing of safety cues contributing to their emotional deficits.

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Poster

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Moore Foundation Grant

Title: Sexually dimorphic neurons control levels of aggressiveness in male *Drosophila* through the neuropeptide Tachykinin

Authors: *K. ASAHINA[1,3], K. WATANABE[1,3], B. J. DUISTERMARS[1,3], E. HOOPFER[1,3,4], C. ROBERTO GONZÁLEZ[2], E. A. EYJÓLFSDÓTTIR[2], P. PERONA[2], D. J. ANDERSON[1]
[1]Biol. and Biol. Engin., [2]Engin. and Applied Sci., Caltech, Pasadena, CA; [3]Howard Hughes Med. Inst., Pasadena, CA; [4]HHMI Janelia Farm Res. Campus, Ashburn, VA

Abstract: Males of most species are more aggressive than females, but the neural mechanisms underlying this dimorphism are not clear. Here we identify a neuron and a gene that control the higher level of aggression characteristic of *Drosophila* melanogaster males. Males but not females contain a small cluster of FruM+ neurons that express the neuropeptide tachykinin (Tk). Activation and silencing of these neurons increased and decreased, respectively, inter-male aggression without affecting male-female courtship behavior. Mutations in both Tk and a candidate receptor, Takr86C, suppressed, while over-expression of Tk potentiated, the effect of neuronal activation. Tk neuron activation overcame reduced aggressiveness caused by

eliminating a variety of sensory or contextual cues, suggesting that it promotes aggressive arousal or motivation. Tachykinin/Substance P has been implicated in aggression in mammals, including humans. Thus, the higher aggressiveness of *Drosophila* males reflects the sexually dimorphic expression of a neuropeptide that controls agonistic behaviors across phylogeny.

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Poster

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CNPq GRANT 14199/2012-7

Title: The anatomical connections of the interpeduncular nucleus: Another interface between the habenula and the mesopontine tegmentum

Authors: L. LIMA[1], L. GONÇALVES[2], F. LEITE[1], *M. METZGER[1]
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Abstract: The medial habenula (MHb) and its major terminal field the interpeduncular nucleus (IP) make part of the dorsal diencephalic conduction (DDC) system that links the limbic forebrain with monoaminergic and cholinergic cell groups in the mid- and hindbrain. Interestingly, two other components of the DCC, the lateral habenula (LHb) and its major target the rostromedial tegmental nucleus, have recently been shown to be central structures of an anti-reward system that encodes aversive conditions and inhibits dopamine and serotonin neurons. Both the MHb and IP contain several subnuclei, with a dorsal group of MHb subnuclei mainly projecting to dorsal parts of the IP, whereas the ventral group of MHb subnuclei massively innervates subnuclei in the ventral IP. Interest in the MHb-IP axis resurged when it was recently discovered that, MHB and IP, as well as probably structures downstream to the IP, are critically involved in mediating the aversive effects of nicotine and the somatic symptoms of withdrawal.

The afferent and efferent connections of the IP have hitherto not been systematically investigated with modern sensitive tracers. Thus, we placed small topic injections of the retrograde tracer cholera toxin subunit b (CTb) or the anterograde tracer Phaseolus vulgaris leucoagglutinin (PHA-L) into different IP subdivisions of male Wistar rats. We found massive reciprocal connections between the IP, the median raphe nucleus, dorsomedial tegmental area, and laterodorsal tegmental nucleus. Moreover, the IP was robustly interconnected with all parts of the supramammillary nucleus. Beside a dominant input from the MHb, the IP received minor inputs from the ventromedial prefrontal cortex, rostral parts of the cingulate cortex, nucleus of the diagonal band, medial and lateral preoptic area, lateral hypothalamus, and medial division of the LHb (LHbM). Dense focal IP outputs were directed to the ventrolateral septum, ventral CA1 hippocampal field, caudal part of the dorsal raphe nucleus, nucleus incertus, and interestingly, LHbM. Regarding a possible topography of IP outputs, we observed that all IP subnuclei have descending projections, whereas dorsocaudal parts of the IP specifically gave rise to most of its ascending projections. In all, our findings indicate that the IP might serve as another relay signaling aversive information from the habenula to serotonergic and cholinergic cell groups in the mesopontine tegmentum, and also as a missing link between the MHb and LHb.

Disclosures: **L. Lima:** None. **L. Gonçalves:** None. **F. Leite:** None. **M. Metzger:** None.

Poster

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Support: R01 MH090264

Title: Ventral striatal projections to the lateral habenula modulate the motivational component of aggressive behavior

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Abstract: Aggressive behavior is commonly associated with a number of neuropsychiatric disorders and is thought to result, in part, from inappropriate reward circuit activation. However, little is known about the neural circuits that modulate the motivational or rewarding components of aggressive behavior. Here we establish a novel behavioral model for investigating the rewarding component of aggression, using aggressive social interaction with an intruder mouse as a reinforcer for the development of conditioned place preference (CPP). In this model, aggressor mice develop a CPP, while non-aggressive mice develop a conditioned place aversion (CPA) to the intruder-paired environment. Further, we identify an inhibitory GABAergic projection from the ventral striatum (vStr) to the lateral habenula (lHb) that acts as a critical modulator of the rewarding component of aggressive behavior. Circuit-specific optogenetic silencing of inhibitory ventral striatal afferents with halorhodopsin (NpHR) in the lHb of aggressive mice abolishes the rewarding properties of aggression. Conversely, activation of these terminals with channelrhodopsin (ChR2) in non-aggressive mice enhances the rewarding properties of the intruder context. Lastly, directly stimulating or inhibiting the lHb cell bodies of aggressive and non-aggressive mice with ChR2 and NpHR, respectively, recapitulates the ventral striatal circuit-specific behavioral phenotypes. These results demonstrate that the vStr-lHb circuit plays a critical role in regulating the rewarding component of aggressive behavior and provides novel mechanistic insight into the neural circuits mediating aggression.

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Poster

563. Motivation and Emotions: Neurocircuitry

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Topic: F.03. Motivation and Emotion

Support: NIH Grant P50 MH086400

Tourette Syndrome Association Postdoctoral Fellowship

Title: Anatomical connections subserving the default mode network in monkeys

Authors: *S. R. HEILBRONNER[1], S. N. HABER[2]
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Abstract: The default mode network (DMN) is a set of brain regions consistently activated at rest and deactivated during task performance. DMN activity is abnormal in many neurological and psychiatric disorders, including major depressive disorder and drug addiction. The two main parts of the DMN are the medial prefrontal cortex (mPFC) and posteromedial cortex (PMC), two areas that are spatially distant and with distinct canonical functions. Functional connectivity within the DMN may result from direct anatomical links, indirect ones, or some combination of both. While there is some evidence of direct connections between these general cortical areas, little is known about their specifics and how they link the network together as a whole. In this study, we delineate how anatomical connections and pathways between mPFC and PMC allow them to operate as a network. Defining these specific DMN connections will establish the circuitry subserving neuroimaging results that demonstrate changes under normal and psychiatric conditions. We used bidirectional and anterograde injections to analyze connections between the PMC and frontal cortex. These connections are substantial. Preliminary stereological analyses and 3-D models indicate that the PMC connects both more strongly (greater density of labeled cells) and to a greater area within the dorsal prefrontal cortex than the ventral prefrontal cortex. That is, anterograde and retrograde connections between the PMC and the dorsolateral prefrontal cortex, anterior cingulate cortex, and dorsomedial prefrontal cortex are more pronounced than the connections between the PMC and the ventromedial prefrontal cortex, subgenual cingulate, orbitofrontal cortex, and ventrolateral prefrontal cortex. In addition, the prefrontal cortex connects to specific nodes within the PMC--defined as overlapping terminal fields and cell patches from multiple DMN regions. These nodes may integrate the multiple streams of information necessary for DMN functions. Interestingly, while this specific pattern of anatomical connections does not closely fit the canonical human DMN, it does roughly match the monkey DMN identified by Mantini and colleagues (2011, Journal of Neuroscience). These results raise interesting questions about the relationship between functional and anatomical connectivity, and possible species divergence in the DMN.

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Poster

563. Motivation and Emotions: Neurocircuitry

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Topic: F.03. Motivation and Emotion

Support: Templeton Foundation Positive Neuroscience Award

IDDRC Grant 5P30HD040677-13

Title: Diffusion tensor imaging of extraordinary altruists: Differences in fractional anisotropy in non-directed living kidney donors

Authors: *K. M. BRETHEL-HAURWITZ[1], E. M. CARDINALE[1], S. A. STOYCOS[1], J. W. VANMETER[2], A. A. MARSH[1]
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Abstract: Altruistic kidney donors volunteer to undergo surgery so that one of their own kidneys can be transplanted into a stranger, an act often described as "extraordinary altruism." The neural basis of this form of extraordinary altruism is not yet well understood. In the present study, we recruited altruistic living kidney donors and healthy controls matched on age, gender, and IQ. Group comparisons of white matter structure were made through analyses of data collected during diffusion tensor imaging (DTI). In particular, it was hypothesized that altruists would have greater fractional anisotropy (FA), a measure of axonal integrity and coherence, in the uncinate fasciculus, a bidirectional white matter tract spanning the frontal and temporal lobes that connects structures of the limbic system, including the amygdala, to the orbitofrontal cortex and temporal poles (Ebeling & Von Cramon, 1992). The amygdala, orbitofrontal/ventromedial prefrontal cortices, and connections between them have been implicated in empathy (Parkinson & Wheatley, 2012) and moral decision-making (e.g., Luo et al., 2006). Further, lower FA in the uncinate fasciculus has been observed in highly antisocial populations (Craig et al., 2009). Voxelwise statistical analysis of the FA data was carried out using Tract-Based Spatial Statistics (TBSS; Smith, 2006), part of FMRIB's software library (FSL; Smith, 2004). TBSS projects all subjects' FA data onto a mean FA tract skeleton, before applying voxelwise cross-subject statistics. Group comparisons of FA in a region of interest (ROI) analysis of bilateral uncinate fasciculus, defined using the Johns Hopkins University probabilistic white matter tractography atlas (Mori et al., 2005; Hua et al., 2008) available in FSL, supported our hypothesis. Altruists exhibited several clusters of greater FA in the left uncinate fasciculus ($p < .05$, FWE). At these thresholds, there were no clusters for which controls exhibited greater FA in the left uncinate fasciculus, and no group differences were observed in the right uncinate fasciculus. Further examination of axial ($\lambda 1$) and radial ($[\lambda 2+\lambda 3]/2$) diffusivities in the left uncinate fasciculus revealed a cluster of greater axial diffusivity in altruists ($p < .05$, FWE), in proximity to the orbitofrontal cortex. This pattern of findings in the white matter structure of demonstrated altruists may have important implications for understanding the neural basis of prosocial behavior, and for understanding the nature of extraordinary altruism.

Disclosures: **K.M. Brethel-Haurwitz:** None. **E.M. Cardinale:** None. **S.A. Stoycos:** None. **J.W. VanMeter:** None. **A.A. Marsh:** None.

Poster

564. Social Behavior: Neuropharmacology

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Topic: F.03. Motivation and Emotion

Support: Morrison Trust

MH086708

GM097632

CDMRP DoD Autism Idea Award

Title: Acute dietary tryptophan manipulation differentially alters social behavior and plasma corticosterone in inbred mice

Authors: W. Q. ZHANG[1,2], P. BARBA-ESCOBEDO[1,3], M. GAMEZ[3], C. M. SMOLIK[1], L. C. DAWS[1], *G. G. GOULD[1]
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Abstract: Metabolism of the neurotransmitter serotonin (5-HT) is abnormal in 20-40% of patients with autism, and in this population elevated levels of whole-blood 5-HT (hyperserotonemia), and higher intestinal 5-HT levels are common. However, there is evidence that brain 5-HT levels and serotonergic neurotransmission may be insufficient for normal brain development and functioning for these autistic individuals. We hypothesize that social behavior impairments may be a product this, and might be ameliorated by increasing brain extracellular 5-HT levels for some patients. Supporting this idea, serum levels of oxytocin, governed by 5-HT transmission, are often reported to be lower in autisitic individuals, selective serotonin reuptake inhibitors improves and tryptophan (TRP; metabolic precursor of 5-HT) depletion worsens all symptoms of autism. However, dietetic studies demonstrate and parents report that unrestricted children with autism consume less TRP than their peers. To examine the impact of varying dietary TRP on social behaviors, we studied the impact of acute (24 h) TRP depletion and supplementation on adult males of three inbred strains of mice. Two of these strains, tufted black

and tan brachyury (BTBRT+/tfJ) and 129S1/SvImJ mice have inherently low levels of social behavior relative to commonly used C57BL/6 mice. We found that TRP depletion significantly worsened the social behaviors of C57BL/6 and 129S mice, while 1% TRP enhancement improved the otherwise impaired social behaviors of BTBR mice ($P<0.05$; N = 8-10) in three-chamber tests. Plasma corticosterone levels following behavior tests were higher in those C57BL/6 and BTBR mice given enhanced TRP diets ($p <0.05$; N=4-10). Marble burying behavior did not differ among strains or treatment groups. Among strains the relative hyperactivity of BTBR and hypoactivity of 129S mice was evident, and these measures were not affected by dietary TRP manipulation. Taken together, our findings support the hypothesis that social behavior is highly sensitive to dietary TRP intake in these mouse strains commonly used in autism research. Based on these findings, studies of conditions under which TRP supplementation may improve sociability deficits, and studies of relevant biomarkers of low central 5-HT stores are warranted.

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Poster

564. Social Behavior: Neuropharmacology

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Topic: F.03. Motivation and Emotion

Support: Bridge Funds

Title: The role of dopamine in social processing and behavior

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Abstract: Social behavior, including attraction to and evaluation of potential mates, is critical for reproduction. While the brain areas implicated in social behavior have been identified, it is unclear how they communicate with areas involved in reward and reinforcement. The ventral striatum, including the shell of the nucleus accumbens (NAc shell) and the olfactory tubercle (OT), has been implicated in the coding of rewarding stimuli. In addition, these areas can be further sub divided into medial and lateral components, the medial areas implicated in social processing, while the core, or lateral, areas are not. We hypothesize that the interactions of these

two networks to regulate appropriate social processing requires dopamine (DA) in the reward and reinforcement circuit. To test this hypothesis, adult male, sexually naïve Syrian hamsters (Mesocricetus auratus) were given systemic intraperitoneal (i.p) injections of cis-(z)-flupenthixol (CZF), a broad spectrum DA antagonist prior to a non-contact partner preference test. Time spent investigating the male or female live stimuli were then recorded. We found that low doses of CZF did not affect opposite-sex preference, while higher doses caused a non-specific reduction in investigation. Centrally, injections of 6-OHDA were targeted to the NAc shell, OT and the NAc core, as a control. Pilot histological data shows DA depletion with 6-OHDA, as measured by a decrease in tyrosine hydroxylase staining; behavioral data is currently being collected on several tests. These include a non-contact partner preference test as well as a contact preference test, where the subjects can interact directly with tethered male and female stimuli. In addition, subjects are tested for possible alterations in odor discrimination or general changes in motivation.

Disclosures: M.J. Levy: None. **A. Petrulis:** None.

Poster

564. Social Behavior: Neuropharmacology

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Support: KAKENHI (23683021)

Takeda Science Foundation

Swiss National Science Foundation 3100A0-117816

Title: Glutamate input in the dorsal raphe nucleus as a determinant of escalated aggression in the male mouse

Authors: *A. TAKAHASHI[1], R. X. LEE[3], T. IWASATO[2], S. ITOHARA[4], H. ARIMA[5], B. BETTLER[6], K. A. MICZEK[7], T. KOIDE[1]
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Abstract: The adaptive nature of aggressive behavior in social animals accounts for its prevalence throughout the animal kingdom. However, when aggressive behavior exceeds the species-typical level, called escalated aggression, it is no longer adaptive for animals. The dorsal raphe nucleus (DRN) has been linked to adaptive and maladaptive aggression, and this area contains the largest accumulation of 5-HT neuronal cell bodies in the brain. However, little is known about the modulation of the DRN by other neurotransmitters when an animal is engaging either in species-typical aggression or escalated maladaptive aggression. In this study, we used genetic and pharmacological tools and *in vivo* microdialysis to identify a key neural modulator of the DRN that determines the level of aggression of male mice. Previously, we have shown that local administration of GABAB receptor agonist baclofen into the DRN escalated aggressive behavior of male mice. Here, by using 5-HT neuron specific GABAB receptor knockout mouse, we first demonstrate that the site of action for baclofen to escalate aggression are the GABAB receptors on non-5-HT neurons. Then, we found that intra-DRN baclofen increases glutamate release within the DRN. Microinjection of L-glutamate into the DRN caused dose-dependent escalation of attack bites. *In vivo* microdialysis revealed that there was an increase of glutamate release in the DRN when animals were engaging in aggressive behavior, and the level of glutamate was further increased when the animal engaged in escalated level of aggressive behavior after social instigation. We conclude that our results demonstrated an enhanced glutamatergic input into the DRN which determines the level of aggression in male mice. We will also report our current attempt to measure the change of 5-HT release during species-typical aggressive behavior and during escalated aggression.

