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Poster

029. Neural Lineage Specification and Plasticity

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 29.01/A1

Topic: A.02. Neurogenesis and Gliogenesis

Support: CIRCNS

SIUC Research Rookies

Title: Rhox8 expression in rodent brains

Authors: H. G. HUFFMAN[1,2], E. D. GRISLEY[1,2], J. A. MACLEAN, II[2], *J. L. CHEATWOOD[1];
[1]Anat., SIU Sch. Med., CARBONDALE, IL; [2]Physiol., SIU Sch. Med., Carbondale, IL

Abstract: Homeobox genes encode transcription factors that govern many processes during development. This 60-amino acid DNA-binding motif associates with promoters by either activating or suppressing the transcription of downstream target genes. Recently, the Rhox, -(X-linked reproductive Homeobox)-, genes were discovered. The Rhox gene set is expressed during embryonic development in the testis, but a select few of these genes continue to show high expression after birth which makes them candidates for controlling postnatal and adult developmental events. At their peak expression in the testis (postnatal day 12), all Sertoli cells express Rhox5, Rhox8, and Sox9, but Rhox5 expression become more restrictive after postnatal day 30. RHOX8 protein is abundant in the testis, epididymis, ovary, and it is weakly detected in the placenta. Of all 33 mouse Rhox genes, Rhox8 is the only one to show expression in somatic cells in the embryonic testis. All others are expressed solely in germ cells. It is a common phenomenon that testis expressed genes exhibit brain-specific splicing or transcripts from alternative promoters. Given Rhox8's potentially unique transcriptional control in the testes, we wanted to determine if Rhox8 was similarly uniquely expressed in the brain. Thus, we examined whether the mRNA of the somatic transcription factors Rhox8, Sox9, and Rhox5 could be detected in adult rat and mouse brain tissue. Rats were euthanized, and brains were removed and flash-frozen. To study the basic expression of the Rhox8, Sox9, and Rhox5 gene in brain tissue, we extracted RNA rat and mouse brains, and then converted the RNA to cDNA in order to perform qPCR (quantitative real-time polymerase chain reaction). Rhox8, Rhox5, and Sox9 were all found to be highly expressed in positive control adult mouse testis tissue. However, only Rhox8 had moderate expression in mouse cortex and hippocampus. As expected we found Rhox8 to have moderate expression in adult rat cerebellum, Sox9 had minimal expression, but

Rhox5 had no detectable expression. These low levels may be due to the use of whole cerebellum in the rat, which contains many cell types which do not appear to express Rhox8 in addition to neurons, some of which were immunopositive for RHOX8 in the mouse brain. Further characterization of RHOX8 expression in adult rats and adult and developing mice was ongoing at the time of abstract submission.

Disclosures: H.G. Huffman: None. **E.D. Grisley:** None. **J.A. MacLean:** None. **J.L. Cheatwood:** None.

Poster

029. Neural Lineage Specification and Plasticity

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 29.02/A2

Topic: A.02. Neurogenesis and Gliogenesis

Support: NIH Grant MH099628

Title: Spatiotemporal dimorphic expression of Xlr3 genes in the developing male and female cortex

Authors: *A. MCDONOUGH[1], J. BERLETCH[2], C. DISTECHE[2], R. HEVNER[3,1];
[1]Ctr. for Integrative Brain Res., Seattle Childrens Res. Inst., Seattle, WA; [2]Dept. of Pathology, [3]Neurolog. Surgery and Pathology, Univ. of Washington, Seattle, WA

Abstract: The high occurrence of neurodevelopmental disorders in males may in part be explained by the inheritance of their X-chromosome from their mothers, particularly if certain genes are maternally imprinted and those genes influence cognitive function. This hypothesis is supported by comparative studies on women with Turner syndrome who have inherited solely a maternal or paternal X-chromosome, who exhibit parent-of-origin effects correlating with differing types of cognitive impairment. Further studies on mouse models of Turner syndrome suggest some X-linked genes may be imprinted and expressed solely from one X-chromosome. The observed sex biases in susceptibility for many neurodevelopmental and neuropsychiatric disorders, and studies on transgenic mice that separate the sex chromosome complement from gonadal sex, suggest differences in brain structure, and thus development and plasticity, may be due to the sex chromosomes rather than gonadal hormones. In preliminary studies we saw high expression of Xlr3 genes in the developing mouse cortex. Based on these findings and previously published research, we hypothesize that these genes function as transcriptional regulators and

they are expressed from the maternal X-chromosome in both males and females, and repressed on the paternal X-chromosome in females. Using *in situ* hybridization and q-PCR we have characterized the expression patterns and levels in the developing male and female cortex during multiple stages of neurogenesis. This knowledge may help explain observed clinical differences in susceptibility to autism and other neurodevelopmental and neuropsychiatric disorders.

Disclosures: **A. McDonough:** None. **J. Berletch:** None. **C. Disteche:** None. **R. Hevner:** None.

Poster

029. Neural Lineage Specification and Plasticity

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 29.03/A3

Topic: A.02. Neurogenesis and Gliogenesis

Support: NSF W05601

Joe and Marie Field Foundation

Title: Stroke injury enhances neurogenesis in multiple new stem cell niches along the ventricular system at sites of high blood-brain-barrier permeability

Authors: *R. LIN[1], J. CAI[1], C. NATHAN[1], X. WEI[1], S. SCHLEIDT[1], R. ROSENWASSER[2], L. IACOVITTI[1];
[1]Farber Inst. of Neuroscience, Dept. of Neurosci., [2]Dept. of Neurolog. Surgery, Thomas Jefferson Univ., Philadelphia, PA

Abstract: Previous studies have established the subventricular (SVZ) and subgranular (SGZ) zones as sites of neurogenesis in the adult forebrain (Doetsch et al., 1999a). Recently, our group also suggested that circumventricular organs (CVOs) serve as adult neural stem cell niches (Bennett et al., 2009, 2010). Since NSC proliferation and differentiation is relatively low in all of these sites in the quiescent brain, in this study, we sought to determine whether these processes might be amplified following perturbation. To do so, rats were subjected either to focal ischemic injury (MCAO) or sustained intraventricular infusion of the cell mitogen bFGF (via Alzet pump). Our data show that both stroke and bFGF induce a long-lasting (14 days) rise in the proliferation ($BrdU^+$) of $nestin^+$, $Sox2^+$, $GFAP^+$ NSCs and increase their differentiation into $Olig2^+$ glial progenitors and Dcx^+ neuroblasts in the SVZ and CVOs but also in several other novel sites along the third (3V) and fourth (4V) ventricles. Importantly, all these sites were supplied by a

rich vasculature of fenestrated capillaries and a blood-brain-barrier (BBB) that was highly permeable to systemically injected sodium fluorescein. These data indicate that stem cell niches exist not only near the lateral ventricle but at multiple sites along the entire ventricular system, consistent with the potential for widespread neurogenesis and gliogenesis in the adult brain. We further suggest that because of their leaky BBB, all of these niches are well positioned to respond to systemic cues following injury which could be important for brain repair.

Disclosures: **R. Lin:** None. **J. Cai:** None. **C. nathan:** None. **X. Wei:** None. **S. Schleidt:** None. **R. Rosenwasser:** None. **L. Iacovitti:** None.

Poster

029. Neural Lineage Specification and Plasticity

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 29.04/A4

Topic: A.02. Neurogenesis and Gliogenesis

Title: Differences in proliferation behaviour and expression of phenotypic markers in embryonic mouse adrenal chromaffin cells and sympathetic neuroblasts

Authors: ***W. CHAN**[1], D. G. GONSALVEZ[1], H. M. YOUNG[1], E. M. SOUTHARD-SMITH[2], K. N. CANE[1], C. R. ANDERSON[1];
[1]Dept. of Anat. and Neurosci., Univ. of Melbourne, Melbourne, Australia; [2]Div. of Genet. Medicine, Dept. of Med., Vanderbilt Univ. Sch. of Med., Nashville, TN

Abstract: Adrenal medullary chromaffin cells and peripheral sympathetic neurons originate from a common sympathoadrenal (SA) progenitor cell. The time course and signalling mechanisms underlying this lineage separation during embryonic development are not fully understood. The present study investigates the expression patterns of phenotypic markers as well as cell cycle dynamics in the embryonic murine adrenal medulla and the neighbouring suprarenal ganglion by immunohistochemical analysis. Transverse sections of the adrenal region in E10.5-16.5 C57BL/6 mice were processed with antibodies against Sox10, tyrosine hydroxylase (TH), phenylethanolamine N-methyltransferase (PNMT) and cocaine and amphetamine regulated transcript (CART). Ki67 immunohistochemistry, coupled with dual-labelling with S-phase markers, bromodeoxyuridine (BrdU) and 5-ethynyl-2'-deoxyuridine (EdU), allowed cell cycle analyses. The noradrenergic marker, TH was detected in cells in both the adrenal and sympathetic region, but with much stronger immunoreactivity (IR) for TH in the adrenal. There was strong CART immunostaining in most neuroblasts, whereas it was detected in only a

handful of adrenal chromaffin cells. Comparison of CART-IR versus TH-IR showed that neuroblasts have low intensity of TH-IR but high intensity of CART-IR, while chromaffin cells in the adrenal medulla had high intensity TH-IR without detectable CART-IR. This phenotypic segregation appeared as early as E12.5 when anatomical segregation of the two cell types is difficult. The adrenergic marker, PNMT, was first detected in a subpopulation of chromaffin cells from E14.5. Chromaffin cell and neuroblasts lineages showed differences in proliferative behaviour from their earliest appearance at E12.5. Initially, 70% of Sox10+ neural crest progenitors were proliferating at E10.5 but dramatically withdrew from the cell cycle at E11.5 as they started to express TH and differentiate into neuroblasts and chromaffin cells. Whereas 88% of TH+ neuroblasts were back in the cell cycle at E12.5, only around 20% of TH+ chromaffin cells were in the cell cycle at E12.5 and subsequent days. Cell cycle length increased modestly for TH+ cells in the ganglion and the adrenal medulla from E12.5. This study identifies the intensity of TH-IR and the expression of CART as phenotypic markers for early discrimination of the chromaffin cells and sympathetic neuroblasts. Developing chromaffin cells also show strikingly less proliferative activity relative to sympathetic neuroblasts.