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Poster

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Topic: F.03. Motivation and Emotion

Support: KAKENHI 23500395

KAKENHI 26430020

Title: Oral intake of monosodium glutamate during the period of development effects on social behavior mediated by gut-brain communication in an ADHD model rat

Authors: *H. HIDA, Y. YOKOYAMA, R. MARUMOTO, Y. SHIMIZU, Y. UEDA, S. MISUMI, A. ISHIDA, C.-G. JUNG
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Abstract: Dysfunction of mesocorticolimbic dopaminergic system such in the amygdala (Amy) and prefrontal cortex (PFC) is related to emotional regulation in attention-deficit hyperactivity disorder (ADHD), which is characterized by hyperactivity, impulsivity and inattention. Using an ADHD model rat, spontaneously hypertensive rat (SHR), we previously revealed that administration of a taste substance for umami increased sociality when rats were grown in single isolated condition with monosodium L- glutamate (MSG) for 5 weeks from postnatal day 25 (P25) to P60: parameters for sociality (sniffing time and riding) were significantly reduced in MSG-treated rats in social interaction test. However, the mechanism of the effect on enhanced sociality by MSG administration during the period of development remains unclear. Expression of dopamine receptor expression (D1R, D2R) and oxytocin receptor (OxtR) was first investigated in the Amy and PFC using real-time PCR. Both D1R and D2R were significantly increased in Amy by MSG intake while those was not changed in PFC. On the other hand, OxtR was significantly decreased in Amy while it increased in mPFC. We then investigated the effect of MSG on brain-gut communication via vagus nerve: transection of gastric branch of vagus nerve (vagotomy) was carried out at P25. In social interaction test, increases of sniffing time and riding number to the same level as H2O-treated control were shown by vagotomy. However, no difference was observed in open-field test. Data suggest that MSG intake during the period of development effects on the formation of emotional behavior by the afferent of vagus nerve from the gastrointestinal tract receptor, relating to altered expressions of D1A and D2A and OxtR in Amy and mPFC.

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Poster

564. Social Behavior: Neuropharmacology

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Topic: F.03. Motivation and Emotion

Support: KAKENHI 23500395

KAKENHI 26430020

Title: Cocaine- and amphetamine-regulated transcript in the central nucleus of amygdala is enhanced by environmental enrichment in ADHD model rat

Authors: *Y. SHIMIZU, Y. YOKOYAMA, S. MISUMI, A. ISHIDA, C.-G. JUNG, H. HIDA
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Abstract: Spontaneously hypertensive rat (SHR) is an animal model for attention deficit hyperactivity disorder (ADHD). We found that environmental enrichment (EE) for 5 weeks from postnatal day 25 (P25) to P60 decreased hyperactivity and anxiety-like behavior in SHR. We also showed that expression of cocaine and amphetamine regulated transcript (CART) is enhanced by EE in the medial frontal cortex (mPFC) and amygdala (Amy) of SHR. As CART peptide is a factor that effects on locomotion activity, anxiety and stress, we challenged to confirm the localization of CART in mPFC and Amy, trying to answer the question how the increase of CART by EE relates to decreased emotional behaviors in SHR. SHR grown for 5 weeks from P25 was fixed with 4% PFA for CART immunostaining. It revealed that many CART-positive cells was observed in the Amy, especially only in the central nucleus of amygdale (CeA), of SHR that were grown in both EE and standard environment (SE). On the other hand, a few CART positive cells was detected in the mPFC only EE-grown rat. Confirmation of the subtype of CART-positive cells revealed that GABAergic cells in the CeA are not immnoreactive to parvalbumin and calretinin. It also revealed that some of CART-positive cell was colocazied with tyrosine hydroxylase positive fibers in the CeA. Data suggested that EE induced CART expression in the mPFA and CeA and that CART in the CeA localized in GABAergic neurons that is not stained for parvalbumin and calretinin, probably relating to decreased hyperactivity and anxiety-like behavior in SHR.

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Poster

564. Social Behavior: Neuropharmacology

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Topic: F.03. Motivation and Emotion

Support: R25 NS080684

DOD/CDMRP Autism Idea Award

Title: Berberine blocks 'uptake 2' and enhances mouse sociability

Authors: *A. SANCHEZ[1], C. SMOLIK[1], T. PHAM[2], K. LALANI[2], G. G. GOULD[1]
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Abstract: Impaired social behavior is a treatment-resistant core symptom of autism. It can manifest in several forms, including indifference to social engagement, social anxiety or empathy deficits. The relative lack of therapeutic efficacy of selective serotonin reuptake inhibitors (SSRIs) to improve social behavior has brought into question their utility as a treatment for autism. Given these limitations associated with treating sociability deficits by targeting the serotonin transporter (SERT), our goal was to characterize the effects of blocking auxiliary 'uptake 2' transporters of serotonin on social and repetitive behaviors in mice. Organic cation (OCT) transporters are among the uptake 2 transporters found in the brain. In three-chamber sociability tests, the uptake 2 transporter blocker decynium-22 (D-22) improves social behavior in otherwise socially-impaired BTBR T+/tf mice. The alkaloid antibiotic berberine has antidepressant-like properties in mice, and also it is both a blocker and substrate of OCTs. Given this, we hypothesized that berberine administration might improve the sociability of BTBR mice, as D-22 does. Indeed, berberine significantly increased BTBR preference for social interactions and social sniffing ($p < 0.05$, N=8-9). Berberine had no impact on the typical sociable behavior of C57BL/6 mice, nor did it alter repetitive marble burying in either strain. We also compared these behaviors in naïve C57BL/6 male mice from different suppliers (Harlan vs. Jackson), and found no significant differences, suggesting that mice from either source can be used interchangeably. Our findings confirm that systemic uptake 2 blockade is a promising strategy for improving social behavior warranting further investigation as a treatment for sociability impairments.

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Poster

564. Social Behavior: Neuropharmacology

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Topic: F.03. Motivation and Emotion

Support: Bridge Funds

Title: GABAergic and glutamatergic connections in the social neural network

Authors: *A. R. BURNS, A. PETRULIS
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Abstract: A conserved network of brain regions is responsible for mediating social behavior in rodents. The bed nucleus of the stria terminalis (BNST), in conjunction with the medial amygdala (MA), is a critical node in this social neural network, integrating hormonal and chemosensory cues. The MA is a heterogeneous structure with the anterior portion (MeA) receiving primarily chemosensory information, whereas the posterior dorsal section (MePD) responds predominately to hormonal cues; this division is preserved in the BNST. The posterointermediate BNST (BNSTpi) receives a large input from the chemosensory MeA whereas the posteromedial BNST (BNSTpm) has numerous steroid-sensitive cells and receives projections from the MePD. However, the chemical nature (GABAergic or glutamatergic) of the connectivity involved in the processing of social information is unknown. To determine if the chemical phenotype of MA projection neurons may encode different social stimuli, we injected the retrograde tracer cholera toxin beta conjugated to Alexa Fluor 594 (CTB) into both the BNSTpm and BNSTpi of male Syrian hamsters. These males were then exposed to one of three conditions, a live male stimulus, a live female stimulus, or no stimulus (a clean cage), in order to induce c-fos expression to identify those neurons responding to a particular stimulus. To our knowledge, there are no available *in situ* hybridization probes for inhibitory or excitatory markers made in hamster. Therefore, we generated Syrian hamster specific mRNA probes for both vGlut2 (a marker of glutamatergic neurons) and GAD65 (a marker of GABAergic neurons). Using these probes, we colocalized labeling for vGlut2 or GAD65 with immunohistochemistry for Fos, and CTB labeled neurons to resolve the chemical nature of neurons involved in processing salient (female) vs. less-salient (male) social cues. Ongoing analyses will determine the percentage of GABAergic and glutamatergic MA neurons that project to the BNST and that are activated by different social cues in male Syrian hamsters.

Disclosures: **A.R. Burns:** None. **A. Petrulis:** None.

Poster

564. Social Behavior: Neuropharmacology

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Title: Adenosine receptor antagonists differentially modulate ethanol-induced impairments on social interaction and recognition in mice

Authors: *M. CORREA[1], L. LÓPEZ-CRUZ[1], N. SAN MIGUEL[1], P. BAYARRI[1], C. CARRATALÁ[1], J. LUCERÓN[1], J. MEDRANO[1], L. DIAZ[1], L. MONFERRER[1], Y. BAQI[2], C. E. MÜELLER[2], J. D. SALAMONE[3]
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Abstract: Caffeine is a non-selective adenosine A1 and A2A receptor antagonist that increases attention and produces anxiety at high doses, while ethanol, which increases adenosinergic tone, has the opposite effects on these behaviors. Adenosine A1 receptors are broadly expressed in the brain, while A2A receptors are localized in specific brain regions such as striatum and olfactory bulbs, which are involved in behavioral activation, exploration, and social interaction in rodents. Social interaction and recognition measures evaluate the natural preference of an animal for exploring conspecific animals versus objects or familiar animals versus novel animals. In the present work several markers of social interaction were evaluated after administration of caffeine, CPT (A1 antagonist) or MSX-3 (A2A antagonist) alone or in combination with ethanol. Anxiolytic doses of ethanol decreased social interaction and impaired recognition of familiar conspecifics the following day. However, caffeine at anxiogenic doses also reduced social interaction and impaired recognition. The selective receptor antagonists CPT and MSX-3 significantly increased social interaction and did not affect recognition. Surprisingly, a dose of ethanol (1.0 g/kg) that reduced social interaction on its own partially reversed the impairing effects of a high dose of caffeine (30 mg/kg). This reversal was also observed for the memory impairments, although at lower doses. MSX-3 completely reversed social interaction and recognition deficits induced by a high dose of ethanol. Although the A1 receptor antagonist CPT had no effect on ethanol-induced social interaction deficits, it had a positive effect on restoring recognition. Thus, A2A receptors seem to be more important than A1 receptors for mediating caffeine-ethanol interactions in social exploration. However, impairments in memory induced by ethanol could be restored by both A1 and A2A receptor antagonism, indicating a difference between these two processes (exploration and memory) in terms of the involvement of specific adenosine receptors and their interaction with ethanol. It is possible that distinct brain circuits mediate social exploration and recognition, and that these circuits are differentially modulated by drugs acting on A1 and A2A receptors.

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Poster

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NIMH (K01MH097841 to IE R00MH085946 and R01MH100292 to VS)

NIH Office of the Director (DP2MH100011)

Title: Excitation of D2-expressing cells in prefrontal cortex leads to reduced social behavior in mice

Authors: *C. K. HANSEN, I. ELLWOOD, T. PATEL, F. DAVATOLHAGH, V. SOHAL
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Abstract: Prefrontal cortex plays a key role in many cognitive functions including social behavior. In layer V of prefrontal cortex (PFC), our group has previously characterized two subtypes of pyramidal neurons which project to different areas and either express D1 or the D2 receptors [1]. The D2-expressing subtype exhibits an increase in excitability following D2 receptor (D2R) activation. Since dopamine is involved in prefrontal function and excessive dopamine and/or D2R activation may believed to contribute to symptoms of schizophrenia, the aim of this study was to determine how excitation of these two cell types affects social behavior. Adult C57BL6 male mice were implanted with guide cannulas, and D1R or D2R agonists were infused into right PFC. D1 Cre or D2 Cre animals were injected with either 750 nl of AAV5-DIO-ChR2-YFP or 1500 nl of AAV5-DIO-eNpHR-YFP into right PFC and implanted with either light fibers or guide cannulas. After 4-6 weeks of virus-mediated expression, animals underwent behavioral testing with 473 nm laser, or 532 nm laser stimulation. We tested animals using a social interaction assay in which we introduce a novel juvenile mouse into the home cage of the subject mouse. Following a 5 min break, we introduced a novel object into the home cage for 4 min period. Local infusion of the D2R agonist quinpirole (3 ug) into PFC of adult mice led to reduced social exploration and increased novel object exploration. Optogenetic excitation of prefrontal D2-expressing cells also disrupted social exploration. Conversely, optogenetic inhibition of prefrontal D2-expressing cells could rescue the quinpirole-induced social dysfunction. Infusion of the D1R agonist, SKF-38393, into PFC did not affect social exploration, but increased novel

object exploration. Optical activation of D1-expressing cells caused no change in social behavior, but increased novel object exploration. In conclusion, our results shows that excitation of D2-expressing - but not D1-expressing - cells in PFC leads to a specific reduction in social function, and suggest a mechanism in which D2R activation excites D2R-expressing neurons. These results are consistent with the hypothesis that excessive activation of D2Rs may contribute to prefrontal dysfunction in disorders such as schizophrenia. 1. Gee, S. *et al. J. Neurosci.* **32,** 4959-71 (2012).

Disclosures: C.K. Hansen: None. **I. Ellwood:** None. **T. Patel:** None. **F. Davatolhagh:** None. **V. Sohal:** None.

Poster

564. Social Behavior: Neuropharmacology

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 564.10/VV38

Topic: F.03. Motivation and Emotion

Support: NIH P50MH100023

Silvio O. Conte Center

Title: Dual, intra-amygdala injection-recording technique to explore oxytocin-mediated changes in social behaviors and neural activity in macaque monkeys

Authors: *P. PUTNAM[1], K. LASZLO[2,3], L. DIECKMAN[3], J.-P. WIEGAND[3], J. ROMAN[3], G. LACY[3], K. GOTHARD[3]
[1]Neurosci., Univ. Of Arizona, Tucson, AZ; [2]Med. Sch., Univ. of Pécs, Pécs, Hungary; [3]Univ. of Arizona, Tucson, AZ

Abstract: The amygdala in primates is critical for normal social behavior, including emotional regulation and evaluation of social stimuli. The amygdala is bidirectionally connected to areas of the primate brain that contain the highest density of oxytocin receptors (nucleus basalis, ventromedial hypothalamus, etc.) Though intranasal administration of oxytocin modulates blood flow in the amygdala of humans performing social tasks, the role of the amygdala in mediating oxytocin-dependent changes in social behavior remains unclear. We have developed a dual recording-microinjection method, allowing delivery of oxytocin into different nuclei of the amygdala while monitoring single neuron activity inside and outside its diffusion radius. This

technique allows us to compare social behaviors and neural activity in the amygdala elicited by intranasal and intra-amygdala administration of oxytocin. We found that intranasal administration of oxytocin enhanced general interest in videos with social content (quantified as total looking time at the monitor) and the frequency of gaze-following saccades in the first monkey tested. Gaze-following saccades were identified based on an ethogram of the viewer's scanpaths, in which we manually identified the region targeted by the saccades of the viewer monkey. Gaze-following saccades met the following criteria: viewers had to first fixate on the eyes of the movie monkey and then saccade within +30 degrees of the movie monkey's line of sight Intra-amygdala administration of oxytocin also increased the frequency of gaze-following saccades and the time spent by the viewer monkeys fixating the eyes of monkeys and humans presented in the videos. The heart rate of the viewer monkey gradually decreased throughout the presentation of videos after receiving intranasal saline but remained elevated after intranasal oxytocin. Ongoing experiments will determine whether intranasal or intra-amygdala administration of oxytocin increases the activity of neurons in the amygdala specialized for processing faces, facial expressions, and eye contact.

Disclosures: P. Putnam: None. **K. Laszlo:** None. **L. Dieckman:** None. **J. Wiegand:** None. **J. Roman:** None. **K. Gothard:** None. **G. Lacy:** None.

Poster

564. Social Behavior: Neuropharmacology

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 564.11/VV39

Topic: F.01. Human Cognition and Behavior

Support: NIH

NARSAD

Stanley

MSCRF

SR/RUSK

Title: Anterior insula mediates social behaviors that are finely regulated by retinoic acid and 5-HT2C receptor in its layer 5 pyramidal neurons

Authors: *S.-H. KIM[1], M. D. RANNALS[2], J. MOORE[1], B. J. MAHER[2], A. SAWA[1]
[1]Psychiatry and Behavioral Sci., Johns Hopkins Univ., BALTIMORE, MD; [2]Lieber Inst., BALTIMORE, MD

Abstract: Our group is interested in how social behavior is mediated by fine regulation of neurochemicals in complex neural networks. Human brain imaging studies have suggested that anterior insula (AI) might be an important hub involved in social and affective behaviors. We hypothesized that molecules that are specifically enriched in this brain region may play a pivotal role in social behavior. From this viewpoint, we underscored Cyp26B1, a retinoic acid (RA)-degrading enzyme. To address our working hypothesis, we locally modulated the expression of Cyp26B1: its knockdown in adult mice resulted in marked increase in RA signaling in the AI and significant behavioral deficits in social recognition/memory paradigms, but not in non-social behaviors. At the electrophysiology level, this genetic manipulation in AI led to a significant reduction of spontaneous excitatory postsynaptic currents (EPSCs) in the layer 5 pyramidal neurons recorded from acute brain slices. To address whether the attenuated neuronal activity of the pyramidal neurons underlies social behavior deficits, we utilized an optogenetic approach and found that deficits in social behaviors elicited by Cyp26B1 knockdown in AI can be normalized by stimulation of layer 5 pyramidal neurons in AI *in vivo*. Furthermore, systemic administration of oxytocin (OT) and an agonist for 5-HT2C receptor could rescue the social behavior deficits, however vasopressin and a 5-HT2C receptor antagonist did not. The 5-HT2C receptor agonist normalized the electrophysiological deficits of the AI pyramidal neurons elicited by knockdown of Cyp26B1, but OT did not. Taken together, we now propose a pivotal role of AI for social behaviors that are finely regulated by RA and 5-HT2C receptor in the layer 5 pyramidal neurons.

Disclosures: S. Kim: None. **M.D. Rannals:** None. **J. Moore:** None. **B.J. Maher:** None. **A. Sawa:** None.

Poster

565. Vocal Communication in Songbirds: Sensory and Motor Mechanisms II

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 565.01/VV40

Topic: F.04. Neuroethology

Support: SFB665

CONACYT

Title: Is the speech phenotype of the R553H FoxP2 mutation really due to haploinsufficiency? Insights from the zebra finch (Taeniopygia guttata) FoxP subfamily

Authors: *E. MENDOZA, N. ARPENIK, U. KOBALZ, C. SCHARFF
Verhaltensbiologie, Freie Univ., Berlin, Germany

Abstract: The heterozygous R553H mutation in the DNA binding domain of the Forkhead transcription factor FoxP2 impairs acquisition of speech and language. The cause of this phenotype is thought to be haploinsufficiency of FoxP2. Transcriptional activity of FoxP2 requires dimerization with FoxP2 or with paralogues FoxP1 and FoxP4. It is thus possible that the R553H mutation not only affects FoxP2 quantity, but also interferes with the ability to dimerize. We therefore studied (1) the co-localization of FoxP2 with FoxP1 and FoxP4, (2) dimerization abilities of zebra finch FoxPs, (3) whether the avian version of the FoxP2-R553H mutation can form dimers with wild type FoxP proteins and (4) if dimers including FoxP2-R553H mutation have transcriptional activity. In the zebra finch striatum significantly more neurons expressed all three FoxPs than other combinations, shown via triple immunohistochemistry. Co-immunoprecipitation assays (Co-IP) *in vitro* and *in vivo* revealed that all FoxP subfamily members of the zebra finch can homo- and hetero-dimerize. Co-IPs with zebra finch FoxP2-R553H or with FoxP2- ΔFoxBox (lacking the Forkhead box) showed that they can interact with wild type FoxP1 or FoxP2 or FoxP4 proteins. Co-transfecions of FoxP2-ΔFoxBox with any of the wild type FoxP proteins in HeK cells rescued the abnormal cytoplasmatic localization of singly overexpressed FoxP2-ΔFoxBox and shifted it to the nuclear localization typical for transcription factors. Luciferase assays revealed that zebra finch FoxP1, FoxP2 and FoxP4 regulate the SV40 promoter in a similar manner as reported for the mouse homologues. Overexpression of the R553H-FoxP2 in combination with wild type FoxPs affected the repression of the transcriptional activity. Together these data provide the first evidence that the FoxP2-R553H mutation, and possibly other FoxP mutations, affect all FoxPs co-expressed in the same neurons through dimerization, leading to an additional dominant negative effect.

Disclosures: **E. Mendoza:** None. **C. Scharff:** None. **N. Arpenik:** None. **U. Kobalz:** None.

Poster

565. Vocal Communication in Songbirds: Sensory and Motor Mechanisms II

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 565.02/VV41

Topic: F.04. Neuroethology

Title: Changes in FoxP2 expression intensities during functional integration of adult-generated neurons into avian basal ganglia circuits

Authors: *J. F. KOSUBEK, C. SCHARFF
Free Univ. Berlin, Berlin, Germany

Abstract: In zebra finches newly generated neurons are recruited constantly to various brain regions during adulthood. Adult-generated medium spiny neurons (MSNs) in the striatal song nucleus Area X and their progenitors in the ventricular zone express the transcription factor FoxP2. Foxp2 is involved in neural circuit formation in mice by regulating the transition of neural precursors to neurons during embryonic cortical development. Foxp2 also orchestrates maintenance and detachment of neuroepithelial progenitors in the spinal cord, pointing to its potential role adult neurogenesis. In juvenile songbirds FoxP2 is highly expressed in Area X during the phase of song learning and virally mediated knockdown in this sensitive period leads to inaccurate song imitation. FoxP2 knockdown in Area X in adult birds prevents social modulation of song variability, supporting its importance in song production. MSNs in Area X show two FoxP2 expression intensities: the proportion of intense FoxP2 immunoreactive MSNs is higher in juvenile birds and in newly recruited MSNs in adults, whereas low-expressing MSNs become more numerous as the birds age. How FoxP2 expression levels shift in newly recruited MSNs during maturation is still unclear. We hypothesized that FoxP2 levels in new MSNs decrease as they integrate into existing neural circuits and get activated during singing. We analyzed newly generated Area X MSNs in adult male zebra finches for FoxP2 intensities and immediate early gene expression as a marker of activation at several time points after receiving a DNA synthesis marker. Preliminary results indicate that the proportion of activated FoxP2-expressing new MSNs increases during maturation as does the proportion of activated new MSNs expressing low levels of FoxP2. These results suggest that high FoxP2 levels are necessary for recruitment, integration and activation of new MSNs into songbird Area X.

Disclosures: J.F. Kosubek: None. **C. Scharff:** None.

Poster

565. Vocal Communication in Songbirds: Sensory and Motor Mechanisms II

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 565.03/VV42

Topic: F.04. Neuroethology

Support: SFB 665

DFG (EXC 257 NeuroCure)

Title: The Reelin receptor VLDLR is a direct target of FoxP2 and regulated developmentally and by singing

Authors: *I. ADAM, E. MENDOZA, U. KOBALZ, S. WOHLGEMUTH, C. SCHARFF
Free Univ. Berlin, Berlin, Germany

Abstract: Mutations of the transcription factor FOXP2 lead to severe speech and language impairments in humans. Structural as well as functional imaging studies indicate that the striatal basal ganglia are especially affected by the mutation. The FOXP2 gene codes for a transcription factor, which is strikingly conserved among vertebrates regarding its sequence as well as its expression pattern. In songbirds it is expressed in a striatal nucleus of the avian basal ganglia, which is necessary for song learning and adult singing. In juvenile zebra finch males FoxP2 is differentially regulated during song learning and in adults by singing. Down-regulation leads to impaired song learning, affects dendritic spine formation and prevents social context induced song plasticity. So far no direct target gene relevant for song learning and song production has been described. We investigated whether the Reelin receptor VLDLR is a direct FoxP2 target gene in zebra finches. VLDLR has been shown to be a direct target of FOXP2 in humans, moreover in songbirds it is regulated by singing. Here we show that FoxP2 can bind to the promoter region of the avian VLDLR gene and activate it *in vitro*. Additionally, VLDLR is co-regulated with FoxP2 during song learning and following adult song performance in zebra finches. Finally we show that experimental down-regulation of FoxP2 in Area X of juvenile males leads to down-regulation of VLDLR. In mice the Reelin pathway is known to be involved in learning and memory e.g. by altering synaptic transmission in the striatum. Vldlr itself is located at the synapse and is able to physically interact with the NMDA receptor-complex. Taken together these results are in line with previous findings suggesting that FoxP2 is involved in synaptic function of striatal spiny neurons. We thus identify a direct target of FoxP2 with putative synaptic function in spiny neurons, which could affect signal propagation through the avian basal ganglia.

Disclosures: I. Adam: None. **E. Mendoza:** None. **U. Kobalz:** None. **S. Wohlgemuth:** None. **C. Scharff:** None.

Poster

565. Vocal Communication in Songbirds: Sensory and Motor Mechanisms II

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 565.04/VV43

Topic: F.04. Neuroethology

Support: KSU

Title: FoxP2 expression in the brain after chick calls

Authors: *C. BESSHO[1], T. TSUZUKI[2]
[1]Dept Physics, [2]Kyoto Sangyo Univ., Kyoto, Japan

Abstract: FOXP2 is the first gene linked to human speech and was more expressed in the striatum nucleus Area X of zebra finch than the surrounding tissue at post-hatch days (S. Haesler et al. ,2004). Haesler et al.(2007) showed that zebra finches with reduced FoxP2 expression levels in Area X imitated tutor songs incompletely and inaccurately . Previously, we reported that l-LTP was induced in Area X of brain slice of juvenile male Bengalese finch by five trains of theta stimulus (5Hz, 1S) every three minutes. We showed that Foxp2 was more strongly expressed in the induced brain slices than control slices (C. Bessho et al .2009). Mice pups vocalize in ultrasonic region when they are removed from their mother. Disruption of the Foxp2 gene of mice caused an absence of ultrasonic vocalizations (ult. voc.) (W. Shu et al. 2005). Foxp2 mediates sex differences in ult.voc. by rat pups (J. Bowers et al. 2013). These two studies indicate that FoxP2 expression has an important role in the separated call from their mother. We showed that the Foxp2 in the brain of Degu pups were more strongly expressed in the separated pups from their mother than control (C. Bessho et al .2012). Chick is a non-song-learning bird but it calls after incubation. To understand a relationship between FoxP2 expression and chick call, we did immunohistochemistry of chick brain. and RT-PCR of mRNA. Each baby chick was taken out of the cage and placed for thirty minutes in another litter. A digital voice recorder was placed over the pup. Two minutes of recording commenced after the pup vocalized. Total RNA isolated from the forebrain and hind brain was reverse transcribed to cDNA and FoxP2 gene was amplified by PCR and amplified DNA were separated on agarose gel. Another half brain was fixed in 10 % formalin Neutral Buffer Solution (pH 7.4) over night at 4°C . Slices , 40μm thick, were reacted with Foxp2 antibody, then washed in PBS and were stained with Alex488 second antibody. Green fluorescence of slices in blue light (490 nm) was observed by fluorescence microscope. Preliminary data showed that there was no difference of FoxP2 expression between separated and control pups.

Disclosures: **C. Bessho:** A. Employment/Salary (full or part-time):; KSU. **T. Tsuzuki:** None.

Poster

565. Vocal Communication in Songbirds: Sensory and Motor Mechanisms II

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 565.05/VV44

Topic: F.04. Neuroethology

Support: NIH Grant GM092842

NIH Grant NS059755

Title: ZEBrA Redux: An improved digital atlas for exploring brain gene expression in the adult male Zebra Finch (www.zebrafinchatlas.org)

Authors: *C. V. MELLO[1], P. V. LOVELL[1], J. B. CARLETON[1], M. WIRTHLIN[1], B. SNIDER[2]
[1]Dept. of Behavioral Neurosci., [2]Ctr. for Spoken Language Understanding, Oregon Hlth. Sci. Univ., Portland, OR

Abstract: The goal of project ZEBrA (Zebra Finch Expression Brain Atlas; www.zebrafinchatlas.org) is to make available to the public an online collection of high-resolution (0.46 μm/pixel) digital images representing the expression patterns for a large set of transcripts in brain of the adult male zebra finches (T. guttata), a representative songbirds species. As a continuously expanding resource, ZEBrA currently houses more than 2,000 images (>100 GB) corresponding to ~500 genes that are expressed in the zebra finch brain, including the major nuclei that comprise the song system. Each gene has been selected based on its relevance for understanding the physiology of the song system (e.g. neurotransmitter receptors, ion channels, signaling systems), or its importance for clarifying issues of vertebrate brain evolution and homology. Following our initial launch in June of 2012, ZEBrA has received thousands of visits from investigators throughout the world. Our on-going mission is to continue to release additional batches of images until we have covered ~1,500-2,000 genes. To facilitate the investigation of brain gene expression, the latest public release of ZEBrA (November 2013) included several major improvements, as well as the addition of new features that have been designed to help users to identify genes of interest, and to explore their expression in the context of songbird neuroanatomy. Major features of ZEBrA include: (1) The *In situ* database - the actual collection of high-resolution *in situ* hybridization images presented along with annotated drawings derived from Karten/Mitra Histological Atlas, (2) A Gene Family Search Page - A feature that facilitates searches for genes based on their membership in specific gene families; (2) A Histological Atlas Browser - A set of 18 annotated drawings prepared in registration with Nissl- and Myelin-stained images of sagittal brain sections derived from the Karten/Mitra atlas, (3) A Neuroanatomical Marker Search Page - A search engine that allows users to retrieve a list of genes that are markers of a given structure, or of multiple structures. ZEBrA represents the

most comprehensive resource available for investigating the brain distribution of genes involved in the physiology, development, and maintenance of functional circuits in the brain of songbirds.

Disclosures: C.V. Mello: None. **P.V. Lovell:** None. **J.B. Carleton:** None. **M. Wirthlin:** None. **B. Snider:** None.

Poster

565. Vocal Communication in Songbirds: Sensory and Motor Mechanisms II

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 565.06/VV45

Topic: F.04. Neuroethology

Support: NIH Grant MH066128

Title: Calcitonin gene related peptide (CGRP) increases neuronal activity in the zebra finch premotor song nucleus RA

Authors: *C. A. WILLIAMS[1], A. F. GARCIA[2], D. J. PERKEL[1,3,2]
[1]Dept. of Biol., [2]Grad. Program in Neurobio. and Behavior, [3]Dept. of Otolaryngology, Univ. of Washington, Seattle, WA

Abstract: An interconnected set of nuclei in the male zebra finch brain underlies the ability to memorize and accurately reproduce vocalizations known as song. These nuclei can be divided into a motor production pathway and a basal ganglia-like loop important for learning, known as the anterior forebrain pathway (AFP). The two pathways split early in the circuit and reconvene at the robust nucleus of the arcopallium (RA), a key candidate site of plasticity for song learning. How the output nucleus of the AFP, the lateral magnocellular nucleus of the anterior nidopallium (LMAN), influences the activity of RA is not well understood. While LMAN releases glutamate onto RA neurons, activating NMDA receptors and affecting song variability, LMAN neurons also express immunoreactivity for calcitonin gene related peptide (CGRP), a neuropeptide that is implicated in pain processing in the spinal cord as well as in migraines. We hypothesized that release of CGRP from LMAN terminals could affect electrical activity in RA. We applied exogenous CGRP to RA neurons in brain slices prepared from adult male zebra finches. Ionotropic glutamate and GABA receptors were blocked with kynurenic acid and picrotoxin, respectively. Extracellular single-unit recordings showed that CGRP (10 – 1000 nM) increased the spontaneous firing rate of RA neurons by 20-150%. The slow time course of the response was consistent with a peptide action. This response was reduced in the presence of either the

peptide CGRP antagonist $CGRP_{8-33}$ or the small molecule antagonist SB268262. In other systems CGRP activates adenylyl cyclase. Application of adenylyl cyclase activator forskolin to RA neurons increased firing rate, mimicking the effect of CGRP. High-frequency (150-400 pulses at 20-100 Hz) stimulation of LMAN afferent fibers resulted in a similar increase in RA neuron firing, rising over approximately 30 seconds and decaying over 10 min. Whole-cell recording showed that activation of LMAN fibers caused a slow inward current. These studies provide evidence that an endogenous neuropeptide in LMAN afferents, putatively CGRP, plays a role in modulating RA activity. It further provides a candidate mechanism to contribute to song plasticity. Finally, and more generally, this connection is a rare example of peptidergic neurotransmission in a cortex-like structure.

Disclosures: C.A. Williams: None. **A.F. Garcia:** None. **D.J. Perkel:** None.

Poster

565. Vocal Communication in Songbirds: Sensory and Motor Mechanisms II

Location: Halls A-C

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Program#/Poster#: 565.07/VV46

Topic: F.04. Neuroethology

Support: R03 HD068960

Busch Biomedical Grant

R01 DC008854

Title: Effects of statins on auditory memories, neurogenesis and neuronal morphology in the zebra finch

Authors: *S. C. TSOI[1], A. QURESHI[2], A. BARRIENTOS[2], U. V. AIYA[3], M. L. PIERCE[3], E. SOYMAN[3], D. STALBOW[2], S. RIBEIRO[2], D. S. VICARIO[3], C. L. PYTTE[1,2], M. L. PHAN[3]
[1]The Grad. Center, CUNY, New York, NY; [2]Queens College, CUNY, Flushing, NY; [3]Rutgers Univ., Piscataway, NJ

Abstract: Statins lower serum cholesterol by inhibiting the HMG-CoA reductase in the cholesterol synthesis pathway in the liver. Statins also cross the blood brain barrier and may decrease cholesterol synthesis in the brain. Statins are thought to be neuroprotective in the damaged brain; however, in the healthy brain, statin use has been associated with impaired learning and memory. One mechanism that might explain these findings is altered neurogenesis,

which requires the continual production of new membrane lipids, including cholesterol. Here, we investigated whether statins impact new neuron incorporation and electrophysiological response properties in the caudomedial nidopallium (NCM) of male zebra finches. NCM is a forebrain auditory region that is involved in discrimination and memory for the complex vocalizations of individual conspecifics and also receives new neurons in adulthood. Male birds were given oral doses (40 mg/kg) of either simvastatin, atorvastatin, pravastatin or water vehicle daily for 60 days. Beginning on treatment day 30, birds received injections of bromodeoxyuridine (BrdU, ~0.078 mg/g, IM, 3x/day for 3 days) to label mitotically active cells. After 60 days of statin/vehicle treatment, we recorded simultaneously from multiple electrodes in NCM of awake, restrained birds and compared multi-unit responses to novel conspecific songs and familiar songs that had been presented 20h earlier. Brains were then processed with immunohistochemistry to label BrdU and the neuron-specific protein Neu-N. A second series of sections was processed to label doublecortin (DCX) which is expressed by young neurons. To determine whether new neuron morphology was altered by statin treatment, we traced DCX-expressing cell bodies using NeuroLucida software and compared morphological measurements of soma size and shape between statin-treated and control birds. Counts of BrdU-labeled (30 day old) neurons or DCX-expressing (<30 day old neurons) in NCM showed no significant difference from vehicle controls for any of the 3 statins. However, in the HVC of the song motor pathway we found significant morphological differences in DCX-expressing cells between atorvastatin-treated and control birds. In statin-treated birds, auditory response magnitudes in NCM did not differ between novel and familiar song stimuli, but did differ as expected in vehicle-treated controls, suggesting that 20h neuronal memory may be impaired in statin-treated birds. These results suggest that the impaired ability to form new auditory memories is not associated with altered new neuron survival. Ongoing work is examining neuronal morphology in NCM.