Disclosures: **W. Chan:** None. **D.G. Gonsalvez:** None. **H.M. Young:** None. **E.M. Southard-Smith:** None. **K.N. Cane:** None. **C.R. Anderson:** None.

Poster

029. Neural Lineage Specification and Plasticity

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 29.05/A5

Topic: A.02. Neurogenesis and Gliogenesis

Support: 1F30MH102002-01

Title: The developmental genetic basis of cortical interneuron identity - the case of chandelier cells

Authors: *S. M. KELLY[1,2], M. HE[1], Z. HUANG[1];
[1]Cold Spring Harbor Lab., Cold Spring Harbor, NY; [2]Stony Brook Univ., Stony Brook, NY

Abstract: Neuronal firing activity within the cerebral cortex is regulated by a diverse assortment of inhibitory GABAergic interneurons, which are able to finely tune circuit activity through a vast array of morphological, molecular, synaptic, and electrophysiological properties. Most cortical interneurons in rodents arise predominantly from three neurogenic zones: the medial and

caudal ganglionic eminences (MGE, CGE), and the pre-optic area (POA). There is evidence that interneuron class identity is determined in part in part by spatial and temporal origin within the subpallium. Furthermore, intrinsic genetic programs and extrinsic cortical signals are both likely to play a role in defining different neuronal subtypes within each class and distributing these cells to cortical layers and regions in the proper density to allow the formation of meaningful circuit motifs. To approach a complete understanding of interneuron development, our studies have focused on characterizing the entire life history of the chandelier cell, arguably the most distinct and uniform GABAergic interneuron yet to be described. Chandelier cells have an unmistakable axon arbor of hundreds of vertically arranged cartridge synapses, each of which specifically innervates the axon initial segment of a pyramidal cell, the site where action potentials are generated. Neocortical chandelier cells (ChCs) are generated between mid-gestation and birth from progenitors in the MGE expressing the homeodomain transcription factor Nkx2.1. Here, using intersectional genetic fate mapping and novel birth dating techniques, we provide an experimental system to examine for the first time the cellular and molecular nature of progenitors that produce several well-defined MGE cell subtypes in cerebral cortex. Further, we have compared the birth timing and progenitor origin of ChCs found in various regions of the cerebrum, such as the hippocampus, piriform cortex, and neocortex. Through these studies, we hope to discover and characterize the mechanism by which Nkx2.1+ progenitors produce and distribute a diverse family of interneurons to be integrated into specific types of cognitive circuits throughout the cerebral cortex.

Disclosures: **S.M. Kelly:** None. **M. He:** None. **Z. Huang:** None.

Poster

029. Neural Lineage Specification and Plasticity

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 29.06/A6

Topic: A.02. Neurogenesis and Gliogenesis

Title: Molecular characterization of human cortical interneurons

Authors: ***J. L. CLOSE**[1], B. LEVI[1], V. MENON[1], S. ANDERSON[2], S. RAMANATHAN[3]; [1]Allen Inst. For Brain Sci., Seattle, WA; [2]Univ. of Pennsylvania, Philadelphia, PA; [3]Harvard Univ., Cambridge, MA

Abstract: We have established a robust protocol for generating cortical interneuron progenitors from human embryonic stem cells. The gene expression profile of these cells has been assayed at

the population, subpopulation and single-cell level to determine progenitor diversity and lineage relationships within heterogeneous pools of interneuron precursors and differentiating neurons. Using reporter lines developed to visualize specific populations and developmental stages, we have determined the gene regulatory networks that underlie the production and maturation of human interneuron subclasses that are crucial to cortical processing.

Disclosures: **J.L. Close:** None. **B. Levi:** None. **V. Menon:** None. **S. Anderson:** None. **S. Ramanathan:** None.

Poster

029. Neural Lineage Specification and Plasticity

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 29.07/A7

Topic: A.02. Neurogenesis and Gliogenesis

Support: NIH NRSA Fellowship 1F31DK097939

NIH R01 Grant 2R01DK078158-04A1

NIH U01 Grant 1U01DK101038-01

Title: Serotonin receptor 5-HT3A in the development of autonomic and sensory innervation of the lower urinary tract

Authors: *E. RITTER[1,3], D. P. BUEHLER[2], H.-H. WU[4], E. SOUTHARD-SMITH[2]; [2]Genet. Med., [1]Vanderbilt Univ., Nashville, TN; [3]Neurosci. Grad. Program, Vanderbilt Brain Inst., Nashville, TN; [4]Dept. of Pediatrics, Keck Sch. of Med. of Univ. of Southern California, Los Angeles, CA

Abstract: Decades of research demonstrate the importance of serotonin (5-HT) signaling in the fetal development and postnatal maturation of various neuronal populations. While most of these studies focused on the brain and enteric nervous system of the gut, very few have inspected the role of 5-HT in development of other peripheral nervous system components. In a gene expression analysis conducted in our lab, we noted increased expression of the type 3A serotonin receptor (5-HT3A) in fetal pelvic ganglia that provide autonomic innervation to the bladder and urethra. This finding, along with reports of 5-HT3A in adult urinary function and visceral pain, led us to pursue the role of this receptor in the development of bladder autonomic and sensory innervation. We aim to define the temporal, spatial, and cell-type specific expression patterns of

the 5-HT3A receptor throughout lower urinary tract development as a basis for interrogating gene function in this system. By employing the Htr3a-EGFP transgenic reporter mouse line and immunohistochemistry, we have characterized expression of the 5-HT3A receptor in a variety of both autonomic and sensory neuronal subtypes within the pelvic and dorsal root ganglia. Expression of 5-HT3A begins early in the development of bladder innervation at 12 days post coitus, which is the earliest time point 5-HT3A has been identified in mouse embryos. 5-HT3A co-localizes with both sympathetic and parasympathetic markers in the pelvic ganglia throughout fetal development, as well as several markers of nociceptive neurons in the dorsal root ganglia and bladder urothelium. Ongoing experiments are focused on determining roles for 5-HT3A signaling in urinary tract function and acute neurogenic inflammation in knockout mice. Given that very few treatment options are available for urinary incontinence and pelvic pain syndromes, elucidating the mechanisms of neurogenesis and maturation in the lower urinary tract lends itself to the identification of novel and specific therapeutic targets for treating these prevalent disorders.

Disclosures: **E. Ritter:** None. **D.P. Buehler:** None. **H. Wu:** None. **E. Southard-Smith:** None.

Poster

029. Neural Lineage Specification and Plasticity

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 29.08/A8

Topic: A.02. Neurogenesis and Gliogenesis

Support: NIH Grant RO1HD055655

NIH Grant R01MH056524

Title: Modeling neurodevelopmental disrutpion and glioblastoma with crispr/cas9 system

Authors: ***F. CHEN**[1], A. CHE[2], A. BECKER[4], J. LOTURCO[3];
[1]Dept. of Physiol. and Neurobio., Univ. of Connecticut, STORRS MANFLD, CT; [2]PNB, [3]Physiol. and Neurobio., Univ. of Connecticut, Storrs, CT; [4]Dept. of neuropathology, Univ. of Bonn Med. Sch., Bonn, Germany

Abstract: The newly developed CRISPR/Cas9 system offers fast, convenient, specific yet efficient way for genome editing. Here we report successful modeling of neurodevelopmental disorder as well as gliaoblastoma multiforme in rat using CRISPR/Cas9 system. *In vivo*

transfection of radial glia with sgRANs targeting PTEN successfully abolished PTEN expression in cells in radial glia lineage i.e. neurons, astrocytes and oligodendrocytes. PTEN-null neurons showed increased soma size, hypertrophic dendrites and axons. PTEN null neurons also showed altered electrophysiological properties such as decreased input resistance, increased sEPSC and mEPSC. These results indicate CRISPR/Cas9 can be used *in vivo* to manipulate gene expression. Moreover, electroporation of three sgRNAs targeting PTEN, NF1 and P53, respectively, into rat radial glia and successfully induced glioblastoma multiforme formation. To our knowledge, this is the first successful demonstration of modeling neurodevelopmental disorder and glioblastoma using CRISPR/Cas9 system.