Disclosures: **S.C. Tsoi:** None. **C.L. Pytte:** None. **A. Qureshi:** None. **A. Barrientos:** None. **D. Stalbow:** None. **U.V. Aiya:** None. **E. Soyman:** None. **M.L. Pierce:** None. **M.L. Phan:** None. **D.S. Vicario:** None. **S. Ribeiro:** None.

Poster

565. Vocal Communication in Songbirds: Sensory and Motor Mechanisms II

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 565.08/VV47

Topic: E.01. Neuroendocrine Processes

Support: NIH R00NS066179

NIH R01NS182179

Title: Song-inducible gene expression in the songbird auditory forebrain: evidence for sex differences, regional differences, and a role for estrogen synthesis

Authors: *A. A. KRENTZEL[1], L. REMAGE-HEALEY[2]
[1]Neurosci. and Behavior, [2]Psychological and Brain Sci., Univ. of Massachusetts Amherst, Amherst, MA

Abstract: Estradiol is an important neuromodulator for auditory processing, especially in vocal learning animals such as songbirds. Previous work has shown that when birds are hearing sounds, estradiol synthesis increases rapidly in the auditory forebrain in both males and females. Exogenous estradiol administration has also been shown to enhance auditory representations in the forebrain, and blocking local estradiol synthesis suppresses auditory processing and disrupts auditory-dependent behaviors. Neuroanatomical evidence is consistent with a role for local estradiol synthesis, since aromatase is expressed within neuron terminals in the auditory forebrain. Males have more terminal aromatase activity and expression in the auditory forebrain as compared to females, although it has not been explored whether there is a functional consequence of this sexual dimorphism. Also, the cellular mechanism for these acute actions is unclear, but available evidence is consistent with a non-classical signaling pathway. To explore these questions, we stained for Egr-1, a song-inducible immediate early gene, in brain sections from sixteen male and female zebra finches that received a single oral dose of fadrozole, an aromatase inhibitor, or saline control prior to song presentation. We observed regional dependent decreases in Egr-1 expression in fadrozole-treated males and females in aromatase-positive regions of the auditory lobule, consistent with a role for central estrogen synthesis in modulating audition. In posterior HVC shelf, we also observed a sex difference in which females responded with elevated Egr-1 immunoreactivity as compared to males, regardless of treatment. In alternate sections we also examined the distribution of phosphorylated CREB, a transcription factor that may regulate Egr-1 expression via non-classical estradiol signaling. Preliminary evidence indicates no significant effects of sex or aromatase inhibition on pCREB expression in the auditory forebrain, indicating that this is an unlikely transcriptional candidate for estradiol's influences on Egr-1 expression. These findings are consistent with a role for brain estrogens in modulating auditory representations in the forebrain, although a cell signaling mechanism remains to be elucidated.

Disclosures: A.A. Krentzel: None. **L. Remage-Healey:** None.

Poster

565. Vocal Communication in Songbirds: Sensory and Motor Mechanisms II

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 565.09/VV48

Topic: F.04. Neuroethology

Title: Song-mediated activation and habituation of the mTOR cascade in the zebra finch auditory cortex

Authors: *S. AHMADIANTEHRANI[1], S. E. LONDON[2]
[2]Dept. of Psychology, [1]Univ. of Chicago, Chicago, IL

Abstract: Inhibition of the highly conserved mechanistic target of rapamycin (mTOR) cascade attenuates learning and memory formation in multiple training paradigms. For adult zebra finches (Taeniopygia gutatta), learning and recognizing the individual songs of other conspecifics is integral to their social lives. Using this naturally occurring model, we set out to determine if song-mediated mTOR activation habituates to multiple presentations of the same song, an indication that this signaling pathway plays a role in song recognition learning, and, potentially, the formation of untrained, biologically relevant memories. We first confirmed the presence of this signaling cascade in the auditory cortex, the site of song recognition learning, using Western blot analysis for mTOR and its downstream target S6 Kinase (S6K). We next asked whether or not hearing song activates this signaling cascade. We found that a short song stimulus elicited a rapid and long-lasting phosphorylation, and thus activation, of both mTOR and S6K in this brain region. The auditory cortex includes higher order sensory processing areas analogous to the mammalian secondary auditory cortex, the caudomedial nidopallium (NCM) and caudomedial mesopallium (CMM). We used immunohistochemistry to determine the degree and anatomical location of mTOR activation in response to familiar and novel song in a song recognition procedure. On the first day, birds were individually exposed to repeated presentations of the training song for three hours. Twenty-four hours later, birds heard thirty-minute playbacks of the same song or a novel song, or were left in silence. Phosphorylation levels of the ribosomal protein S6, a downstream substrate of S6K, was lower in the NCM and CMM of birds who heard familiar, versus novel, song playbacks. Activation of mTOR following novel but not familiar song playbacks indicates that this cascade habituates in response to recognized song, and suggests that the mTOR cascade is involved in song recognition learning. The mTOR-mediated phosphorylation of S6 facilitates synaptic protein synthesis, a key component of learning and memory. Therefore, these findings lay the groundwork for investigations into the cellular mechanisms that drive mTOR-dependent learning and memorization of song and other socially meaningful behaviors.

Disclosures: S. Ahmadiantehrani: None. **S.E. London:** None.

Poster

565. Vocal Communication in Songbirds: Sensory and Motor Mechanisms II

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 565.10/VV49

Topic: F.04. Neuroethology

Support: NSF OMA-0835976

Burroughs Welcome Fund

Medical Foundation Smith Family Award

The Ministry of Trade, Industry and Energy (Grant number: 10041629)

Title: Motor and auditory coding of sub-syllabic transitions in zebra finch song

Authors: *Y. LIM[1,4], J. MARKOWITZ[2], T. J. GARDNER[3]
[2]Cognitive and Neural Systems, [3]Biol., [1]Boston Univ., Boston, MA; [4]Ctr. for Bionics, Biomed. Engin. Res. Inst., Korea Inst. of Sci. and Technol., Seoul, Korea, Republic of

Abstract: A recent study has indicated that the representation of time is non-uniform in the pre-motor cortex area HVC of zebra finches (*1*). However, the prior study concluded that inhomogeneities of spike probability are not easily explained in acoustic terms, but rather reflect extrema of air pressure and muscular tension controlling the syrinx. In this study, we defined 4 different automated methods for localizing points of high acoustic contrast in zebra finch syllables. We then examined how well these measures correlate with the firing times of HVC interneurons and HVC_X projection neurons. We found a significant correlation between HVC firing and the acoustic contrast scores. We next examined correlations between these acoustic contrast scores and firing times of neurons in the primary auditory cortex, area L2a. The recordings were performed in un-anesthetized male zebra finches listening to unfamiliar conspecific songs. We found that measures that perform well in explaining firing inhomogeneities in the HVC also perform well in predicting the firing times of primary auditory neurons. These results suggest that the primary auditory cortex may encode syllables in chunks that reflect the timing of motor commands that would be required to produce the same song. This result provides a low-level analog in the zebra finch for the motor theory of speech perception in humans (*2-6*). 1. A. Amador, Y. S. Perl, G. B. Mindlin, D. Margoliash, *Nature* **495**, 59-64 (2013). 2. A. M. Liberman, F. S. Cooper, D. P. Shankweiler, M. Studdert-Kennedy, *Psychological Review* **74**, 431-461 (1967). 3. A. M. Liberman, I. G. Mattingly, *Cognition* (1985). 4. A. M. Liberman, I. G. Mattingly, *Science* (1989). 5. A. M. Liberman, D. H. Whalen, *Trends*

Cogn Sci (Regul Ed) **4**, 187-196 (2000). 6. B. Galantucci, C. A. Fowler, M. T. Turvey, *Psychonomic Bulletin & Review* **13**, 361-377 (2006).

Disclosures: Y. Lim: None. **J. Markowitz:** None. **T.J. Gardner:** None.

Poster

565. Vocal Communication in Songbirds: Sensory and Motor Mechanisms II

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Topic: F.04. Neuroethology

Support: NIH Grant NS075044

Title: Recurrent circuitry shapes a sparse premotor neural sequence

Authors: *G. KOSCHE, D. VALLENTIN, M. A. LONG
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Abstract: In some neural systems, only a small fraction of neurons participate in each percept or movement (Wolfe et al., 2010), and this sparse coding scheme has been proposed to provide important theoretical (Barlow, 2009) and practical (Fiete et al., 2004) advantages for the network. In the song production nucleus HVC (proper name) of the zebra finch (Taeniopygia guttata), premotor (HVC(RA)) cells typically generate only one high-frequency burst at an invariant moment during the song (Long et al., 2010). Different HVC(RA) neurons burst at different moments, and together they form a sparse representation of time (Hahnloser et al., 2002) that ultimately leads to the patterned activation of song-producing muscles. The precise coordination of neural activity is essential to this process, but the circuit mechanisms that underlie the generation of sparse firing and the resulting behavioral sequences remain unclear (Gibb et al., 2009; Li and Greenside, 2006). Here we show that the activity of local circuit interneurons is crucial for establishing precise premotor commands as well as normal singing behavior. In agreement with previous findings (Mooney and Prather, 2005), we find that HVC(RA) neurons and interneurons are densely interconnected. Further, we demonstrate that the high-frequency bursts of HVC(RA) cells during singing strongly engage inhibitory interneurons through highly pervasive and facilitating synaptic connections, leading to a structured pattern of spiking that can dictate principal neuron firing times. These results suggest that inhibitory circuits can play a crucial role in defining the serial order and precise timing of neurons involved in generating skilled action.

Disclosures: **G. Kosche:** None. **D. Vallentin:** None. **M.A. Long:** None.

Poster

565. Vocal Communication in Songbirds: Sensory and Motor Mechanisms II

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W.M. Keck Foundation

Title: Contributions of higher-order auditory cortical areas to adult song maintenance in the zebra finch, Taeniopygia guttata

Authors: *H. SHOENHARD[1,2], N. F. DAY[3], Z. BURKETT[3], M. J. COLEMAN[2]
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Abstract: The brain must process raw sensory input until it is fit to inform adaptive behavior. In the case of the male zebra finch, Taeniopygia guttata, auditory input of the song that the bird is producing must feed back into the song motor control area, HVC, such that song can be reliably produced. Large scale changes in the auditory inputs via deafening lead to alterations in song. In addition, perturbations of distinct auditory input lead to changes in HVC activity (Roberts et al., 2012). Higher auditory processing occurs in a network of areas, including nucleus interfacialis (NIf) and the caudomesopallium (CM), which both project directly to, and together provide most of the auditory input to, HVC. In addition, CM contains a subregion, nucleus avalanche (Av), that is reciprocally connected to HVC and NIf (Akutagawa and Konishi, 2010). In an attempt to elucidate how higher-order processing in NIf and non-Av regions of CM could inform motor output, we bilaterally lesioned one or both of these areas and measured the effects upon long-term song production as compared with deafened and sham-deafened birds. As shown previously, long-term loss of NIf showed little effect upon song motor output. However, non-Av CM lesions produced a variety of alterations in song, including merging of adjacent syllables, addition of syllables, and loss of the last syllable of the motif. These effects sometimes took

several months to emerge after lesioning. CM lesions' effects upon syllable characteristics varied between birds. This heterogeneity could be due to differences in lesion location, although this is difficult to determine because CM's borders are not easy to distinguish. Our current analysis focuses on the effects of lesioning Av on song. We hope to discover how each of these areas' higher-order processing of auditory information informs the motor output entrained by HVC. This may ultimately help us discover to how highly specific sensory processing allows animals to accurately produce adaptive motor behaviors.

Disclosures: H. Shoenhard: None. **N.F. Day:** None. **Z. Burkett:** None. **M.J. Coleman:** None.

Poster

565. Vocal Communication in Songbirds: Sensory and Motor Mechanisms II

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Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 565.13/VV52

Topic: F.04. Neuroethology

Support: HHF #23103

Title: Hearing the call: HVC lesion disrupts auditory processing of conspecific calls in Bengalese finches

Authors: A. E. ASTON, C. M. URBANO, *B. G. COOPER
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Abstract: Auditory processing and vocal production in humans and songbirds are inextricably linked. We sought to explore whether frequency selective manipulations of sound production can induce frequency specific changes in auditory perception in adult male Bengalese finches (*Lonchura striata domestica*). Mean peak frequency of Bengalese finch song typically falls between 3-4 kHz. Damage to the left HVC (letters used as proper name) in Bengalese finches lowers the mean peak frequency of their learned song by 40-50% and they produce predominately lower frequency (< 2.2 kHz) syllables; whereas right HVC lesion results in a 20% decrease in peak frequency and a greater retention of syllables above 2.2 kHz compared to left HVC lesion birds. Thus, one can manipulate motor control of song frequency and evaluate whether such changes systematically alter auditory perception. If vocal production modulates auditory perception then lesion to left HVC would bias auditory processing toward lower frequencies. Birds with left or right HVC damage, or sham-treated control birds, were socially isolated for 24 hours. The birds were then exposed to four types of female contact call: a normal

call, a low-pass filtered (<2.2 kHz), a high pass filtered (>2.2kHz), and a synthetic, tonal high frequency (3-5 kHz) contact call. We measured mean number of calls and latency to call within 20s after presentation of each stimulus. We predicted a lesion by call type interaction effect, but this was not observed ($p = .75$). We found the mean number of calls produced by both lesion groups was significantly lower than sham birds ($p<.002$). There were also significant differences in mean number of calls as a function of calltype. Compared to the synthetic call, birds called significantly less to the normal call and the low frequency call ($p<.004$, .003, respectively). Latency to call was not significantly affected by HVC lesion. However, birds responded with the shortest latency to synthetic calls compared to all of the other three calltype categories ($p<.037$, $p<.039$, $p<.004$). Therefore, frequency of the contact call modulates response latency and mean number of calls produced. HVC lesion only reduces the number of calls produced after hearing a conspecific contact call and did not affect latency to call. We suggest that this pattern reflects the importance of HVC in auditory perception, but not sensory processing, of the contact call. These data complement the findings on song discrimination tasks, in which the functional integrity of HVC is required to identify songs, and further illustrate the importance of HVC in auditory processing of conspecific calls.

Disclosures: **A.E. Aston:** None. **B.G. Cooper:** None. **C.M. Urbano:** None.

Poster

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Topic: D.02. Auditory

Support: NIH DC02524

NSF

Title: A novel cell type mediates motor to auditory interactions necessary to feedback-dependent song plasticity

Authors: *E. HISEY, M. TANAKA, R. MOONEY
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Abstract: The transmission of motor-related signals to sensory regions of the brain is widely speculated to facilitate the learning and maintenance of complex motor skills. For example, in

humans, information flowing from speech motor cortex to the auditory cortex is theorized to facilitate speech learning and maintenance. Songbirds resemble humans in their capacity for vocal learning, but the identity and function of pathways that convey information from motor to auditory regions of the brain remain poorly understood. We recently described a novel cell type in the song premotor nucleus HVC of zebra finches that forms axon terminals in part of the auditory telencephalon (Avalanche, or Av). To begin to better characterize these Av-projecting HVC cells (i.e., HVC_{AV} cells), we made targeted whole cell current clamp recordings from HVC_{Av} cells and other HVC projection neuron (PN) types in brain slices. These recordings revealed that HVC_{AV} cells exhibited intrinsic electrophysiological properties that were distinct from other types of HVC PNs, consistent with their playing a functionally specialized role. Moreover, HVC_{Av} cells receive excitatory input from HVC PNs that project to downstream song motor regions, and thus HVC_{Av} cells could convey motor-related signals to the auditory system. One possibility is that HVC_{Av} cells transmit information to the auditory system that is used for the auditory feedback-dependent modification of song. If this idea is correct, then selectively killing HVC_{Av} cells should not affect previously learned songs, but should prevent song modification in response to changes in auditory feedback. To test this idea, we used an intersectional viral gene targeting method to selectively ablate HVC_{Av} cells in adult zebra finches that had learned to produce highly stable songs. Ablating HVC_{AV} cells had negligible effects on adult song structure, indicating that these cells do not play an essential role in song motor control even though they are embedded in a song premotor structure. However, whereas blocking auditory feedback by deafening triggers rapid degradation in spectral and temporal features of adult song, adult finches in which HVC_{Av} cells were ablated prior to deafening were able to maintain stable syllable sequences and individual syllables also exhibited reduced spectral degradation. These findings are consistent with a forward model in which HVC_{Av} cells transmit motor-related signals to the auditory system to facilitate the feedback-dependent modification of learned vocalizations.

Disclosures: **E. Hisey:** None. **M. Tanaka:** None. **R. Mooney:** None.

Poster

565. Vocal Communication in Songbirds: Sensory and Motor Mechanisms II

Location: Halls A-C

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Topic: F.04. Neuroethology

Support: NSERC

Title: Song-control system or vocal-control system? HVC is active during production of learned aggressive calls

Authors: *S. K. MISCHLER[1,2], E. KARLIN[1], S. A. MACDOUGALL-SHACKLETON[1,2]
[1]Psychology, Univ. of Western Ontario, London, ON, Canada; [2]Advanced Facility for Avian Res., London, ON, Canada

Abstract: Birds' learned vocalizations play an important role in the social interactions that are integral for the viability of flocks, whether it be by communicating movements or producing alarm calls when a predator is near. The neurobiology of such bird calls has been neglected in comparison to the neurobiology of bird songs. Birdsong is used in a reproductive context and tends to be more complex than calls, albeit this distinction is often unclear. Calls are often also assumed to not require imitative vocal learning, but we now know that a variety of calls are plastic. The song-control system in the avian brain is instrumental for the learning, production and maintenance of song is songbirds. This circuit includes the brain regions HVC, the robust nucleus of the arcopallium (RA) and area X. Little work has addressed whether these brain regions are involved in the production of calls. Black-capped chickadees produce a variety of highly complex vocalizations that they use in different contexts. The fee-bee song is the primary long-distance song most often produced by males during the breeding season to defend a breeding territory and attract mates. Production of this song is highly seasonal, but seasonal plasticity of HVC in this species is minimal, which suggests that HVC may be maintained year-round for the production of complex learned non-song vocalizations (e.g. the gargle and the chick-a-dee call). Specifically, the gargle call is an aggressive vocalization used to indicate the propensity of attacking, the chick-a-dee call is used to coordinate group movements within a flock and sometimes as an indicator of mild alarm and the tseet call is understudied and it's function is unclear. In this study, we used motor-driven immediate-early gene expression to assess whether song-control regions were active during the production of a variety of call types by black-capped chickadees. Birds were trapped in London, Ontario and exposed to behavioural manipulations in order to elicit production of the fee-bee song, gargle call, chick-a-dee and tseet calls. Following a session of vocalizing, brains were processed by immunohistochemistry to visualize immediate-early gene (ZENK) expression in the song-control regions of the brain. The birds that produced the gargle call consistently showed significantly more ZENK activation in HVC than birds that produced the fee-bee song, chick-a-dee and tseet calls. This suggests that HVC should be considered a vocal-control brain region rather than strictly a song-control region. Therefore HVC is crucial not only to learning, production and maintenance of song, but other learned vocalizations as well.

Disclosures: S.K. Mischler: None. **E. Karlin:** None. **S.A. MacDougall-Shackleton:** None.

Poster

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Topic: F.04. Neuroethology

Support: HHF #23103

Title: Breathing to sing: HVC modulates air pressure during song

Authors: *C. M. URBANO, B. G. COOPER
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Abstract: Birdsong is a learned vocal behavior that requires precise coordination between the respiratory and syringeal (vocal organ) motor systems. Subsyringeal air pressure is precisely regulated during phonation to control the acoustic features of song syllables; air pressure amplitude is determined by the combined effects of expiratory muscle activation and syringeal gating of airflow. HVC (used as a proper name) is a premotor nucleus that is important for the control of syllable sequence and song timing. Disruption of HVC neural activity through either ablation or inactivation affects temporal features of song in both juvenile and adult songbirds. Prior research in our lab has shown that unilateral HVC lesion in male Bengalese finches (*Lonchura striata domestica*) reduces air pressure amplitude and increases the duration of intersyllable intervals. These changes in song respiratory features suggest that eliminating HVC activity may influence song tempo by disrupting learned motor coordination of the respiratory and/or syringeal motor systems. Here we explore whether HVC damage transiently or permanently disrupts air pressure patterns and the resultant changes in expiratory muscle activation. Immediately after the lesion, the amplitude of expiratory and inspiratory pulses decreased significantly and there was also a reduction in slope at the onset of the pressure pulse. To investigate how long this reduction in air pressure amplitude persisted, we measured air sac pressure before unilateral HVC lesion and six months postsurgery. Six months after the lesion, we found that air pressure amplitude of expiratory pulses remained attenuated, suggesting that it does not recover. Therefore, without normal bilateral HVC activity, typical subsyringeal air pressure levels cannot be achieved. To determine how expiratory muscle activation changed as a function of HVC damage, we are recording electromyograms of the expiratory muscles (*m. obliquus externus abdominis*) before and after unilateral HVC lesion. These data illustrate the importance of neural control in maintaining temporally precise muscle activity and coordinating independent physiological systems during the production of complex, learned vocalizations.

Disclosures: **C.M. Urbano:** None. **B.G. Cooper:** None.

Poster

565. Vocal Communication in Songbirds: Sensory and Motor Mechanisms II

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Topic: F.04. Neuroethology

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Title: Aberrant song alters new neuron survival in Area X and NCM in the adult male zebra finch

Authors: ***A. PEREZ**[1], K. WASNER[2], S. RIBEIRO[2], A. LOPEZ[2], E. RODRIGUEZ[3], S. BIENSTOCK[2], C. PYTTE[2]
[1]Grad. Center, City Univ. of New York, New York, NY; [2]Queens Col., Flushing, NY; [3]Duke Univ., Durham, NC

Abstract: Adult neurogenesis provides a continual source of new neurons to a limited number of brain regions. However, the mechanisms regulating new neuron survival are poorly understood. In the adult male zebra finch, the quality of song produced is positively correlated with numbers of new neurons incorporated into the song motor pathway nucleus HVC (proper name), consistent with the idea that appropriate matching between received feedback and expected feedback contributes to new neuron survival. We tested this idea in adult male zebra finch basal ganglia nucleus Area X and in the auditory processing region caudomedial nidopallium (NCM). Both Area X and NCM respond to playback of the bird's own song, demonstrated by electrophysiological responses and fMRI respectively; and both regions receive new neurons throughout adulthood. Area X is required for song learning and is part of the anterior forebrain pathway that generates song variability in adulthood. NCM stores a memory of the tutor's song and also plays a role in conspecific song discrimination. To test whether new neuron survival in these regions is influenced by the quality of the bird's own song, we altered song structure by unilaterally denervating the syrinx and quantified new neurons in Area X and NCM in both hemispheres. Mitotically active cells were labeled with bromodeoxyuridine (BrdU, 3x/day) for four consecutive days. Two weeks after BrdU injections, song was altered by unilaterally sectioning the tracheosyringeal portion of the hypoglossal nerve (NXIIts), which innervates the syrinx. Control birds received sham surgery. Pre and post-treatment songs were compared using Sound Analysis Pro. Two weeks after surgery, birds were perfused and brains were processed

using immunohistochemistry to label BrdU and the neuron-specific protein Hu. We found that left and right NXIIts nerve section decreased the number of new neurons in Area X bilaterally relative to control birds. Interestingly, we found hemispheric asymmetry in effects of treatment on new neurons in NCM. Left NXIIts nerve section decreased the number of new neurons in both left and right NCM. Right NXIIts nerve section decreased the number of new neurons in left NCM and increased new neuron numbers in right NCM, relative to left and right neuron number in NCM of controls. These results suggest that the quality of song production plays an important role in the survival of new neurons and these effects differ by brain region and hemisphere.

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Poster

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Topic: F.04. Neuroethology

Support: R01 MH066128

Title: A glutamatergic neuron influences pallidal firing patterns in zebra finch Area X

Authors: *A. BUDZILLO[1], D. J. PERKEL[2]
[1]Neurobio. and Behavior, [2]Biol. and Otolaryngology, Univ. of Washington, Seattle, WA

Abstract: Area X is the striato-pallidal component of a basal ganglia loop essential for juvenile song learning and adult song plasticity in the songbird. Area X contains several cell types: three GABAergic interneurons, cholinergic interneurons, novel putative glutamatergic interneurons, and pallidal projection neurons. Pallidal neurons are GABAergic and highly spontaneously active. Subtle disruptions or brief pauses in their firing can increase the probability of firing in the downstream thalamic nucleus DLM. Area X receives excitatory projections from pallial nuclei HVC and LMAN and dense dopaminergic innervation from the midbrain. Dopamine D1 receptors in Area X have been implicated in mediating changes in song variability in an adult social context dependent form of song plasticity. Previously we have described strong, periodic spontaneous excitatory postsynaptic currents (EPSCs) in Area X. These EPSCs arise locally, suggesting the presence of a novel glutamatergic neuron in Area X. Here we explore further the

functional role of these neurons. We made cell-attached recordings from pallidal neurons, which can be easily identified by their high spontaneous firing rates (mean ISI=27.25 ms; n=9) and large cell bodies, in brain slices from adult male zebra finches. Area X was surgically isolated from HVC and LMAN to focus on local glutamatergic signaling. Application of the AMPA receptor antagonist NBQX (10 μM) did not change mean firing rate (mean ISI=26.01; n = 9), but did result in a significant decrease in variability of firing (baseline cv ISI=0.2367; NBQX cv ISI=0.1513; n=9; P=0.039). Thus, local glutamatergic synaptic activity within this microcircuit could alter pallidal cell firing statistics, which in turn could affect downstream signal transmission to DLM. We previously reported that the novel glutamatergic cell in Area X participates in a microcircuit motif that results in tightly coupled EPSC-IPSCs, with an inter-event lag of less than 5 ms. We have recorded spontaneous synaptic activity in whole cell voltage clamp mode in pallidal cells from isolated Area X slices. Further quantification of these coupled events shows that EPSCs are followed within 5 ms by inhibitory postsynaptic currents (IPSCs) 18.1% of the time (n=24 pallidal cells). However, the proportion of events that appear to participate in this motif varied from 0 to 60.4% across cells (n=24). The D1 receptor agonist SKF-38393 (10 μM) increased the overall rate of IPSCs, as well as the relative proportions of coupled EPSCs and IPSCs (n=3 pallidal cells), suggesting the existence of a novel synaptic mechanism for dopaminergic modulation of Area X output in the songbird.

Disclosures: A. Budzillo: None. **D.J. Perkel:** None.

Poster

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Topic: F.04. Neuroethology

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Title: *In vivo* two-photon targeted single-cell labeling in the zebra finch

Authors: *S. BENEZRA[1], R. T. NARAYANAN[2], M. OBERLANDER[2], M. A. LONG[1]
[1]Neurosci., NYU Sch. of Medicine, Dept. of Physiol. and Neurosci., New York, NY; [2]Computat. Neuroanatomy, Max Planck Inst., Tübingen, Germany

Abstract: Humans have the ability to learn and execute a wide range of movement sequences that are integral to everyday life, from playing the piano to speaking, but little is known about how these behaviors are represented within the brain. Songbirds offer an excellent experimental model to study the organization of identified cortical circuits underlying a complex learned motor behavior. Towards that end, we focused on the cortical premotor nucleus HVC (proper name), which contains the neural circuitry enabling song production (Vu et al., 1994; Hahnloser et al., 2002; Long and Fee, 2008). HVC premotor neurons send primary axons to the forebrain motor nucleus RA (robust nucleus of the arcopallium) which ultimately drive song. In addition to these efferent outputs, RA-projecting HVC neurons also send axon collaterals within HVC (Gurney and Katz, 1981; Mooney, 2000), which are thought to form a synaptic chain capable of enabling precise sequences (Long et al, 2010). Despite their implications for a range of HVC circuit models (Fee et al., 2004; Amador et al., 2013), the fine structure of these processes is poorly understood. To address this issue, we examined the morphology of premotor neuron axons by delivering an intracellular tracer (Neurobiotin) *in vivo* using juxtacellular (loose patch) stimulation (Pinault, 1996; Narayanan et al., 2014), coupled with 2-photon imaging (Margrie et al., 2003) in order to specifically target premotor neurons in that nucleus. The axonal arbors were then reconstructed using a semi-automated process (Oberlaender et al., 2007). In addition to a quantification of the arborization patterns and putative target neurons, we are also interested in whether the projection patterns of single premotor neurons can suggest topographic structure, especially in light of recent evidence suggesting that connectivity within HVC may exhibit a directional bias (Stauffer et al., 2012; Poole et al., 2012; Day and Nick, 2013).

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Topic: F.04. Neuroethology

Title: Unilateral cooling differentially affects song timing and syntax in Bengalese finch

Authors: *Y. ZHANG, A. A. KOZHEVNIKOV
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Abstract: Singing in songbirds is a learned complex motor behavior controlled by the nuclei that are symmetrically present in both hemispheres. How the two hemispheres coordinate to produce complex motor sequences that follow certain syntactic rules is unclear. We investigated this question by unilaterally changing temperature in the left or right HVC (proper name, formerly high vocal center) of a Bengalese finch. Song timing is affected primarily by changing temperature in left HVC but not right HVC, suggesting that timing control is lateralized to the left hemisphere. The variable number of syllable repeats is affected by changing temperature unilaterally in HVC, but which hemisphere depends on the syllable. Additionally, some of the syllable branching points change their transition probabilities with temperature change in either hemisphere.

Disclosures: **Y. Zhang:** None. **A.A. Kozhevnikov:** None.

Poster

565. Vocal Communication in Songbirds: Sensory and Motor Mechanisms II

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Topic: D.02. Auditory

Title: Sensorimotor feedback maintains auditory objects formation

Authors: *S. MA[1,2], M. GAHR[1,2]
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Abstract: In an international cocktail party, people are able to identify those who speak the same languages from background noise easier than those who speak other languages. It suggests that there must be neural mechanisms for listener to maintain the language sensitivity of his own languages. Reports in human studies that patients with damage to the Broca's area suffered from aphasia, and also had difficulty in speech comprehension, imply that sensorimotor feedback may influence speech perception. In zebra finch, the sensorimotor nucleus HVC (higher vocal center) corresponds functionally to the Broca's area. Both HVC and Broca's area were mostly studied in motor domain, whereas feedback from motor domain to auditory domain in speech perception is less known. The aim of this study is to investigate the influence of HVC on auditory object formation with regard to the international cocktail party problem. We employed electrophysiological methods to examine responses to auditory objects in both male and female auditory cortices. We found that the habituation rate to auditory objects was higher in male than

in female. Inactivation of HVC in male resulted in decrease of habituation rate. Our results provide direct evidence that sensorimotor integration affects auditory object formation.

Disclosures: **S. Ma:** None. **M. Gahr:** None.

Poster

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Topic: D.02. Auditory

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Title: Temporal cue cognition of the song auditory information processing in auditory forebrain of zebra finch

Authors: M. ARAKI[1], M. M. BANDI[2], C. P. CONNAUGHTON[3], *Y. YAZAKI-SUGIYAMA[1]
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Abstract: As in human speech, avian vocalization is generated and cognized as a sequence of sound patterns. Male zebra finches learn specific songs, from their auditory experience during the juvenile period, and employ it in courtship behavior and individual identification. Songs of zebra finches generally consist of a few acoustical elements, called syllables, which emerge in a stereotyped sequence separated by small silent gaps (~30 ms). In the zebra finch brain, neurons in circuits that are necessary for song learning and production, called the 'song system', show selective auditory responses to conspecific songs, songs they have heard from their own species, or their own songs, depending on the identity of the circuits. In this study we investigated how song auditory information is processed and cognized in the auditory circuit, which projects to the 'song system', especially in the primary auditory area, field L (homologous to the mammalian primary auditory cortex). In subregion L2a (homologous to layer 4 in the mammalian auditory cortex), where neurons receive thalamic inputs, neurons showed auditory responses to continuous white noise (WHN) as well as to conspecific songs (CON), while neurons in the downstream layer, L3, did not respond to WHN. Interestingly, L3 neurons responded to WHN when it was presented with the same durations and intervals as syllable elements of CON. When stimulated with same CON syllables repeatedly, L3 neurons decreased their responses during

repetition with shorter intervals, but not with longer intervals. Moreover, these neurons in the auditory cortex showed the same response magnitudes to CON, even when the frequency phase was scrambled within each syllable. These results suggest that within the auditory information processing for song cognition, temporal cues of syllable onset and offset provide necessary information in the primary auditory cortex.

Disclosures: M. Araki: None. **M.M. Bandi:** None. **C.P. Connaughton:** None. **Y. Yazaki-Sugiyama:** None.