Disclosures: **F. Chen:** None. **A. Che:** None. **A. Becker:** None. **J. LoTurco:** None.

Poster

029. Neural Lineage Specification and Plasticity

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 29.09/A9

Topic: A.02. Neurogenesis and Gliogenesis

Support: CIHR Grant

Title: Primitive neural stem cells at the top of the neural stem cell hierarchy

Authors: *S. YAMMINE[1], R. LEEDER[2], D. VAN DER KOOY[1];
[1]Mol. Genet., [2]Inst. of Med. Sci., Univ. of Toronto, Toronto, ON, Canada

Abstract: Primitive neural stem cells (pNSCs) are Oct4+GFAP- cells that can be isolated from the mouse embryo as early as E5.5, and persist throughout development into adulthood where they are a rare population residing in the subependymal zone of the lateral ventricles. pNSCs are LIF-dependent and can be clonally passaged *in vitro* to self-renew or give rise to definitive (d)NSCs, which are GFAP+ and dependent on EGF and FGF. Given that pNSCs arise earlier in development than dNSCs and are upstream dNSCs *in vitro*, we asked whether this hierarchy is true *in vivo*, and what the role of an upstream pNSC population is in the embryo and adult mouse brain. We have previously shown that expression of the pluripotency gene Oct4 is an exclusive marker for pNSCs, so we used Oct4fl/fl;Sox1Cre mice to assess the requirement of Oct4 in pNSCs. The forebrain of adult mice homozygous for the Oct4 conditional knockout did not give rise to any pNSC-derived neurospheres *in vitro*, while dNSC-derived neurospheres were not affected by Oct4 loss. This indicates a requirement for Oct4 expression in either the survival or

proliferation of pNSCs, and offers a mouse model for loss of pNSC function. We hypothesized that rare pNSCs serve as a reserve pool of NSCs in the adult brain. To test this, we ablated dNSCs and downstream progenitors by administering 7 days of AraC followed by 3 days of ganciclovir (GCV) to Oct4fl/fl;Sox1Cre;GFAP-tk mice by intraventricular infusion. While no dNSC-derived neurospheres form immediately after this ablation paradigm, the level of dNSC-derived neurospheres from control mice without the loss of Oct4 recover to 50% naive abundance 4 weeks post-treatment. In mice homozygous for the loss of Oct4, however, we observed a significantly reduced recovery of dNSCs following AraC/GCV infusion to only 6% 4 weeks post-treatment. Using Oct4fl/fl;Sox1Cre mice, we have observed that pNSCs in the perinatal brain also are sensitive to the loss of Oct4. Next we asked whether pNSCs are required for the initial establishment of the NSC lineage in the early embryo, as they are for repopulating a depleted NSC lineage in the adult. Preliminary results using mice with tamoxifen-inducible Oct4 excision suggest that pNSCs in the early embryo also are Oct4-dependent, providing a novel loss of function model to study the role of pNSCs at the onset of brain development. In conclusion, pNSCs in the early embryo, perinatal and adult brain are dependent on Oct4. Further, pNSCs are responsible for the repopulation of the depleted dNSC pool in the adult brain, strongly suggesting pNSCs are at the top of the neural stem cell lineage *in vivo* and may be responsible for the initial establishment of the NSC lineage in the early embryo.

Disclosures: **S. Yammine:** None. **R. Leeder:** None. **D. van der Kooy:** None.

Poster

029. Neural Lineage Specification and Plasticity

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 29.10/A10

Topic: A.02. Neurogenesis and Gliogenesis

Support: EMBO ALTF 1295-2012

Title: Dispersion of clonally related interneurons

Authors: *C. MAYER[1], X. H. JAGLIN[1], C. STREICHER[2], J. DIMIDSCHSTEIN[1], S. HIPPENMEYER[2], L. CEPKO[3], G. J. FISHELL[1];
[1]Physiol. & Neurosci., NYU Neurosci. Inst., New York, NY; [2]Inst. of Sci. and Technol. Austria, Klosterneuburg, Austria; [3]Departments of Genet. and Ophthalmology and HHMI, Harvard Med. Sch., Boston, MA

Abstract: Unlike pyramidal cells, GABAergic interneurons originate in subpallial germinal zones and migrate tangentially to reach the cortex. The majority of GABAergic interneurons originate from the medial ganglionic eminence (MGE), which in addition to cortical interneurons also generates interneurons destined for the striatum and hippocampus, as well as projection neurons for the globus pallidus, amygdala, and septum. It is poorly understood whether these cell types are generated from distinct intermixed progenitors, or from the same progenitors through a stochastic or temporally regulated program. To determine the relationship of MGE-derived interneurons, we specifically marked precursor cells in the MGE by *in utero* intraventricular injections of a retroviral library encoding GFP, and we identified sibling cells by DNA sequencing of a diverse set of viral DNA tags. Specificity of viral infection was achieved through the selective expression of the receptor for this retrovirus being confined to the MGE in a Cre-dependent manner. After cell migration, virally labeled neurons originating from the MGE were widely spread throughout the telencephalon. Clonally-related interneurons rarely exhibited spatially isolated clusters, as had been previously observed for cortical pyramidal clones. Instead, sibling neurons where spread across functional cortical areas and brain structures, including the hippocampus, striatum and cortex. Our results support the hypothesis that environmental cues rather than intrinsic programs determine the spatial distribution of interneurons. We will present our findings on the spatial spread and cell type differentiation of clonally-related interneurons.

Disclosures: **C. Mayer:** None. **X.H. Jaglin:** None. **C. Streicher:** None. **J. Dimidschstein:** None. **S. Hippenmeyer:** None. **L. Cepko:** None. **G.J. Fishell:** None.

Poster

029. Neural Lineage Specification and Plasticity

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 29.11/A11

Topic: A.02. Neurogenesis and Gliogenesis

Support: NIH Grant NS044080

Title: The role of Gsx2 in the generation of the septum-derived olfactory bulb interneurons

Authors: *S. QIN[1], H. CHAPMAN[3], S. M. WARE[4], R. R. WACLAW[2], K. CAMPBELL[1]; [1]Developmental Biol., [2]Exptl. Hematology and Cancer Biol., Cincinnati Children's Hosp. Med. Ctr., Cincinnati, OH; [3]Cell Biol. and Human Anat., Univ. of California, Davis, Davis, CA; [4]Pediatrics and Med. and Mol. Genet., Indiana Univ., Indianapolis, IN

Abstract: The homeobox gene Gsx2 is expressed in the ventricular zone (VZ) of the developing ventral telencephalon. Previous studies have suggested that Gsx2 is crucial for the specification of distinct neural cell types, including olfactory bulb (OB) interneurons, which originate from the dorsal lateral ganglionic eminence (dLGE) and septum and migrate rostrally toward the OB, where they radially migrate to populate different layers and terminally differentiate. Gsx2 was shown to be important for the specification of dLGE-derived OB interneurons; however, whether it is involved in the generation of septum-derived OB interneurons is unclear. In this study, we conditionally inactivated Gsx2 in the septum without drastically affecting it in the dLGE using Olig2Cre mice and examined whether septum-derived OB interneurons were impaired. Our results using a Zic3-LacZ reporter, which is expressed by many septal cells and their OB derivatives, suggest that septal progenitors give rise to a subpopulation of Sp8+ and calretinin (CR)+ OB interneurons in the glomerular layer (GL). Moreover, conditional inactivation of Gsx2 using Olig2Cre impaired the generation of septum-derived Sp8+ and CR+ OB interneurons in the GL. To investigate the cause(s) of the phenotype, we analyzed control and Gsx2 conditional knockout (Gsx2 cKO) embryos and found reduced specification of Sp8+ neuroblasts in the mutant septum. By analyzing proliferation markers Ki67 and BrdU, we observed reduced number of intermediate progenitors (INPs) as well as increased cell cycle exit in the subventricular zone (SVZ) of the mutant septum, suggesting Gsx2 is crucial for the generation and/or expansion of INPs in the SVZ of the septum. In summary, our results support the notion that Gsx2 is required for the normal specification and expansion of septum progenitors that give rise to a subpopulation of OB interneurons.

Disclosures: **S. Qin:** None. **H. Chapman:** None. **S.M. Ware:** None. **R.R. Waclaw:** None. **K. Campbell:** None.