Poster

565. Vocal Communication in Songbirds: Sensory and Motor Mechanisms II

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 565.23/VV62

Topic: D.02. Auditory

Support: NIH Grant R00NS066179

NIH Grant R01NS182179

Title: The role of norepinephrine in acute auditory processing of learned vocalizations

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Abstract: Neuromodulator signaling is important for many cognitive functions, including processing information from the environment. Auditory signal processing is modulated least in part by the actions of catecholamines. From previous studies in rodent and primate auditory cortex, norepinephrine is known to alter neuron firing properties and tone frequency tuning. However, it is not clear how norepinephrine modifies processing of complex vocalizations. Songbirds are one of the few animal taxa in which individuals learn vocalizations and thus provide a good model for studying auditory processing for complex features. In the songbird brain, catecholamine levels and immediate early gene expression in catecholaminergic neurons are elevated in response to songs, suggesting a role for acute norepinephrine modulation of audition. We use zebra finches to investigate the role of norepinephrine in song processing in caudomedial nidopallium (NCM), a region homologous to the secondary auditory cortex in mammals. Previously, in the NCM, we showed that norepinephrine significantly enhances

single-unit auditory-evoked responses relative to baseline firing activity. Here, we test whether the changes in normalized auditory-evoked responses are due to the changes in firing and/or burst frequencies. The results revealed that norepinephrine significantly decreases spontaneous but not stimulus-evoked firing frequency. Moreover, the changes in spontaneous firing frequency during norepinephrine infusion significantly correlated with the average change in stimulus-evoked firing frequency. The spontaneous firing frequency also significantly correlated with the ratios between spontaneous and song-evoked firing, suggesting that norepinephrine acts to change baseline activity to enhance the signal-to-noise ratio for auditory coding. In fact, for all auditory stimuli, ratios between spontaneous and stimulus-evoked firing were increased by norepinephrine treatment. These results together indicate that norepinephrine has an important role in auditory information coding for complex vocalizations. Ongoing analyses are directly testing whether norepinephrine can enhance encoding precision of NCM neurons for time-variant complex vocalizations. Together, this study contributes to the understanding of the role of neuromodulators on complex information processing. Support from NIH R00NS066179 and R01NS182179.

Disclosures: **M. Ikeda:** None. **D. Jeon:** None. **L. Remage-Healey:** None.

Poster

566. Optical Methods for Studying Neural Pathways

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Program#/Poster#: 566.01/VV63

Topic: G.04. Physiological Methods

Support: NIMH

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NIDA

Simons Foundation Fellow of the Life Sciences Research Foundation

NSF Graduate Fellowship

Fannie and John Hertz Foundation Graduate Fellowship

National Defense Science & Engineering Graduate Fellowship

Title: Functional connectivity and nonlinear information propagation in visual cortical circuits revealed by all-optical neurophysiology in the awake mouse

Authors: *J. H. MARSHEL[1], S. YANG[2], W. E. ALLEN[3], R. PRAKASH[3], I. KAUVAR[2], C. RAMAKRISHNAN[1], K. DEISSEROTH[4,1,5]
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Abstract: Activity flowing through neural circuits may be refined, amplified, and modulated to more robustly represent new information and more effectively carry this information to downstream circuitry. Understanding how circuits could adapt their activity patterns to transform and transmit information may require the ability to precisely manipulate activity patterns and measure effects with single-cell resolution. Here, we combine two-photon Ca^{2+} imaging using GCaMP6M with single-cell optogenetics using $C1V1_{T/T}$ to manipulate active layer 2/3 visuocortical subnetworks in awake mice viewing defined visual stimuli. Experimental parameters were determined to yield high imaging and stimulation quality while circumventing overlap of $C1V1_{T/T}$ and GCaMP6M excitation spectra and maintaining single-cell resolution for both. We reliably stimulated individual neurons at up to 20 Hz (~2 ms per single-spike stimulus), observing a consistent linear increase in GCaMP6M response with stimulation rate. We employed two methods for multi-neuron stimulation with single cell resolution *in vivo*. One method stimulates neurons in a sequence, rapidly moving among neurons (at most 180 microsecond lag between most distal neurons in the field of view (FOV)). The second method employs two-photon excitation with a phase spatial light modulator (SLM) to stimulate groups of neurons simultaneously. We stimulated groups of orientation-selective neurons (1-9 per group) that preferred the same direction, while the mouse viewed drifting gratings that varied in orientation and direction. When stimulated neurons preferred the orientation presented, their responses were observed to be amplified above the sum of baseline stimulation response (during grey screen) and visual response to the grating without optogenetic stimulation. Stimulating during oblique orientation presentation led to linear summation. Strikingly, when stimulation paired with orthogonal visual stimuli, responses were suppressed below baseline stimulation response. We also found indirectly stimulated neurons (ISNs) that reliably increased responses when other neurons were stimulated in the FOV, revealing excitatory functional connectivity that varied in modulation amplitude with network state. Modulation was strongest when indirect stimulation occurred during presentation of the ISN's preferred direction, and weakest during its oblique and orthogonal orientations. These results reveal nonlinear network mechanisms favoring selective flow of accurate information, compared with mismatched or orthogonal information, through the network, a potentially fundamental property of cortical circuitry.

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Poster

566. Optical Methods for Studying Neural Pathways

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Program#/Poster#: 566.02/VV64

Topic: G.04. Physiological Methods

Title: All-Optical physiology: Combining two-photon calcium imaging with single-cell optogenetic stimulation *in vivo*

Authors: *J. M. DOERING, G. PRAMANIK, E. ROSALES, A. TOSE, Z. BARGER, A. STROH
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Abstract: Establishing a spatially resolved connectivity map of cortical microcircuitry requires both monitoring and modulation of neuronal activity with single-cell specificity. Here, we combine optogenetic stimulation and calcium imaging with single-cell resolution. Integrating an optogenetic approach with simultaneous *in vivo* two-photon calcium imaging remains challenging, due to the interference of high intensity light pulses used for optogenetic stimulation with the optical readout. To overcome these limitations, we conduct spectrally non-overlapping two-photon calcium imaging with simultaneous two-photon optogenetic stimulation using an Optical Parametric Oscillator (OPO) to test the feasibility of "all-optical physiology". AAVs encoding for the two-photon-excitable opsin C1V1mCherry, under the control of the calcium/calmodulin-dependent protein kinase II (CaMKII) promoter expressed exclusively in excitatory neurons were injected in layer 2/3 of mouse visual cortex. Employing VGLUTII and GAD65/67 immunofluorescence for characterization of the opsin-expressing neurons we observed strong, membrane-bound expression of C1V1mCherry in excitatory neurons. For network analysis, Oregon Green BAPTA-1 (OGB-1) was pressure-injected in the C1V1-expressing region (V1). The calcium indicator (OGB-1) was excited using a Ti:Sa laser at 800 nm while C1V1 was simultaneously excited using the OPO at wavelengths ranging between 1100-1300 nm. *In vivo*, we demonstrate simultaneous two-photon calcium imaging and two-photon optogenetic stimulation without cross-talk. This approach allows for causally investigating the impact of single neuron excitation on microcircuit activity.

Disclosures: J.M. Doering: None. **G. Pramanik:** None. **E. Rosales:** None. **A. Tose:** None. **Z. Barger:** None. **A. Stroh:** None.

Poster

566. Optical Methods for Studying Neural Pathways

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Program#/Poster#: 566.03/VV65

Topic: G.04. Physiological Methods

Support: FIRST program (Head: Hideyuki Okano)

Title: A combination study of optogenetics and optical imaging to identify unknown cortico-cortical projection patterns in macaque

Authors: *Y. NAKAMICHI[1], M. HASHIMOTO[2], N. KITAMURA[1], K. HAGIYA[1], T. SATO[1], M. TANIFUJI[1]
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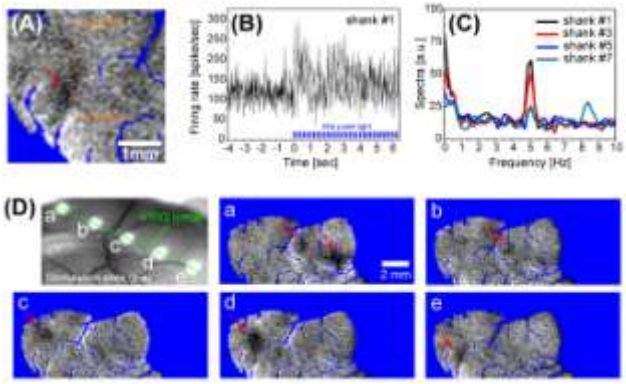
Abstract: To understand neural circuit mechanisms of signal processing across cortical areas, we have to identify connected pairs of cells (or cortical sites) between cortical areas and then to analyze difference and similarity in response property of the pairs. This strategy, however, is usually not available since it is difficult to find the pairs in large area of cortices *in vivo*. To achieve this goal, here, we establish a technique to identify unknown cortico-cortical projection pattern in macaques by combining optogenetics and optical intrinsic signal imaging (OISI): We used optogenetics to stimulate neurons in one cortical area and OISI to detect the response in the area receiving the projections. We used interhemispheric connections at V1/V2 border region as a model system to test feasibility of our technique. We first crated a vector, AAV9- CaMKIIa-hChR2(ETTC)- EYFP- MBD. MBD, myosin binding domain, was inserted to make channel rhodopsin 2 (ChR2) expression localized mainly in soma and dendrite but not in axon (Lewis TL, 2009). In this way, we can stimulate somas and dendrites with minimum stimulation on passing axonal fibers. In fact, ChR2 expression in axon was decreased by insertion of MBD in mouse cortex. ChR2 expression took about 70 days after the injection to reach plateau and be stable more than 10 months in macaques by monitoring EYFP fluorescence on the cortical surface. Optical stimulation at V1/V2 border of one hemisphere elicited statistically significant intrinsic signals in the other hemisphere reproducibly (Fig. A, arrow). We verified the result by recording multi-unit activities around the activation spot using electrode array (Fig. A, circles) as shown in PSTH and its spectrum (Fig. B and C). To our surprise, we found that optical stimuli in regular spatial intervals along the border elicited activation spots in irregular spacing and number around V1/V2 border in the other hemisphere (Fig. D).



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Poster

566. Optical Methods for Studying Neural Pathways

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Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 566.04/VV66

Topic: G.04. Physiological Methods

Title: Expanding the dynamic range of genetically-encoded calcium indicators for monitoring high frequency neuronal activity in *Drosophila*

Authors: ***Y. SUN**, J. A. STROTHER, J. P. HASSEMAN, G. TSEGAYE, B. FOSQUE, E. R. SCHREITER, A. NERN, M. B. REISER, K. SVOBODA, L. L. LOOGER, V. JAYARAMAN, D. S. KIM
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Abstract: Genetically-encoded calcium indicators (GECIs) have become increasingly popular for monitoring neural activity in genetic model organisms. We recently developed the GCaMP6 family of GECIs that enables single-trial single action potential (AP) detection under favorable conditions. GCaMP6 sensors have relatively high calcium affinities (Kd range: 144-375 nanomolar). They saturate at high firing rates (>50Hz) and have relatively slow off-rates. GCaMP6 variants thus cannot track instantaneous firing rates in neurons with high spike rates. We mutated the calcium-binding domains of GCaMP6 proteins and screened variants in solution and in the *Drosophila* larval neuromuscular junction (NMJ). We identified low affinity (Kd

range: 600 nanomolar to 370 micromolar) variants with high dynamic range. In the NMJ these indicators responded monotonically to spike rates from 1Hz to 160Hz. These GCaMP variants also exhibit improved linearity. Interestingly, some variants have significantly faster on- and off-rates. The off-kinetics of one variant is several-fold faster than those of GCaMP6f, the fastest GCaMP6 variant. We further tested low affinity variants in adult *Drosophila* in visual and olfactory circuit assays and observed improved discriminability in high activity regimes. These next generation, low-affinity GCaMP variants will be useful for monitoring fast-spiking neurons across a wide range of species.

Disclosures: **Y. Sun:** None. **J.A. Strother:** None. **J.P. Hasseman:** None. **G. Tsegaye:** None. **B. Fosque:** None. **E.R. Schreiter:** None. **A. Nern:** None. **M.B. Reiser:** None. **K. Svoboda:** None. **L.L. Looger:** None. **V. Jayaraman:** None. **D.S. Kim:** None.

Poster

566. Optical Methods for Studying Neural Pathways

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Topic: G.04. Physiological Methods

Support: Human Frontiers in Science Program

Beckman Institute

Mallinckrodt Foundation

Gordon and Betty Moore Foundation

Pew Charitable Trust

Michael J. Fox Foundation

Kimmel Foundation

Title: Developing a genetically encoded voltage sensor for visualizing neuronal connectivity *in vivo*

Authors: ***N. FLYTZANIS**[1], C. BEDBROOK[2], H. CHIU[1], S. MCISAAC[3], C. XIAO[1], K. CHAN[1], M. ENGQVIST[3], L. HERWIG[3], P. STERNBERG[1], F. ARNOLD[3], V. GRADINARU[1]
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Abstract: The current needs of neuroscience to link the activity of defined brain circuits with behavior are not fully met by currently available tools and techniques, e.g. electrophysiology or functional magnetic resonance imaging. The development of optical methods for the detection of activity within a population of neurons aims to fill the gap in scale between single unit recordings and whole brain scanning. Examples include genetically encoded indicators (GEI) of either cell membrane potential or intracellular calcium. Currently available GEIs suffer from either slow kinetics, low signal-to-noise ratio (SNR), or low baseline fluorescence. Our efforts have focused on the optimization of the microbial rhodopsin voltage sensor, Arch. While Arch exhibits large changes in fluorescence in response to changes in voltage with sub-millisecond kinetics, it suffers from very low baseline fluorescence and light-activated currents that hyperpolarize the cell. Past efforts to abolish these photocurrents have been successful, but have also adversely affected the speed of the sensor. Here, we report two Arch variants, Arch-11 and Arch-22, with larger dynamic range of sensitivity, faster kinetics and higher baseline fluorescence (4-5x and 2-3x respectively) than Arch WT and previously reported mutants. Both variants show greatly reduced photocurrents with 655 nm exposure (the wavelength used for fluorescence excitation) compared to Arch WT (55x and 99x lower respectively). For Arch-11, the combination of these improvements results in a fluorescent voltage sensor that is able to detect action potentials throughout a neuron (cell body and processes) at 40 Hz firing rate. In addition, we used Arch-11 to monitor spontaneous firing and circuit connectivity of multiple mammalian neurons. We show that Arch-11 can be used as an *in vivo* tool by expressing it in worm sensory neurons and tracking fluorescence changes in response to an odorant stimulus. The ability to follow neuronal activity in systems like *C. elegans*, where electrophysiological recordings are inherently difficult, will enable mapping of functional connectivity. With these improvements, Arch-11 provides a basis for further evolution of rhodopsin voltage sensors.

Disclosures: **N. Flytzanis:** None. **C. Bedbrook:** None. **H. Chiu:** None. **S. McIsaac:** None. **C. Xiao:** None. **K. Chan:** None. **M. Engqvist:** None. **P. Sternberg:** None. **F. Arnold:** None. **V. Gradinaru:** None. **L. Herwig:** None.

Poster

566. Optical Methods for Studying Neural Pathways

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Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 566.06/VV68

Topic: G.04. Physiological Methods

Support: Howard Hughes Medical Institute

Title: Improved red fluorescent genetically-encoded calcium indicators for *in vivo* imaging

Authors: *H. DANA, Y. SUN, J. P. HASSEMAN, G. TSEGAYE, B. F. FOSQUE, E. R. SCHREITER, B.-J. LIN, S. D. BRENOWITZ, V. JAYARAMAN, L. L. LOOGER, K. SVOBODA, D. S. KIM
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Abstract: Genetically-encoded calcium indicators (GECIs) are widely used for *in vivo* imaging of neural activity in specific cell types in various animal models. Currently, the emission spectra of most popular GECIs are in the green range (green GECIs). GECIs with emission in the red wavelength regime (red GECIs) would enable (1) imaging deeper in the brain, (2) combining functional microscopy with optical modulation of neuronal activity using channelrhodopsin-2 or other effectors with blue action spectra, and (3) dual color activity monitoring using both green and red GECIs. Here we report on improved red GECIs and their performance *in vivo*. We made thousands of variants of two red GECIs, RCaMP1h and R-GECO1.0, using structure-guided mutagenesis and tested their responses to action potentials in cultured neurons. *In vivo* performance of selected improved variants were studied in the mouse visual cortex, fly neuromuscular junction, and fly visual system. We discuss the advantages of improved red GECIs and their compatibility with other optical indicators and effectors.

Disclosures: H. Dana: None. **Y. Sun:** None. **J.P. Hasseman:** None. **G. Tsegaye:** None. **B.F. Fosque:** None. **E.R. Schreiter:** None. **B. Lin:** None. **S.D. Brenowitz:** None. **V. Jayaraman:** None. **L.L. Looger:** None. **K. Svoboda:** None. **D.S. Kim:** None.