Poster

029. Neural Lineage Specification and Plasticity

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 29.12/A12

Topic: A.02. Neurogenesis and Gliogenesis

Support: NIH R01NS045702

Title: Netrin-1 promotes neuroblast lineage plasticity in the subventricular zone after neonatal brain injury

Authors: *M. RAYMOND, S. FOX, P. LI, V. GALLO;
Children's Natl. Med. Ctr., Washington, DC

Abstract: Infants born preterm and with very-low-birth-weight are susceptible to hypoxia-induced diffuse white matter injury (DWMI) leading to loss of oligodendrocytes, myelin abnormalities, and long term behavioral disorders. Progenitor cells residing in the developing brain hold great potential as a means to prevent or reverse DWMI caused by hypoxic events. Using a model of chronic hypoxia in the perinatal rodent, resulting in DWMI similar to what is observed in preterm infants, we use genetic lineage tracing to demonstrate that distinct progenitor/stem cell populations are capable of regenerating oligodendrocytes in WM after injury, including Glial Fibrillary Acidic Protein (GFAP)-, Platelet Derived Growth Factor Receptor-α (PDGFRα)-, Ascl1 (Mash1)-, and Glutamate Decarboxylase-65 (GAD65)-expressing cells. We also demonstrate hypoxia-induced increase in the expression of the chemotropic factor netrin-1 (NTN1) in both the subventricular zone (SVZ) and choroid plexus (CP). GAD65-expressing progenitors express the NTN1 receptors deleted in colorectal cancer (DCC), uncoordinated homolog 2 (Unc5H2), and Unc5H4. Using both *in vivo* and *in vitro* methods, we demonstrate a NTN1- mediated migratory effect on GAD65-expressing progenitors of the SVZ resulting in an increase in GAD65-derived glia in the overlying WM. We also show that NTN1 plays a role in lineage determination, as treatment of SVZ progenitors with NTN1 lead to the generation of oligodendrocytes and astrocytes derived from GAD65-expressing cells. Our study demonstrates novel sources of glia in the developing brain following DWMI, and reveals NTN1 as a molecular signal associated with GAD65-progenitor migration and lineage plasticity in the postnatal brain. Supported by NIH R01NS045702.

Disclosures: **M. Raymond:** None. **S. Fox:** None. **P. Li:** None. **V. Gallo:** None.

Poster

029. Neural Lineage Specification and Plasticity

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 29.13/A13

Topic: A.02. Neurogenesis and Gliogenesis

Support: Bundesministerium für Bildung und Forschung (01GN 1009A)

Deutsche Forschungsgemeinschaft (BE 4182/2-2)

Title: Distinct lineages for neuro- and oligodendrogliogenesis in the adult subependymal zone

Authors: *F. ORTEGA[1], S. GASCON[2], G. MASSERDOTTI[2], A. DESHPANDE[2], C. SIMON[2], J. FISCHER[3], L. DIMOU[2], C. LIE[4], T. SCHROEDER[5], B. BERNINGER[1];
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Abstract: The adult mouse subependymal zone (SEZ) harbors a population of adult neural stem cells (aNSCs) known as type B cells that give rise to neuronal and oligodendroglial progeny. However it is not known how the aNSCs behave during their lineage progression towards the neurogenic or oligodendrogliogenic fate. Likewise is not known whether the same aNSC can give rise to neuronal and oligodendroglial progeny or whether these distinct progenies constitute entirely separate lineages. Continuous live imaging and single cell tracking of aNSCs and their progeny isolated from the mouse SEZ revealed the lineage progression of the aNSCs. Moreover we confirmed that aNSCs exclusively generate oligodendroglia or neurons, but never both within a single lineage. Furthermore, activation of canonical Wnt signaling selectively stimulated proliferation within the oligodendrogliogenic lineage, resulting in a massive increase in oligodendrogliogenesis without changing lineage choice or proliferation within neurogenic clones. *In vivo* activation or inhibition of canonical Wnt signaling respectively increased or decreased the number of Olig2 and PDGFR-a positive cells, suggesting that this pathway contributes to the fine tuning of oligodendrogenesis in the adult SEZ.

Disclosures: **F. Ortega:** None. **S. Gascon:** None. **G. Masserdotti:** None. **A. Deshpande:** None. **C. Simon:** None. **J. Fischer:** None. **L. Dimou:** None. **C. Lie:** None. **T. Schroeder:** None. **B. Berninger:** None.

Poster

029. Neural Lineage Specification and Plasticity

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 29.14/A14

Topic: A.04. Stem Cells

Support: Télevie, FNRS, Belgium

Fonds Spéciaux, ULg, Belgium

Title: Characterization of neural crest stem cells isolated from human adult bone marrow

Authors: *S. WISLET, C. COSTE, V. NEIRINCKX, B. ROGISTER, A. GOTHOT;
Univ. of Liège, Liège, Belgium

Abstract: Adult neural crest stem cells (NCSC) are of extraordinary high plasticity and promising candidates for a use in regenerative medicine. Several locations like skin, dental pulp or bone marrow have been described in rodent. However, very few information is available concerning their correspondence in human tissues. The main objective of this project was then to isolate and characterize NCSC from adult human bone marrow. In order to reach that goal, we decided to compare three different sources of adult human stem cells: 1) MSC isolated from adipose tissue, considered as negative control for NCSC; 2) NCSC from dermis, considered as positive control; 3) MSC isolated from iliac bone marrow. Those three cell types were compared using several methods: 1) cell culture: we evaluated all cell types to be cultivated in clonal, adherent or sphere conditions. 2) cell differentiating abilities: we compared all cell types to differentiate into adipocytes, osteocytes, chondrocytes, melanocytes, and Schwann cells. 3) Expression profile: we evaluated the expression of stem cell and NCSC markers like Sox2, Notch1, Oct3/4, AP2, Ascl1, Brn3a, FoxD3, Msi1, Ngn1, P75NTR, Pax3, Pax6, Snail1, Sox1, Sox9, and Sox10. 4) Injection into chick embryos: in this last experiment we analyzed the ability of all cell types to migrate and follow migration pathway of chick NCSC. Altogether, it appeared that the ability of cells to growth as spheres seemed to be linked to their embryonic origin (neural crest). NCSC were confirmed to be present in human dermis, in human adult bone marrow, but also and surprisingly, in human adipose tissue. NCSC from all tested tissues were FoxD3, Msi1, Ngn1, P75NTR, Slug and Twist-positive, but negative for all other tested makers. Finally, all NCSC injected into chick embryos, were able to migrate and differentiate into mature cell types classically observed for NCSC, while MSC were quiescent on the site of injection.

Disclosures: **S. Wislet:** None. **C. Coste:** None. **V. Neirinckx:** None. **B. Rogister:** None. **A. Gothot:** None.

Poster

029. Neural Lineage Specification and Plasticity

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 29.15/A15

Topic: A.04. Stem Cells

Support: KHIDI Grant A120202

Title: Effects of zinc on adipose-derived mesenchymal stem cell (AD-MSCs) proliferation and differentiation

Authors: *H. KIM[1], B. LEE[1], J. KIM[1], B. CHOI[1], I. KIM[1], S. LEE[2], M. SOHN[3], S. SUH[1]; [1]Dept. of Physiol., Hallym Univ., CHUNCHEON, Korea, Republic of; [2]Dept. of Med. biology, Hallym Univ., Chuncheon, Korea, Republic of; [3]Dept. of Nursing, Inha Univ., Incheon, Korea, Republic of

Abstract: Zinc is an essential element required for cell division, migration and proliferation. Zinc is highly localized in the synaptic vesicle of mossy fiber terminals of the dentate granule cells where neural cell proliferation is most active in the adult brain Especially, in zinc deficient conditions, cell proliferation and differentiation of neural progenitors are impaired significantly. Our previous study demonstrated that zinc chelation reduced hypoglycemia- or seizure-induced neurogenesis in adult animals. Mesenchymal stem cells (MSCs) are multi-potent stem cells that have the capacity to differentiate into osteoblasts, chondrocytes, adipocytes, and neuron-like cells. Since MSCs are derived from various sources including adipose tissue which is abundant and easily obtained, it could be an ideal candidate for cell replacement therapy. Several studies have revealed that MSCs can differentiate into neurons when exposed to the appropriate soluble external factors, which can be supplied in cell culture medium. Thus, the objective of this study was to evaluate the effect of zinc on adipose-derived mesenchymal stem cell proliferation and differentiation. To evaluate the role of zinc on cellular proliferation, adipose-derived MSCs (AD-MSCs) were cultured in 96-well plate at 5×10*2 cells per well. After the cells had attached they were treated with culture media containing zinc at the following concentrations: 0, 10, 30, 50, 100 and 300μM. Zinc treated medium was changed every 3 days for 14 days. AD-MSCs proliferation was analyzed at day 3, 6, 9 and 14 with cell counting kit (CCK-8) assay. AD-MSCs proliferation was significantly increased by zinc supplement in a dose-dependent manner except 300μM. In 300μM zinc survival rate was only 35% and 8% at day 3 and day 6, respectively, compared with control. To investigate whether extracellular zinc chelation affects mesenchymal stem cell proliferation, cultured AD-MSCs were treated with CaEDTA(1mM) or clioquinol(CQ)(50μM) in medium. CaEDTA had no effect on AD-MSCs proliferation compared with control. However, in CQ treated AD-MSCs were dead within 24 hours. Taken together, the present study found that zinc is a vital element for AD-MSCs proliferation and differentiation but high concentration of zinc is harmful.

Disclosures: **H. Kim:** None. **B. Lee:** None. **J. Kim:** None. **B. Choi:** None. **I. Kim:** None. **S. Suh:** None. **S. Lee:** None. **M. Sohn:** None.