Poster

566. Optical Methods for Studying Neural Pathways

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Topic: G.04. Physiological Methods

Support: DARPA Neuro-FAST

NIMH

NIDA

NSF

SFARI

Title: Identification of cellular-activity timing relationships spanning large tissue volumes during behavior

Authors: *L. GROSENICK[1], M. BROXTON[2], C. K. KIM[1], C. LISTON[1], P. KALANITHI[1], B. POOLE[2], S. YANG[3], A. ANDALMAN[1], T. ANDERSON[4], L. C. LEUNG[5], E. SCHARFF[1], E. FERENCZI[1], J. T. VOGELSTEIN[8], N. COHEN[3], A. LEVSKAYA[1], Z. ZHANG[3], O. YIZHAR[1], R. MADELAINE[5], C. RAMAKRISHNAN[1], S. GANGULI[6], A. MUTO[9], K. KAWAKAMI[9], P. MOURRAIN[5], S. J. SMITH[4], P. SUPPES[7], M. LEVOY[2], K. DEISSEROTH[1]
[1]Bioengineering, [2]Computer Sci., [3]Electrical Engin., [4]Cell. and Mol. Physiol., [5]Psychiatry and Behavioral Sci., [6]Applied Physics, [7]Philosophy, Stanford Univ., Stanford, CA; [8]Dept. of Statistical Sci., Duke Univ., Durham, NC; [9]Natl. Inst. of Genet., Div. of Mol. and Developmental Biol., Mishima, Shizuoka, Japan

Abstract: Tracking the coordinated activity of individual cellular events across volumes of intact tissue is a major challenge in biology that has inspired considerable technological innovation. However, synchronous measurement of the high-speed activity of individual neurons across three dimensions in brain tissue during behavior has not yet been possible. Here we develop a computational imaging approach (SWIFT3D) that integrates high-dimensional, structured statistics with light field microscopy to allow the synchronous acquisition of single-neuron resolution activity throughout intact tissue volumes at up to 100 Hz during behavior. We first show that this methodology allows fast, simultaneous activity mapping across the entire brain of transparent zebrafish during prey-capture behavior. More surprisingly, we then find that the statistical deconvolution intrinsic to SWIFT3D allows deep high-speed imaging in mammalian brains using conventional wide-field illumination (a modality otherwise unsuitable for light-scattering mammalian tissue). Together with the single-snapshot volume acquisition and large field of view of SWIFT3D, this property enables quantitative characterization of cellular-resolution dynamics simultaneously spanning hippocampal subfields CA1, CA2, and CA3 during contextual learning in awake behaving mice. Finally, we extend SWIFT3D to cellular-resolution high-speed imaging in human brain volumes; we map volumetric dynamical motifs within and across human cortical layers, statistically identifying dependence relationships between individual cells while simultaneously taking into account the dynamics of all other neurons detected in the volume. This ability to record synchronously from volumes of many cells across layers, subfields, and regions opens the door to identifying dynamical motifs and timing dependencies among coordinated cell assemblies during adaptive, modulated, or maladaptive physiological processes. LG, MB, CKK, and CL contributed equally to this work. **Support:** DARPA Neuro-FAST, NIMH, NIDA, NSF, SFARI.

Disclosures: L. Grosenick: None. **M. Broxton:** None. **C.K. Kim:** None. **C. Liston:** None. **P. Kalanithi:** None. **B. Poole:** None. **S. Yang:** None. **A. Andalman:** None. **T. Anderson:** None. **L.C. Leung:** None. **E. Scharff:** None. **E. Ferenczi:** None. **J.T. Vogelstein:** None. **N.**

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Poster

566. Optical Methods for Studying Neural Pathways

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Topic: G.04. Physiological Methods

Support: Human Frontiers in Science Program

the Beckman Institute

the Mallinckrodt Foundation

the Gordon and Betty Moore Foundation

the Pew Charitable Trust

the Michael J. Fox Foundation

Kimmel Foundation

Title: Optogenetic tracing of cell-type specific projections from the pedunculopontine nucleus to midbrain in the rat

Authors: *C. XIAO, J. T. TREWEEK, S. R. KUMAR, K. CHAN, S. L. MCKINNEY, B. YANG, V. GRADINARU
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Abstract: The pedunculopontine tegmental nucleus (PPN) provides robust cholinergic (ChAT) and glutamatergic (Glu) excitatory inputs to the midbrain and drives striatal dopamine release, which implicates the PPN in the control of reward processing and voluntary movement. However, validating PPN as a target to treat drug addiction and movement disorders requires better understanding of the precise nature of the PPN to midbrain projections. Herein, cell-type specific optogenetic tools enable reversible and bidirectional activity control in distinct PPN neuron populations with high temporal resolution. Utilizing optogenetics, we selectively tagged 1) PPN ChAT neurons in ChAT-Cre rats with Cre-dependent ChR2-YFP, and 2) PPN Glu

neurons with CaMKIIa-driven ChR2-YFP. By performing acute brain slice patch clamp recordings, we found that in comparison with Glu neurons, ChAT neurons exhibited higher input-resistance, stronger responses to depolarization current injections, and greater maximal firing rates. Blue-light stimulation of ChR2-expressing PPN neurons (10 ms at 10 Hz and 20 Hz) reliably evoked spikes within 5-10 ms after the initiation of light pulse with narrow variations (standard deviation 90% trials in ChAT neurons, but in only <60% trials in Glu neurons. These parameters could inform optogenetic tagging *in vivo* to match waveforms with tagged cell-types. By tracing ChR2-YFP labeled axons, we observed that both ChAT and Glu neurons in PPN project unevenly to midbrain nuclei. The density of ChAT fibers decreased in the following order: the ventral part of the substantia nigra pars compacta (SNc), the ventral tegmental area (VTA), dorsal SNc, and the substantia nigra pars reticulata (SNr). Glu fibers were most abundant in the SNc and VTA, but sparse in the SNr. By delivering light to the midbrain slice, we stimulated axon terminals of opsin-expressing PPN Glu neurons, and recorded time-locked Glu postsynaptic currents in 64% (7/11) of midbrain neurons. In contrast, stimulating ChAT axon terminals in this fashion induced slowly developed nicotinic inward currents (~15 pA) and increased the frequency of spontaneous excitatory postsynaptic currents by ~60% in 30% (6/20) of midbrain neurons. In summary, the projections of PPN ChAT and Glu neurons to midbrain exhibit distinct anatomical and physiological patterns, suggesting a possibility that these neuronal subsets could play distinct roles in reward processing and motor behaviors.

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Poster

566. Optical Methods for Studying Neural Pathways

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Program#/Poster#: 566.09/VV71

Topic: G.04. Physiological Methods

Support: NIMH

NSF

DARPA Neuro-FAST

NIDA

SFARI

Title: Physical and chemical properties of CLARITY brain-polymer hybrids

Authors: *A. TOM[1], A. MALKOVSKIY[2], Z. BAO[3], K. DEISSEROTH[1,4,5]
[1]Bioengineering, [2]Biomaterials and Advanced Drug Delivery Lab., [3]Chem. Engin., [4]Psychiatry & Behavioral Sci., Stanford Univ., Stanford, CA; [5]Howard Hughes Med. Inst., Chevy Chase, MD

Abstract: CLARITY enables unprecedented resolution of structural and molecular information of intact biological systems via the formation of transparent polymer-tissue hybrids. In order to maximize the utility of CLARITY for application to a variety of tissue sources and types, and to understand the underlying chemistry and physical parameters, a thorough investigation of CLARITY hybrid properties must be conducted. In this study, monomer to cross-linker ratios were varied to determine resulting polymer localization and relative density within mouse cortex, hippocampus, and thalamus using confocal Raman spectroscopy and mechanical compression testing. Furthermore, the influence of polymer crosslinking on tissue morphology was investigated via fluorescence microscopy, scanning electron microscopy, and protein quantification assays. In these investigations, we identify Raman correlates of CLARITY hybrid formation, which may be used to track and quantify tissue clarification. The synthesis of CLARITY hybrids may be tunable for different applications using quantitative descriptors such as these, which may also further enhance our understanding of how polymers form and crosslink within tissue.

Disclosures: **A. Tom:** None. **A. Malkovskiy:** None. **Z. Bao:** None. **K. Deisseroth:** None.

Poster

566. Optical Methods for Studying Neural Pathways

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Program#/Poster#: 566.10/VV72

Topic: G.04. Physiological Methods

Support: NIMH

NSF

NIDA

Title: Applying CLARITY-based methods for structural interrogation of intact biological systems to diverse peripheral and primate tissues

Authors: *B. HSUEH[1], J. EPP[2], P. PAUERSTEIN[1], L. YE[1], R. TOMER[1], Y. NIIBORI[2], S. KIM[1], D. LYONS[1], A. SCHATZBERG[1], P. FRANKLAND[2], S. JOSSELYN[2], K. DEISSEROTH[1]
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Abstract: A key challenge in biology is obtaining high-resolution structural and molecular information from complex systems while simultaneously preserving global spatial and functional perspective. To address this problem, we developed CLARITY, a technique based on chemical transformation of biological tissue into a hydrogel polymer network, enabling high-resolution light-microscopy imaging and labeling of cleared biological tissues without the need for mechanical deconstruction of samples. Though originally developed with the central nervous system in mind, the advantages of investigating large-scale tissues in the intact, three-dimensional state are not limited to questions of the brain. We developed modified CLARITY methods to biological systems beyond the mouse brain, including spinal, cardiac, musculoskeletal, dermatologic, pulmonary, endocrine and gastrointestinal tissue. CLARITY-based immunostaining was found to enable visualization and tracing of complex, tortuous neurovascular networks in the developing mouse heart, lung, pancreas and hair follicles, and revealed novel pancreatic islet migration defects in Neuropilin-2 knockout mice. CLARITY methods also were found to enable visualization of mitotic markers such as BrdU or Ki67 both in hippocampus and in intestinal crypts, creating new opportunities for studying neurogenesis and stem cell biology in structurally intact niches in health and disease. Finally, the advantages of CLARITY-based methods were extended to non-human primate and human studies, in glucocorticoid-receptor mapping of squirrel monkey cortex, and in islet-mapping and vasculature-tracing in fetal human pancreas. Using this highly diverse set of application domains, we demonstrate that clarified tissue is compatible with an extensive range of immunofluorescence staining techniques, and enables new ways of studying intact system development and function throughout major fields of biomedical science.

Disclosures: B. Hsueh: None. **P. Pauerstein:** None. **L. Ye:** None. **R. Tomer:** None. **S. Kim:** None. **D. Lyons:** None. **A. Schatzberg:** None. **K. Deisseroth:** None. **J. Epp:** None. **Y. Niibori:** None. **S. Josselyn:** None. **P. Frankland:** None.

Poster

566. Optical Methods for Studying Neural Pathways

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NSF

NIDA

Title: CLARITY-based whole brain activity mapping with immediate early gene TRAP

Authors: *L. YE[1,2,3], R. TOMER[1,2,3], B. HSUEH[1,3], C. J. GUENTHNER[2,4], L. LUO[2,4], K. DEISSEROTH[1,2,3]
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Abstract: CLARITY enables transformation of a mammalian brain into a fully assembled hydrogel-hybridized structure that is transparent to both light and macromolecules. This allows visualization of long-range projections, local circuit wiring, and subcellular structures throughout the whole brain without sectioning, providing a platform for detailed intact-circuit analysis and mapping. We now have developed a high-throughput platform to apply CLARITY to brains from mice carrying a signal of past behavior: genetically engineered with an inducible, immediate early gene-based TRAP (Targeted Recombination in Active Populations) system. In a fear conditioning paradigm, we were able to pharmacologically specify 3-hour time windows in which neuronal activity was precisely and permanently labeled with fluorescence signals at the single cell level throughout the whole brain. By combining CLARITY with COLM (CLARITY-optimized light-sheet microscopy), a fully assembled whole brain activity map at subcellular resolution could be rapidly obtained in a truly unbiased manner, registering both well-known and previously uncharacterized anatomical regions of interest to a regions active in an affective state (fear) that was behaviorally verified in the very same animal. CLARITY's unique compatibility with deep molecular phenotyping enabled multiple rounds of molecular characterization of the identified regions, and together, the combination of CLARITY and TRAP illustrates a brain-wide, high-resolution, multimodal activity-mapping methodology.

Disclosures: L. Ye: None. **R. Tomer:** None. **B. hsueh:** None. **C.J. Guenthner:** None. **L. Luo:** None. **K. Deisseroth:** None.

Poster

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Title: Single-cell phenotyping within transparent intact tissue through whole-body clearing

Authors: *B. YANG[1], C.-K. CHEN[1], B. E. DEVERMAN[1], R. P. KULKARNI[2], J. B. TREWEEK[1], V. GRADINARU[1]
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Abstract: Advances in tissue clearing methods have made molecular phenotyping of brain tissue possible. By matching the refractive index of the brain tissue with its surrounding media, deep brain structures can be imaged and visualized with conventional microscopy. Building on the previously published CLARITY method, we've developed: 1) PACT, for PAssive CLARITY Technique, a practical protocol for passive clearing and staining of up to 3mm thick brain slices; 2) PARS, for Perfusion-assisted Agent Release *in situ*, a whole body clearing method for simultaneous clearing of brain and peripheral organs; and 3) RIMS, for Refractive Index Matching Solution, an affordable FocusClear alternative index matching media for imaging cleared tissue slices or up to 300 μm-thick PFA fixed brain sections. With PARS, all relevant steps including tissue preservation, clearing, labeling and refractive index matching can be performed *in situ* prior to the extraction of the tissue. PARS is scalable both in terms of the number and size of animals that can be processed simultaneously. We can significantly limit the amount of tissue expansion during the clearing procedure, which will facilitate comparing brains across experimental cohorts. Validated for rodent whole-organ and whole-body clearing, PACT, PARS, and RIMS are compatible with endogenous-fluorescence, immunohistochemistry, long-term storage, and microscopy with cellular and subcellular resolution. As a demonstration of the power of this technique, we show PARS-cleared whole brain and peripheral organ images of

mice injected systemically with AAV-eGFP. PARS provides cellular-level reporter expression data from within whole organs, eliminating the need for sectioning and thereby greatly speeding up screening for AAV vectors with desired tropisms. In addition, these methods are relevant to studying central and peripheral nerve anatomy (including connectivity, myelination), tumor margins and heterogeneity, stem cell distribution, all within intact organs or bodies.

Disclosures: **B. Yang:** None. **C. Chen:** None. **B.E. Deverman:** None. **R.P. Kulkarni:** None. **J.B. Treweek:** None. **V. Gradinaru:** None.

Poster

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NIDA

SFARI

Stanford Bio-X

Title: Optogenetic investigation of dopamine D2-receptor signaling in risk-preference

Authors: *K. ZALOCUSKY[1,2], C. RAMAKRISHNAN[1], B. KNUTSON[3], K. DEISSEROTH[1,5,4]
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Abstract: The midbrain ascending dopamine system has long been implicated in substance abuse and addiction. More recent work also links dopaminergic signaling to other forms of behavioral addiction, including compulsive over-eating (Johnson et al., 2010) and pathological gambling (Dodd et al., 2005; Grosset et al., 2006; M. Tippmann-Peikert et al., 2007). These studies point to a role of dopamine D2 receptor (D2R) signaling, in particular, in mediating the switch to behavioral addiction. In a novel operant task to examine risk-preference or gambling

behavior, we observed that systemic administration of D2R/D3R agonist pramipexole (PPX) increased risk-seeking choices in a linear, dose-dependent fashion (n = 4-6 per dose, doses range from 0.075 to 0.3mg / kg, r2 = 0.99). To determine which D2R/D3R-expressing brain regions mediate this behavioral change, we infused PPX bilaterally into the nucleus accumbens (NAc) or orbitofrontal cortex (OFC) through permanently-implanted cannulas 30min before the gambling task. We found that D2R/D3R agonism in the NAc, but not the OFC, was sufficient to increase risk-seeking choices (n = 5 in OFC, 6 in NAc; 0.75uL/side of 10µg/µL PPX; p = 0.006). To optogenetically examine (at specific timepoints in the gambling task) the behavioral role of D2R-expressing medium spiny neurons (MSNs), the likely targets of our pharmacological manipulations, we needed to genetically target this cell population in the rat. To this end, we developed a D2R promoter that can be expressed in an adeno-associated viral (AAV) vector and targets opsin expression to D2R-expressing MSNs with 98% specificity (112/114 cells expressing enhanced yellow fluorescent protein (eYFP) were also labeled by anti-D2R antibody). Using this novel construct, we expressed eChR2-eYFP fusion protein specifically in the D2R-expressing cells of the NAc. We found that stimulating these cells with 15mW, 473nm light at 20Hz for 1s during the decision period of the task significantly decreased risky choices in risk-seeking, but not risk-averse, rats as compared to eYFP-expressing controls (n = 17 ChR2, n = 18 eYFP animals; p = 0.008). Finally, to test the duration of the impact of D2R-MSN activity on decision-making, we restricted optical excitation to a pseudo-random subset of trials, with a minimum of 6 trials between light stimulation. Using this protocol, we demonstrated single-trial control of risk-seeking choices with optogenetic stimulation of NAc D2R-expressing MSNs (n = 6, p = 0.046). These findings reveal a highly temporally-specific role for this genetically and topographically-defined population of neurons in risk-seeking behavior.

Disclosures: K. Zalocusky: None. **C. Ramakrishnan:** None. **B. Knutson:** None. **K. Deisseroth:** None.

Poster

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NIDA

NSF

SFARI

Title: Top-down bidirectional control of learned fear responses

Authors: *A. ADHIKARI[1], J. FINKELSTEIN[2], T. N. LERNER[2], L. A. GUNAYDIN[2], S. PAK[2], A. LEI[3], K. DEISSEROTH[4,5]
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Abstract: Previous studies showed electrical stimulation of the ventral medial prefrontal cortex (vmPFC) during cued fear extinction increases extinction retrieval, while stimulation of the dorsal medial prefrontal cortex (dmPFC) has the opposite effect in rats. As fear conditioned responses are dependent on the central amygdala, it has been hypothesized that the opposing effects of these regions on fear is due to their distinct projections to the amygdala. The vmPFC is thought to activate inhibitory intercalated cells in the amygdala, which inhibits the central amygdala, while the dmPFC projects to the basolateral amygdala, which in turns excites the central amygdala, presumably leading to increased fear. These data led to a model in which projections from the vmPFC and the dmPFC to the amygdala decrease and increase, respectively, freezing during extinction retrieval. However, this model has not been directly tested. Here, we tested these ideas directly by optogenetically activating vmPFC and dmPFC cell bodies, as well as their projections to the amygdala during cued and contextual fear extinction. Animals were injected with viral vectors encoding ChR2-YFP or YFP under the CamK2α promoter in the vmPFC or the dmPFC, and fiberoptic cannulae were implanted either in the mPFC or above the amygdala. On day 1 mice were fear conditioned to an auditory tone or to a context by pairing these stimuli with foot shocks. On day 2 mice were exposed to these stimuli without foot shocks to extinguish conditioned freezing fear responses, while receiving optogenetic activation. Lastly, on day 3 mice were tested for retrieval of fear by exposing them again to the conditioned stimuli. We report that optogenetic activation of vmPFC cell bodies ($n=7$ YFP and 7 ChR2, $p<0.05$) during fear extinction decreased freezing specifically during cued, but not contextual, fear extinction retrieval. On the other hand, activation of dmPFC cell bodies during fear extinction did not change freezing in either paradigm ($n=6$ YFP and 6 ChR2). Remarkably, activation of the vmPFC-amygdala projection only during fear extinction decreased freezing both during extinction and retrieval of extinction, for both cued and contextual conditioning ($n=7$ YFP, 10 ChR2, $p<0.05$). Lastly, activation of dmPFC-amygdala projections ($n=12$ YFP and 13 ChR2, $p<0.01$) during extinction selectively increased freezing only during retrieval of cued extinction. These data suggest the vmPFC-amygdala projection robustly inhibits fear elicited by both simple/discrete and complex/contextual fear-conditioned cues, while the dmPFC-amygdala projection selectively increases fear to discrete fearful stimuli.

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Poster

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ARC Future Fellowship FT110100887

NHMRC Project Grant 1066887

Title: Decoding zebrafish neural circuits using optogenetics and patterned excitation

Authors: ***A. W. THOMPSON**[1], L. A. HEAP[1], I. A. FAVRE-BULLE[1,2], H. RUBINSZTEIN-DUNLOP[2], E. K. SCOTT[1]
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Abstract: Understanding the structure and function of neural circuits remains a fundamental goal of modern neuroscience. We address this problem by observing correlated patterns of activity across broad, defined populations of neurons within a circuit using the zebrafish model organism. Signalling in the optic tectum of 7dpf zebrafish larvae is detected using a genetically-encoded calcium indicator, GCaMP5, in combination with selective-plane illumination microscopy (SPIM). We have paired this imaging technique with a piezo-driven z-stage to capture high-speed, high-resolution scans of the larval zebrafish tectum in 3-dimensions. We have identified stereotypical tectal cells that are active in response to defined, external input including visual and auditory stimuli. In order to determine how tectal circuits encode information from internal sources, we have also imaged tectal GCaMP5 in response to stimulation of upstream brain regions including the cerebellum and thalamus. This is achieved by genetic targeting of the optogenetic protein, channelrhodopsin-2 (ChR2), to these areas. ChR2 stimulation is further restricted using a spatial light modulator (SLM) to induce neural firing in only subsets of cells in these regions, while resulting GCaMP5 activity is imaged in the optic

tectum. These techniques have allowed us to determine a spatio-temporal model of the functional circuits within the larval zebrafish tectum.

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Poster

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Support: NIMH

DARPA

Gatsby

DAAD

Title: Advancements in neuronal silencing using inhibitory chloride-conducting channelrhodopsins

Authors: *A. BERNDT[1], S. LEE[2], C. RAMAKRISHNAN[2], K. DEISSEROTH[2]
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Abstract: In optogenetics, neuron inhibition is typically achieved by utilizing light activated pumps such as halorhodopsins or archaeorhodopsins. However, the inherent biophysical properties of these tools dictate that only one ion is transported per absorbed photon. Therefore, these pumps are less efficient compared to the excitatory channelrhodopsins, which conduct several hundreds or thousands of ions during each photocycle. For this reason, it has been a major goal of optogenetic engineering to design a channelrhodopsin with altered ion selectivity to inhibit neural activity upon light stimulation. A crucial step towards this objective has been obtaining the x-ray structure of the channelrhodopsin hybrid C1C2, because it allows precise structure-guided engineering of this class of proteins for the first time. Guided by the C1C2 structure, we replaced nine amino acids along the ion-conducting pathway of C1C2 to create an ion-conducting pore more suitable for transporting negatively charged ions. We revealed that this new construct (iC1C2) is not selective for cations, but conducts chloride ions and residual

amounts of protons. Consequently, expression in cultured neurons from rat hippocampus shifted the reversal potential from - 7 mV in C1C2 to -64 mV in iC1C2. This value lies between the average spiking threshold (-55 mV) and the resting potential (-69 mV) of all tested neurons. Unlike other inhibitory tools, iC1C2 transports chloride ions passively over cell membranes utilizing physiological ion gradients. Once activated by light, iC1C2 reduced the input resistance of expressing cells by 50%, which resulted in a spike inhibition probability of 88%. To enable bi-stable inhibition of neuronal activity and greater light-sensitivity of expressing cells, we further engineered iC1C2 by replacing cysteine 167 by alanine. This created a Step-Waveform-Inhibitory-Channelrhodopsin (SwiChRCA) which maintains a negatively shifted reversal potential of -68 mV, and conferred extended lifetime of the conducting state (tau_off = 160 s). As a result, cultured neurons remained inhibited for many seconds after brief blue light stimulation at low intensities. Furthermore, SwiChRCA was immediately deactivated by red light (tauoff = 200 ms) which allowed precisely-timed switching of neurons back into the excitable state. We prepared AAVs coding for CamKII-iC1C2_eYFP and CamKII-SwiChRCA_eYFP, injected mouse pre-frontal cortex, and mapped the ability of these constructs to inhibit generation of action potentials in various intact-tissue settings. Additionally, we present strategies for expanding this next generation of chloride conducting channelrhodopsins.

Disclosures: **A. Berndt:** None. **S. Lee:** None. **C. Ramakrishnan:** None. **K. Deisseroth:** None.

Poster

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DARPA

Gatsby

Title: Achieving dual and separable optogenetic inhibition of different neuronal subtypes within microcircuits

Authors: *S. LEE[1], A. BERNDT[1], C. RAMAKRISHNAN[1], L. FENNO[1], R. PRAKASH[1], F. ZHANG[2], K. DEISSEROTH[1]
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Abstract: Optogenetic activation of different neuronal cell types, using multiple excitatory opsins within the same brain region, has revealed new insights into the circuit elements crucial for behavioral and pathological states such as information processing and behavioral dysfunction (Yizhar et al. 2011 Nature). However, this level of circuit dissection has not yet been explored with inhibitory optogenetic tools. Here, we report the development and characterization an inhibitory opsin, eOxy, derived from the proton pump of the marine dinoflagellate Oxyrrhis marina. We first added trafficking sequence motifs necessary to facilitate the expression of eOxy in neurons, allowing for its usage for optogenetic experiments. eOxy showed strong photocurrents (~1 nA) in cultured neurons with a peak activation wavelength at ~510nm, which is further blue-shifted than the currently available inhibitory pumps. Even more interestingly, it showed minimal activity at the red wavelength (633 nm), presenting new opportunities for combining eOxy with other optogenetic tools. To explore the possibility of combinatorial optogenetic inhibition with eOxy, we injected PV-Cre mice with a virus cocktail consisting of a mix of CaMKIIa-eOxy and DIO-NpHR in the CA1 hippocampus or mPFC of PV-Cre mice. In addition, to enable delineation of individual opsin-expressing cells within the densely-packed CA1 pyramidal layer, we added a p2A linker sequence between the opsin and fluorophore, demonstrating that this strategy can work for inhibitory pumps (Prakash et al. 2012 Nature Methods). We performed simultaneous dual whole-cell recordings in acute hippocampal slices with CaMKII-positive excitatory pyramidal neurons (expressing eOxy) and PV (parvalbumin)-positive fast-spiking interneurons (expressing NpHR) using different wavelengths (510 nm and 633 nm). Our experiments showed that we could inhibit electrically-evoked spiking in both cell populations, or specifically in just the PV+ interneuron population. We also performed *in vivo* optrode recordings in the mPFC of injected animals, where eOxy showed robust ability to inhibit spontaneous multi-unit activity while the inhibition of PV cells with NpHR demonstrated a small increase in spontaneous activity. In conclusion, we present here the first demonstration of combinatorial optogenetic inhibition with a new inhibitory optogenetic tool, eOxy. The dual or selective silencing of distinct neuronal populations will allow for a refined study and dissection of neuronal microcircuits.

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Poster

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NSF

NIDA

Berry Postdoctoral Fellowship

Title: Subsecond cholinergic dynamics underlying hippocampal network state in freely-behaving rats

Authors: *T. J. DAVIDSON[1], E. B. ANDERSON[3], T. N. LERNER[1], C. RAMAKRISHNAN[1], J. MATTIS[1], L. M. GROSENICK[1], I. V. KAUVAR[1], L. M. FRANK[3], K. DEISSEROTH[2]
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Abstract: The role of the neuromodulator acetylcholine (ACh) in hippocampus (HPC)-dependent memory is of long-standing research and clinical interest. *In vitro* and behavioral data suggest that varying ACh levels may dynamically tune HPC circuits for encoding (higher [ACh]) or retrieval (lower [ACh]) (ref. 1). ACh has also been hypothesized to regulate subsecond, behavior-linked changes in HPC network connectivity (ref. 2). However, the activity of identified HPC-targeting ACh neurons in freely-behaving animals has never been reported, and no causal link between rapid ACh dynamics and HPC function has been established. We have used optical methods in freely-moving rats to both record from and stimulate a genetically-defined population of ACh neurons in the medial septum/diagonal band (MSDB) that provides the major ACh projection to HPC. We had previously developed a transgenic Cre driver rat line to target ACh neurons, and here we have used a viral strategy to express the excitatory opsin ChR2 or the genetically-encoded calcium indicator GCaMP6F in these cells. In slice physiology recordings, ChR2-expressing neurons displayed characteristic features of ACh neurons (broad spike and slow after-hyperpolarization potential) and were reliably driven by blue light pulses (473nm) at firing rates of at least 20Hz. To record the activity of this population, we used fiber photometry (ref. 3) to monitor population fluorescence in GCaMP6F-expressing cells during open field exploration. We recorded transients of up to 30% $\Delta F/F$, and signal-to-noise ratios >10 under minimally-bleaching illumination (~100μW). Consistent with predictions of graded ACh modulation of HPC network state, we observed a strong correlation between fluorescence and log(running speed), ($R^2 = 0.22$-0.50, $p \ll 0.001$). Finally, to test the effects of these dynamics on HPC, we optogenetically stimulated ACh cells in MSDB while recording local field potentials in dorsal HPC. 8Hz stimulation markedly reduced the rate of sharp-wave ripple complexes

(SWR) in HPC (0.8 to 0.1 Hz, p<10^-4). This decrease was complete ~250 ms after light onset, and recovered ~1 s after light offset. Our results therefore are consistent with a role for septohippocampal ACh in rapidly modulating HPC network activity. 1. Hasselmo et al. Prog. Brain Res. 145, 207-231 (2004). 2. Kemere, C., Carr M. et al. PLoS ONE 8, e73114 (2013). 3. Gunaydin L., Grosenick L. et al., Cell, in press (2014).

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Poster

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DARPA Neuro-FAST

NIDA

SFARI

Title: Brain-wide imaging of an anhedonic state using awake optogenetic functional MRI (ofMRI)

Authors: ***E. FERENCZI**[1], C. LISTON[2], K. ZALOCUSKY[1], K. KATOVICH[3], M. R. WARDEN[2], D. AMATYA[2], B. PATENAUDE[4], L. GROSENICK[1], C. RAMAKRISHNAN[2], P. KALANITHI[5], A. ETKIN[4], B. KNUTSON[3], G. H. GLOVER[6], K. DEISSEROTH[7]
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Abstract: We combined optogenetics and functional magnetic resonance imaging (ofMRI) to examine whole-brain spatiotemporal patterns of Blood Oxygen Level Dependent (BOLD)

activity during stimulation of medial prefrontal cortex (mPFC) pyramidal neurons in awake rodents. Human neuroimaging studies have demonstrated that mPFC activity (subcallosal cingulate gyrus, also known as area 25) is elevated in patients with depression (Mayberg et al, 2005) and rodent investigations have shown that increased excitability of mPFC excitatory neurons reduces social interaction in mice (Yizhar et al, 2011). Here, we assessed the causal role of mPFC in regulating the hedonic response to sucrose in rats. We expressed a stable step function opsin (SSFO) driven by the calmodulin kinase IIα promoter to target pyramidal neurons in the mPFC of wild type Sprague Dawley rats. We found that stimulation of mPFC pyramidal neurons by SSFO caused anhedonic behavior, manifested by a small (8-9%) but significant decrease in sucrose preference ($n = 8$ for SSFO, $n = 10$ for YFP controls, $p < 0.01$ for interaction in 2-way ANOVA). We performed awake functional MRI scans (7T magnet) to assess brain-wide patterns of BOLD activity arising from this stimulation. Awake scanning was enabled by a 5-10 day habituation protocol in a MRI simulation environment. Scans were performed using a single shot (0.5 s TR) variable density spiral in-out pulse sequence to obtain functional images with 470 μm in-plane spatial resolution. As a positive control, the BOLD response to a natural sensory (visual) stimulus was monitored through unilateral retinal illumination with green light. Activation of mPFC (2 s continuous 470 nm light) yielded a robust BOLD response at the fiber site ($n = 6$, $p < 0.01$ corrected), which was absent in YFP controls ($n = 5$). We then performed resting state scans to assess changes in whole-brain functional connectivity following mPFC activation by SSFO (5 s continuous 470 nm light at the start of a 5 min resting state scan). We observed significant increases in positive BOLD signal correlation between mPFC and a number of brain regions including ventral striatum and medial orbital cortex ($p < 0.01$ corrected, $n = 4$ for SSFO and $n = 4$ for YFP). *In vivo* multi-site electrophysiological recordings suggest that mPFC activation by SSFO modulates the spectral profile of the local field potential by increasing the ratio of high to low gamma power in functionally connected targets of mPFC. We discuss the potential role of these changes in whole brain network dynamics in contributing to psychiatric symptoms related to the altered experience of reward, as seen in depression, schizophrenia and addiction.

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Poster

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CURE Challenge Award

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5R01 NS034774

Title: Thalamic driving of cortical spindles and seizures with on demand loss of consciousness

Authors: *J. PAZ[1], J. SOROKIN[2], T. J. DAVIDSON[3], K. DEISSEROTH[3], J. R. HUGUENARD[2]
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Abstract: Neural interactions between cerebral cortex and thalamus mediate sensation, perception, and consciousness and generate rhythms associated with sleep and epilepsies. The causal roles of thalamus in the control of cortical rhythms has been controversial. To probe the role of thalamocortical neurons in cortical rhythmogenesis, we combined *in vitro* and *in vivo* electrophysiology and closed-loop optogenetic control of neural circuits in freely behaving rats and mice. Driving synchronized burst firing mode at 8-12 Hz of thalamic relay neurons was sufficient to drive cortical sleep-spindles, absence type seizures and loss of consciousness. Destabilizing thalamocortical output by reduction in firing with halorhodospin was sufficient to desynchronize cortical rhythms and abruptly terminate seizures in real time. These approaches provide a toolbox for reliable real-time control of thalamocortical rhythms of therapeutic interest for psychiatric and neurological disorders.

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Poster

566. Optical Methods for Studying Neural Pathways

Location: Halls A-C

Time: Tuesday, November 18, 2014, 8:00 AM - 12:00 PM

Program#/Poster#: 566.21/VV83

Topic: F.03. Motivation and Emotion

Support: NIH Grant 2PN2EY018241-09

Title: Mechanisms of neuromodulatory control of the zebrafish acoustic startle response: Optogenetic control with the light-controlled metabotropic glutamate receptor 2 (limglur2)

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Abstract: Great inter-individual variation is present in the acoustic startle behavior (ASR) in wild-type populations of zebrafish. In invertebrates, neuromodulators play a role in behavioral diversity by diversifying the output of fixed neuronal networks. Neuromodulators act through G protein-coupled receptors (GPCR) and affect intracellular signaling pathways in neurons. To connect GPCR activity in specific circuits to behavioral control we re-engineered the mammalian Gi/o-coupled receptor mGluR2 to make it light-sensitive with a photoswitchable tethered ligand (PTL). The light-controlled metabotropic glutamate receptor 2 (LimGluR2) is turned ON/OFF when exposed to light of different wavelengths (380nm vs. 490nM) (Levitz et al., 2013). We showed that optical control of the Gi signaling pathway in distinct Gal4-expressing neuronal populations differentially modulates the ASR behavior. Specifically, LimGluR2 activation in a Gal4 pattern that includes the Mauthner cell induced a decrease in ASR probability that was not reversible within minutes. In contrast, activation of LimGluR2 in a pan-neuronal pattern reversibly increased the ASR probability. Animals expressing LimGluR2 did not behave significantly different from siblings not expressing the receptor. We speculate that behavioral control by the Gi pathway might be part of the native control of the ASR during ethologically-relevant situations and may contribute to behavioral diversity.

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