Poster

029. Neural Lineage Specification and Plasticity

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 29.16/A16

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

Support: DFG grant SFB 780/TP5

DFG grant MA2410/1-3 and 1-4

DFG grant FR 620/12-1

NIH grant R37 HL63762

American Health Assistance Foundation

Consortium for Frontotemporal Dementia Research

Bright Focus Foundation

Title: Characterization of a novel creert transgenic line suitable for inducible gene manipulation in cerebellar granule cells

Authors: *I. T. POHLKAMP[1,2,3], L. STELLER[3], P. MAY[4], T. GUENTHER[5], R. SCHUELE[5], J. HERZ[2,3], M. FROTSCHER[6], H. H. BOCK[4];
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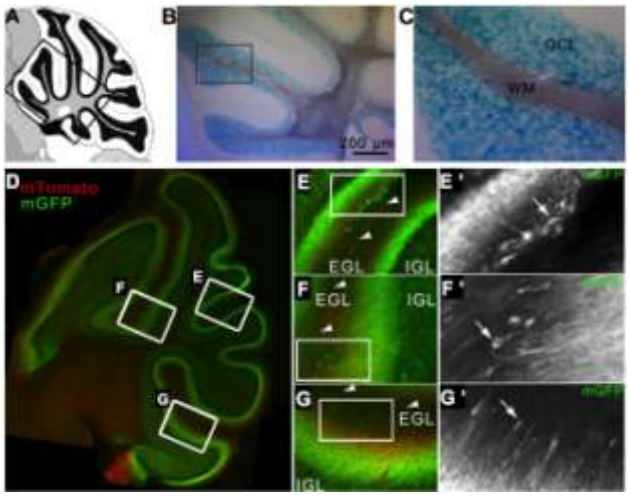
Abstract: We created an Nse-CreERT2 mouse line expressing the tamoxifen-inducible CreERT2 recombinase under the control of the neuron-specific enolase (Nse) promoter. By using two different Cre reporter lines we could show that this Nse-CreERT2 line has recombination activity in the granule cells of all cerebellar lobules (Fig.1 A-C) as well as in postmitotic granule cell precursors (GCP) in the external granular layer of the developing cerebellum (Fig.1 D-G). A few hippocampal dentate gyrus granule cells showed Cre-mediated recombination as well. Cre

activity could be induced in both the developing and adult mouse brain. The established mouse line constitutes a valuable tool to study the function of genes expressed by cerebellar granule cells in the developing and adult brain. In combination with reporter lines it is a useful model to analyze the development and maintenance of the cerebellar architecture including granule cell distribution, migration, and the extension of granule cell fibers *in vivo*. Figure 1: CreERT2 activity in the cerebellum is restricted to granule cells. A-C: NSE-CreERT2 mice were bred to a R26-LoxP-stop-LoxP-LacZ reporter line and tamoxifen was injected at adult age. A: Schematic overview, B: LacZ staining (blue) is restricted to the GCL, one lobule is shown enlarged in (C). D-G: NSE-CreERT2 mice were bred with a R26-CAG-LoxP-mTomato-LoxP-mGFP reporter mouse, injected at P3 and sacrificed at P8. D: overview of the cerebellum, GFP immunoreactivity illustrates cells in that recombination has occured, mTomato is expressed in non-recombined cells. Frames E-G are shown enlarged, E'-G' = further enlargement to show mGFP-immunoreactive granule cell precursors in the inner EGL. Arrowheads in E-G point to the fissure between two lobules. Arrows point to precursors in the EGL, presumable migrating radially (E') or tangentially (F',G'). WM = white matter, GCL = granule cell layer, mGFP = membrane tagged GFP. mTomato = membrane tagged Tomato. IGL = internal granule cell layer, EGL = external granule cell layer.



Disclosures: **I.T. Pohlkamp:** None. **L. Steller:** None. **P. May:** None. **T. Guenther:** None. **R. Schuele:** None. **J. Herz:** None. **M. Frotscher:** None. **H.H. Bock:** None.

Poster

029. Neural Lineage Specification and Plasticity

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 29.17/A17

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

Support: NIH/NINDS/OD RC2NS069488

Title: Time, place and significance of protein-protein interactions within the brain of intact animals

Authors: N. SHARIFAI[1], T.-C. DENG[1], M. BOULINA[1], H. SAMARAJEEWA[1], R. PRABHAKA[2], S. WUCHTY[3], *A. CHIBA[1];
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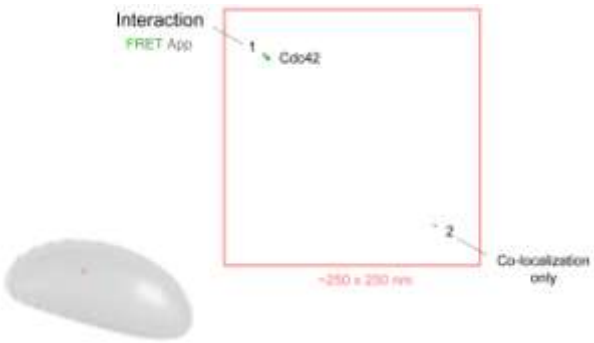
Abstract: The current landscape of interactome is either a static network of proteins reconstructed from observations made outside endogenous context or a dynamic network of proteins implicated through genetic interactions occurring inside intact animals without evidence of physical association between proteins. Here, we describe strategies to visualize dynamic protein interactions within living animals. Using optically transparent organisms and Förster resonance energy transfer as a proxy for physical association between proteins (FRET App), we quantitate when and where a ubiquitously expressed molecular switch signals through broadly connected partners. Freely dispersing, the proteins physically associate in a fraction of the time and place where they co-localize. Their restrictive signaling captured at the level of whole brain and single neurons is consistent with the knockout phenotype that emerges during neuronal network formation. Imaging the dynamic protein network within intact animals supplements analyses of protein function and provides insights to how molecular integration underscores complex cellular networks.



Disclosures: **N. Sharifai:** None. **A. Chiba:** A. Employment/Salary (full or part-time):; University of Miami. **M. Boulina:** None. **T. Deng:** None. **H. Samarajeewa:** None. **R. Prabhaka:** None. **S. Wuchty:** None.

Poster

029. Neural Lineage Specification and Plasticity

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 29.18/A18

Topic: A.04. Stem Cells

Support: PROMEP/103.5/10/7697

CONACYT CB2011-167821

CONACYT scholarship (CVU/Becario): 265757/218228

FAI2011 (C12-FAI-0362.62)

Title: Effects of cytomegalovirus infection in human neural precursor cells depend on differentiation state

Authors: *H. M. GONZÁLEZ[1], C. SALAZAR-ALDRETE[1], A. HERNÁNDEZ-SALINAS[2], M. JIMÉNEZ-CAPDEVILLE[1], D. NOYOLA[2], C. CASTILLO[1];
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Abstract: Cytomegalovirus (CMV) is the most common cause of congenital infection in developed countries and a major cause of developmental brain disorders in children, including mental retardation and hearing loss. However, the mechanisms that produce central nervous system damage during congenital CMV infection have not been clearly elucidated. Some *in vitro* models have demonstrated that neural precursor cells (NPCs) show the greatest susceptibility to CMV infection in developing brains. We sought to establish an *in vitro* model of CMV infection of the developing brain in order to analyze the cellular events associated to invasion by this virus. Two different NPCs were used, the human SK-N-MC neuroblastoma cell line and an immortalized neural cell line, hNS-1. The aim was to investigate the effect of the differentiation stage in relation to the susceptibility by comparing the neuroblastoma cell line with the multipotent cell line hNS-1. We infected both cell lines with the human CMV laboratory strain AD169 at multiplicity of infection of 0.1 and 0.01. Viral infection was corroborated by detection of the CMV glycoprotein B using an indirect immunofluorescence assay. We found that the effects of the virus were more severe in the neuroblastoma cell line where a notorious loss of cultured cells was noticed and low viral concentrations induced cellular proliferation in a transitory way, in addition to possible differentiation stimulation. Furthermore, we induced hNS-1 to differentiate and evaluated the effect of CMV in these differentiated cells. Like SK-N-MC cells, hNS-1 differentiated cells were also susceptible. Viability of differentiated hNS-1 cells decreased after CMV infection in contrast with undifferentiated cells. In addition, differentiated

hNS-1 cells showed an extensive cytopathic effect whereas the effect was scarce in undifferentiated cells. Our results show that NPCs are permissive to CMV infection and the differentiation degree is an important factor for virus susceptibility, those characteristics make CMV able to produce changes in the developing brain and also it may contribute to establish a critical period time where the infection may be more severe.

Disclosures: H.M. González: None. **C. Salazar-Aldrete:** None. **A. Hernández-Salinas:** None. **M. Jiménez-Capdeville:** None. **D. Noyola:** None. **C. Castillo:** None.

Poster

029. Neural Lineage Specification and Plasticity

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Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 29.19/A19

Topic: A.04. Stem Cells

Support: CONACYT (MEXICO) POSDOCTORAL FELLOWSHIP

Title: Effects of gdnf and et3 on proliferation and differentiation of postnatal neural progenitors in murine model

Authors: *A. CARREON-RODRIGUEZ[1,2], R. HARTMANN[4], H. HORDER[4], J. BELKIND-GERSON[3], A. M. GOLDSTEIN[2];
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Abstract: **Purpose:** The aim of this study was to characterize enteric neural stem cells (ENSC) of the postnatal murine intestine using the neurosphere culture system. We demonstrated the effects of GDNF, Et3 and their combination on the development and maturation of ENSCs isolated from postnatal murine muscularis and mucosa-submucosa-layer *in vitro* **Materials & Methods:** Large intestine of postnatal mice (P19 – P35) was separated into mucosa-submucosa and muscularis layer. The tissue was minced and digested with dispase and collagenase and mechanically dispersed and filtered. The cells were grown in Proliferation NeuroCult Medium supplemented with EGF, bFGF and heparin in low adherence plates and divided into control or groups treated with GDNF, Et3 or their combination. Cells were incubated at 37^{o}C and neurosphere-like bodies (NLB) developed in suspension. At day 5, the number and diameter of NLB's (Feret's diameter) were determined by phase-contrast microscopy taking 50 um as lower

limit, and then NLBs were dispersed, and plated in a suspension with Differentiation Neurocult Medium. Each control or treated group was split again in four groups control or treated with GDNF, Et3 or their combination (GE) during differentiation phase for 1 week, at the end of which expression of TuJ1 (neural cell body and neurites) and GFAP (glial) was determined by immunocytochemistry (ICC). Statistical analysis were performed by Anova followed by Fisher LSD. **Results:** The muscularis-derived cells treated with combination of GE gave the highest number of NLB's compared to other groups. In contrast treatment with GE on muscularis-derived cells produced smaller NLB's compared to other groups but not affecting the number of them. Regarding differentiation capacity, a difference was found in muscularis-derived vs. mucosa-submucosa-derived neurospheres. A higher cell density, sprouting and networks were more pronounced in the muscularis-derived populations within the muscularis culture, pretreatment with Et3 gave rise to TuJ1+ neuronal cells migrating out of the neurosphere and forming dense networks of cells. **Conclusions:** Et3 on its own seems to have a stronger impact on number of postnatal mucosa-submucosa spheres vs embryonic cultures. Our results of ICC against GFAP and TuJ1 also demonstrate an important supporting role of Et3 pretreatment during proliferation with the effect to stimulate migratory processes and neuronal phenotype during differentiation in postnatal muscularis-derived enteric neurospheres. In contrast,mucosa-submucosa-derived spheres seem to benefit of GDNF pretreatment during proliferation which later enhances neuronal growth during differentiation.

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Poster

029. Neural Lineage Specification and Plasticity

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 29.20/A20

Topic: A.04. Stem Cells

Support: CONACYT 140917

CONACYT 130627

INPER 21041

INPER 21081

Title: Expression of cortical neuron markers in cells derived from human amniotic epithelial cells

Authors: D. AVILA-GONZALEZ[1,2], I. L. GARCIA-CASTRO[1], A. MOLINA-HERNANDEZ[1], G. GARCIA-LOPEZ[1], *N. F. DIAZ[1];
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Abstract: The human pluripotent stem cells (PSC), i.e. embryonic stem cells and induced PSC, have the capacity to differentiate into all cell types that conform the embryo. These cells express the transcription factors OCT4, SOX2 and NANOG as well as TRA 1-60, SSEA-3 and -4, surface PSC-specific antigens. Furthermore, in recent years significant advances have been made in differentiation of PSC into a specific neural type. However, there are several issues to be solved before its clinical therapeutic applicability, i.e: immune rejection, chromosomal and epigenetic aberration, possible formation of tumors, etc. On the other hand, the human amniotic epithelial cells (hAEC) have been postulated as a reservoir source of PSC. Several laboratories have described that hAEC share characteristics with PSC and can differentiate into specialized cells. Also, these cells synthetize and release brain-derived neurotrophic factor and neurotrophin 3, which are involved in neuron differentiation. Finally, hAECs have several properties that can be useful in the clinic, such as low immunogenicity, anti-viral and anti-bacterial effects, etc. The main objective of this study was to analyze if hAEC have the ability to differentiate into cortical neurons. To this end, we used a reproducible protocol to obtain hAEC from normal pregnancy at term. The tissue was collected in accordance to institutional ethics committee guidelines. After mechanic separation and enzymatic treatment, hAEC were cultured in standard medium adding epidermal growth factor until they reached confluence. As a first approach, we analyzed the presence of the pluripotent stem cell markers in our cultures. Our results showed that the cells were positive to pluripotency transcription factors and surface antigens, as well as, E-cadherin (cell adhesion molecule). Then, we determined if these cells were able to differentiate into neural types of the cerebral cortex. In a first stage, we utilized the fibroblastic growth factor type 2 and an inhibitor of Activin/Nodal signaling (SB431542) in a chemically defined medium to generate neural progenitor cells from hAEC. Then, we withdrew all factors from the medium to induce cortical fate differentiation. Until now, our results have shown the presence of neural progenitor (PAX6) and intermediate progenitor (Tbr2) markers in our cultures. According to these results, hAEC would be a good model to study the differentiation of human neuronal lineages.

Disclosures: **D. Avila-Gonzalez:** None. **N.F. Diaz:** None. **A. Molina-Hernandez:** None. **G. Garcia-Lopez:** None. **I.L. Garcia-Castro:** None.

Poster

029. Neural Lineage Specification and Plasticity

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 29.21/A21

Topic: A.04. Stem Cells

Support: The Institute of Industrial Technology Grant, Toyo University

Title: Investigation of the relationship between iPS and C2C12 cells regarding both diffusible factors and cell-cell contacts

Authors: *T. SHINGEN, T. NEDACHI, H. KAWAGUCHI;
Toyo Univ., Itakura, Japan

Abstract: Induced pluripotent stem (iPS) cells have the capability to differentiate into various types of cells induced by diffusible factors *in vitro*. However, we expect a new method by which iPS cells directly differentiate into various organs *in vivo*. The development of this method requires the elucidation of the relationship between iPS cells and other types of surrounding cells regarding both diffusible factors and cell-cell contacts. After C2C12 cells, a cell line derived from mouse myoblastoma, were cultured on 35-mm dishes, the supernatant was collected and filtered to prepare C2C12 cell conditioned medium (C2C12-CM). We found that iPS cells originated from the mouse skin efficiently differentiated into neuronal cells by the addition of C2C12-CM (level of significance, $p < 0.05$), analyzing the intensity of fluorescence by immunofluorescent staining using an anti-βIII-tubulin antibody. Subsequently, iPS and C2C12 cells were co-cultured separately on 35-mm dishes using a cloning ring, which was removed after 1 day of cultivation. We also found that the direction of the outgrowth of neuritis derived from the induced neuronal cells tended to be opposite to that of C2C12 cells after cultivation for 12 days. Therefore, the detailed time course of the differentiation of induced neural cells was observed via time-lapse videomicroscopy using an incubator microscope during the co-culturing of iPS and C2C12 cells. The time course of the expression of various mRNA, such as βIII-tubulin, Lim-3, and NeuN, in differentiated iPS cells by the addition of C2C12-CM was also investigated using RT-PCR. This study was partially supported by the Institute of Industrial Technology Grant, Toyo University.

Disclosures: **T. Shingen:** None. **H. Kawaguchi:** None. **T. Nedachi:** None.

Poster

029. Neural Lineage Specification and Plasticity

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 29.22/A22

Topic: A.04. Stem Cells

Support: This work was supported by NIH/NINDS Intramural Research Program

Title: Definitive multiplex biomarker identification of embryonic rat cortical neural stem cell phenotype reveals that these cells are ontogenetically restricted only to early embryonic development but can recapitulate their embryonic seminal properties both *in vitro* and *in vivo* after implantation into adult rat brain

Authors: *D. MARIC, N. POTHAYEE, Y. H. CHANG, A. SEDLOCK, K. SHARER, N. BOURAOUD, J. L. BARKER, A. KORETSKY;
NINDS/NIH, Bethesda, MD

Abstract: Embryonic cortical neural stem cells (ecNSCs) in the rat emerge from telencephalic neuroepithelium (NE) at the onset of neural tube closure at 11 days of gestation (E11), where they undergo both symmetrical divisions to maintain their self-renewal and asymmetrical divisions to begin initial differentiation along the neuronal and glial cell lineages. Mediating this critical transition is the interplay of at least two transcription factors, Sox2 and Pax6, with increased expression of the latter being crucial in driving ecNSCs toward lineage commitment and promoting expression of LeX antigen (aka SSEA-1 or CD15), one of the earliest phenotypic markers identifying embryonic cortical neuroglial progenitors (ecNGPs). Using these established neural cell lineage delimited properties, we developed a comprehensive multiplex fluorescence immunostaining method combining up to 30 relevant biomarkers to phenotypically identify bona fide ecNSCs, their intermediates and differentiated endpoints both *in situ* and *in vitro*. Applying this phenotyping method to sagittal rat brain sections staged over E11-22 and over perinatal-adult period of rat cortical development, it was evident that ecNSCs uniquely express a $Nestin^{+}CD29^{+}PCNA^{+}$ $Sox2^{+}Pax6^{-}Tbr2^{-}Tbr1^{-}Olig2^{-}LeX^{-}GLAST^{-}S100^{-}GFAP^{-}Doublecortin^{-}$ $CD57^{-}PSA\text{-}NCAM^{-}$ phenotype, thus identifying them as authentic self-renewing NSCs. Over E12-15, Pax6 is progressively up-regulated in ecNSCs initially promoting their asymmetrical divisions into $Nestin^{+}CD29^{+}Sox2^{+}Pax6^{+}LeX^{+}$ ecNGPs, which give rise both to $Tbr2^{+}LeX^{+/-}$ $Doublecortin^{+}$ neuronal progenitors and $Tbr2^{-}LeX^{+/-}GLAST^{+}$ radial glia. After E17, the above ecNSC phenotype is no longer detectable in the cortical NE or other purported NSC niches implying a very limited ontogenetic existence of these cells during embryonic cortical development. Similarly, in adult rat brain, neither ependymal nor subependymal cells lining the SVZ, a purported source of adult cortical NSCs (acNSCs), nor their putative NSCs counterparts in the dentate gyrus of hippocampus exhibited the unique phenotype of ecNSCs, implying that neurogenic and gliogenic potentials in adult rat brain are derived from a separate seminal pool of neural stem/progenitor cells rather than embryonic-like NSCs. However, using optimized multiplex biomarker cell identification methods to phenotype live ecNSCs and preparative FACS

cell sorting to isolate a pure population of these cells, it was possible to recapitulate their embryonic seminal properties both in short-term cultures under permissive growth conditions and after implantation of these cells into lateral ventricles of 3-week-old rat brains.

Disclosures: **D. Maric:** None. **N. Pothayee:** None. **Y.H. Chang:** None. **A. Sedlock:** None. **K. Sharer:** None. **N. Bouraoud:** None. **J.L. Barker:** None. **A. Koretsky:** None.

Poster

029. Neural Lineage Specification and Plasticity

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 29.23/A23

Topic: A.04. Stem Cells

Title: Neurotrophins and trk receptors expression in preinduced rat bone marrow stromal cells into neuron phenotype with selegiline

Authors: *A. ROEINTAN[1], T. TIRAIHI[2,3], A. ABDANIPOUR[4], T. TAHERI[2];
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Abstract: Cell therapy is one of the approaches for treatment of locomotive deficits in spinal cord trauma and neurodegenerative disorders. Neural stem cells (NSCs) derived from bone marrow stromal cell (BMSCs) are considered as a feasible option for cell therapy. NSCs can be used as autologous without mutagenic and ethical problems. *In vitro* study showed that several chemicals could induce BMSCs into neural phenotypes, while some of these chemicals were reported to have mutagenic, teratogenic or carcinogenic properties. In this study, we have investigated the feasibility of using of Selegiline as an efficient inducer for neuronal differentiation of rat BMSCs and its effect on gene expression of neurotrophins and their receptors during neural differentiation of rat BMSCs. Selegiline has multiple effects which makes it a good candidate for neuroprotection as well as induction of neurotrophic factors. The optimal concentration of Selegiline was selected by dose response and time course studies using the percentage of viable cells and percentages of immunoreactive cells to Nestin and Neurofilament 68 (marker for neural progenitor), while the markers of neuron such as Tyrosine Hydroxylase, Neu-N and Neurofilament 200 were also evaluated. The expression profile of neurotrophins including NGF, BDNF and NT-3 and their receptors (TrkA, TrkB, TrkC and

p75NTR) receptors was evaluated. The results indicated that the NSCs are immunoreactive for Neurofilaments 68, Nestin, Tyrosine Hydroxylase but they are not immunoreactive to Neurofilaments -200 and Neu-N. According to results of RT-PCR, NGF and BDNF but not NT-3 are expressed in both un-differentiated as well as the induced BMSCs. In contrast, TrkA and TrkB is expressed just in neurally differentiated cells, while TrkC is not expressed in these cells either before or after differentiation. Interestingly, p75NTR expression is absent in un-differentiated cells but is initiated upon the induction of neural differentiation. Based on these results, Selegiline is an inducer for BMSCs. Moreover, the expression of neurotrophin genes and their receptors suggested the autocrine mode of action of induced BMSCs.

Disclosures: **A. Roeintan:** A. Employment/Salary (full or part-time):; full. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Shefa Neuroscience Research center, Khatam Ol Anbia Hospital. **T. tiraihi:** None. **A. Abdanipour:** None. **T. taheri:** None.

Poster

030. Neuronal Differentiation

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 30.01/A24

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

Support: HUCM BFPSAP

Title: Neuronal and vascular anatomy in the zebrafish brain

Authors: *S. RAHMAT, E. GILLAND, T. HEINBOCKEL;
Howard Univ., Washington, DC

Abstract: Although embryonic and larval vasculature in zebrafish has been described, few studies have documented brain vessels and related neuroanatomy beyond two weeks of development. Transgenic zebrafish, between one and twelve weeks old, expressing GFP in cranial motor neurons were injected with fluorescent vascular dye and examined with confocal angiography to demonstrate maturation of cerebral vasculature. A segmental series of midline hindbrain central arteries (CAs) in early larvae connect the basilar artery to bilateral venous channels by extending intra-rhombomerically through the mid portion of rhombomeres (r) r3-8, medial to the reticulospinal neuronal clusters in each segment. Each midline CA divides into paired trunks near the ventricular surface at the level of the medial longitudinal fasciculus

(MLF), continues branching within the hindbrain neuroepithelium and ends as numerous small, unbranched channels that traverse nuclei and fiber tracts before draining into a laterally-located pial venous plexus. While hindbrain CAs in younger stages (week one) were roughly similar along the series, the r3 and r8 stems became progressively larger than the others during weeks two and three, with more elaborate branching and increased neurovascular territorial supply. By 75 days, the r3 CA was effectively the arterial supply of the entire rostral hindbrain, including branches that supply the cerebellum, the trigeminal motor nucleus in r2 and the migrated facial motor nucleus in r7. Since small r4-7 CAs are present in mature brains, it is likely that the increased territory of the r3 CA resulted from angiogenic sprouting of existing branches rather than capture of neighboring arterial trees by vessel fusion. In contrast, other major cerebellar, midbrain and forebrain arterial vessels supplied roughly the same neuronal territories in mature animals as in early larvae, but with greatly increased branching. Zebrafish hindbrain vascular and CNS elements are patterned in relation to the early rhombomeric segmental framework, with some aspects of the segmental plan being maintained in adult brains, while other, clearly non-segmental features arise during post-larval brain maturation.

Disclosures: **S. Rahmat:** None. **E. Gilland:** None. **T. Heinbockel:** None.

Poster

030. Neuronal Differentiation

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 30.02/A25

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

Support: NIH R01 NS065054

Bob Allison Ataxia Research Center

Title: Development of serotonergic neurons in the larval zebrafish spinal cord

Authors: *J. E. MONTGOMERY[1], T. D. WIGGIN[1], B. CORWIN[1], C. LILLESAAR[2], L. BALLY-CUIF[3], M. A. MASINO[1];
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Abstract: In vertebrates, much of the neural circuitry that is required for coordinated locomotion is contained within the spinal cord. The output of this circuitry is modulated by serotonin, which is derived from two distinct sources in vertebrates; 1) descending projections originating in the raphe and 2) a population of intraspinal neurons. The number and distribution of the intraspinal serotonergic neurons (ISNs) vary between species, as mammals possess a relatively small number of ISNs when compared to fish and amphibians. Although the functional properties of the ISNs have not been established in zebrafish, morphological evidence suggests that they innervate motor neurons and thus modulate their activity. Study of ISN development and morphological properties will likely provide insight into the role of 5-HT in establishing mature locomotor behavior in zebrafish larvae. A 3.2kb fragment of the zebrafish *pet1* promoter was used to drive expression of fluorescent reporters in the ISNs of developing zebrafish larvae. Confocal imaging of EGFP expression *in vivo* was used to quantify ISN cell number and location along the rostro-caudal and dorso-ventral axes of the spinal cord. We selectively photoconverted single Kaede-expressing ISNs with a UV laser to visually isolate and characterize individual neurons. Projection distance, neurite length, and arborization were measured in photoconverted ISNs and tracked from 3-10 days post fertilization (dpf). ISN cell number and morphological characteristics exhibited the greatest changes between 3 and 4 dpf. The timing of these morphological changes corresponds with the switch in zebrafish swimming behavior from long, infrequent swimming episodes at 3 dpf to shorter, more frequent swimming episodes at 4 dpf. Future work will test the functional maturation of the ISNs as well as their involvement in modulating locomotor activity.

Disclosures: **J.E. Montgomery:** None. **T.D. Wiggin:** None. **B. Corwin:** None. **C. Lillesaar:** None. **L. Bally-Cuif:** None. **M.A. Masino:** None.

Poster

030. Neuronal Differentiation

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 30.03/A26

Topic: A.02. Neurogenesis and Gliogenesis

Title: Hairy and enhancer of split 6 (Hes6) gene modulates the neuroblast and astrocytes differentiation in the dentate gyrus without any influences on cell proliferation and integration into mature neurons

Authors: *S. NAM[1], J. KIM[1], J. KIM[2], I. HWANG[1], J. SEONG[1], Y. YOON[1];
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Abstract: Hes6 is a member of hairy-enhancer of split homologs (Hes) and it functions in interaction with other Hes family genes. During development, Hes genes are expressed in neural stem cells and proliferating cells. However, role of Hes6 is demonstrated as opposite to role of other Hes family in cell culture studies and developing embryo or postnatal studies. Hes6 promotes neuronal differentiation and suppresses glial differentiation. Also, overexpressed Hes6 induced neuronal apoptosis. Like neurogenesis in developing brain, neural stem cells in a dentate gyrus of the adult hippocampus have the ability to proliferate and differentiate to neuronal cells or glial cells. Hes6's participation in the process of neurogenesis during the developing stage has been demonstrated. Until now, unlike its unveiled role in the development stage, Hes6 participation and its effect on adult hippocampal neurogenesis in not demonstrated. Therefore, in present study, we investigated the effects of the Hes6 on the process of the adult hippocampal neurogenesis by comparing the Hes6 knockout and its wild mice. To do this, we immunstained the relevant marker protein of proliferating cell(Ki67 and BrdU), post-mitotic neuroblast (DCX), mature neuronal cell (NeuN) and astrocyte (GFAP). Additionally, we injected 5-bromo-20-deoxyuridine to trace the fate of the mitotic cell at that time. Condition of the Hes6 knockout negatively affects adult hippocampal neurogenesis, in the aspect of neuronal differentiation. On the contrary, the astrocyte differentiation was increased in the knockout group compared to the wild group. Proliferating cell was slightly reduced in the knockout group, but the difference was not statically significant between two groups. Unlike the slight reduction pattern of Ki67 or BrdU cell, the ration of the BrdU and NeuN double positive cell was slightly higher in the knockout group. These results suggest that Hes6 is related with the regulation of the neuronal and glial differentiation even in the process of adult hippocampal neurogenesis.

Disclosures: **S. Nam:** None. **J. Kim:** None. **Y. Yoon:** None. **J. Seong:** None. **I. Hwang:** None. **J. Kim:** None.

Poster

030. Neuronal Differentiation

Location: Halls A-C

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Program#/Poster#: 30.04/A27

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

Support: Spanish Ministry of Science and Innovation (SAF2012-37417)

Fondo de Investigaciones Sanitarias, Instituto de Salud Carlos III (RD12/0019/0002)

CHDI Foundation Inc. Funds

Title: New insights in striatal development: Characterization of Znf521 in mouse and human brain

Authors: M. PARDO[1], N.-N. VINH[2], L. MARION-POLL[3], J.-A. GIRAULT[3], R. MARTÍN-IBÁÑEZ[1], A. ROSSER[2], *J. M. CANALS[4];
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Abstract: Many factors involved in striatal development remain uncharacterized. Here we describe Znf521 expression during mouse and human brain development, a transcription factor (TF) first identified in B cell development. During mouse development, Znf521 is expressed in brain structures such as the Lateral and Medial Ganglionic Eminences (LGE and MGE, respectively), olfactory bulb, septum, thalamus, cortex and cerebellum. In the LGE, Znf521 protein is detected in the mantle zone (MZ) at E12.5 and is expressed until adulthood. Znf521 expression changes during development, being homogeneous at embryonic LGE stages but becoming more lateral at postnatal ages. Furthermore, most Znf521 expressing cells do not colocalize with the proliferative marker Ki67. This suggests that Znf521 is expressed by postmitotic neurons, which was verified by NeuN colocalization. Interestingly, Znf521 also colocalizes with MSN markers such as Ctip2 and FoxP1 whereas its expression does not coincide with interneuron markers such as parvalbumin and choline acetyltransferase (ChAT). Since MSNs can be subdivided into two subpopulations, positive for either enkephalin or Substance P, we next performed double *In situ* hybridization-immunohistochemistry for enkephalin or Substance P and Znf521. Our results show preferential colocalization with Substance P suggesting that Znf521 is involved in direct pathway neuron generation. We also analyzed Znf521 in the direct pathway by localizing Znf521 in D1- or D2-GFP mouse models. We and others have defined a set of LGE specific TFs that participate in MSN development. For this reason we studied the relationship between Znf521 and striatal TFs including Ebf1, Helios, and Ikaros. Our results indicate that Znf521 and Helios are expressed by different MSN populations, while Ikaros and Znf521 only colocalize in the lateral LGE. We next analyzed Znf521 expression and localization during human fetal LGE development in fetuses aged 7 to 13.5 weeks post conception (wpc). As in mouse development, Znf521 expression was observed in the LGE/striatum, internal capsule, choroid plexus, and septum. In the basal ganglia Znf521 is expressed in the MZ with a lateral pattern of expression from 8 wpc onwards. Most Znf521

positive cells were found in the MZ and do not colocalize with Ki67, although a few Znf521 positive proliferating cells are detected in the SVZ. Our results also demonstrate that Znf521 is expressed by human MSNs during development since it colocalizes with Ctip2 and some FoxP1 positive cells. These results demonstrate that Znf521 is expressed by a subset of MSNs during mouse and human development, suggesting that it may be involved in striatal neurogenesis.

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Poster

030. Neuronal Differentiation

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 30.05/A28

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

Support: NIH R01 MH090740

Title: Sox8 is required for the formation of the striatonigral pathway

Authors: ***P. MERCHAN SALA**[1], T. L. SCHAEFER[3], A. A. ASHWORTH[3], M. WEGNER[4], K. CAMPBELL[2];
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Abstract: The striatum is the major component of the basal ganglia. It receives information from different areas of the cortex and once integrated it sends its outputs to either the globus pallidus or the substantia nigra pars reticulata (SNr) which ultimately feedback to the premotor cortex via the thalamus. Thus the striatum plays key roles in the control of motor behavior as well as in learning and other cognitive functions. The striatal output pathways are classified into indirect (striatopallidal) and direct (striatonigral) pathways. Imbalances in the activity of these pathways are thought to underlie the behavioral abnormalities observed in a number of neurological disorders including attention deficit hyperactivity disorder (ADHD) and Parkinson's disease. Little is known about the molecular genetic mechanisms that control the formation and function of the direct and indirect pathways. In this respect, the SoxE family member, Sox8, is expressed in the developing and adult striatum. Moreover, using GENSAT Sox8-EGFP mice we have found that the direct pathway is specifically labeled from embryonic to adult stages. Using

Sox8lacZ mice, we found that there is a dramatic reduction in the formation of striatonigral pathway in both homozygous and heterozygous animals. This is despite the fact that the striatum appears normal in size. Our preliminary findings suggest that substance P-positive fibers abnormally terminate in the globus pallidus of Sox8 mutants suggesting that Sox8 is required for long axon growth of direct pathway fibers. We are currently attempting to identify downstream effectors of Sox8 using RNA-seq and are particularly interested in identifying dosage-sensitive genes that may explain the haploinsuffient phenotype. Moreover, we have developed a gain-of-function approach using Dlx5/6-tTA mice to drive a tetO-Sox8-IRES-EGFP transgene, in order to examine whether Sox8 is sufficient to instruct a direct pathway phenotype. Finally, we have conducted several behavioral tests on the Sox8 mutants and our preliminary results indicate that both the homozygous and the heterozygous mice display increases in basal locomotor activity as well as impaired cognition and social behavior.

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Poster

030. Neuronal Differentiation

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

Support: NIH grant 1K99NS085037

NIH grant NS062822

HHMI

Title: Hox genes govern the development of respiratory circuits

Authors: ***P. PHILIPPIDOU**[1], L. JEANNOTTE[2], J. S. DASEN[1,2];
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Abstract: Breathing is a basic motor behavior that is essential in all terrestrial vertebrates. The frequency and amplitude of respiratory contractions are driven by neural networks residing in the brainstem that coordinate the activation of dedicated sets of spinal motor neurons (MNs). Motor neurons within the spinal cord directly drive the activity of inspiratory and expiratory muscles, including the diaphragm and intercostals. Despite the complexity of the neural networks that

regulate respiratory rhythms, diaphragm contraction is controlled by a single motor input supplied by MNs within the Phrenic Motor Column (PMC). We identified a transcriptional network orchestrated by two Hox transcription factors (Hoxa5 and Hoxc5) that controls critical aspects of respiratory circuit assembly. Selective deletion of Hox5 genes from MNs in mice leads to perinatal lethality due to respiratory failure. In Hox5 mutant mice, there is an extinction of PMC identity, characterized by a loss of PMC molecular determinants, progressive loss and disorganization of phrenic MNs, axonal misrouting, and, most strikingly, a dramatic loss of synaptic contacts at the diaphragm muscle. Several PMC-specific genes, including cell adhesion molecules and trophic factors, are downregulated in Hox5 mutants, differentially contributing to the multiple aspects of this phenotype. Erosion of PMC identity from cervical MNs also results in aberrant breathing cycles, indicating that establishing phrenic MN identity through Hox5 gene activity is an obligate step in the wiring of mammalian respiratory networks.

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Poster

030. Neuronal Differentiation

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 30.07/A30

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

Support: ARISTEIA II (4257) to LZ

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Title: The role of Pitx2 in the development of spinal ventral interneurons

Authors: I. ROZANI, *D. A. MANGOURA, L. ZAGORAIOU;
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Abstract: In the ventral spinal cord, interneuron specification is controlled by the combinatorial expression of certain transcription factors. We have identified a small interneuron population that expresses the paired-like homeodomain transcription factor Pitx2. We undertook an analysis of Pitx2 deficient embryos to determine how loss of Pitx2 affects interneurons that express it. Pitx2 is involved in the development of many organs. In the CNS, Pitx2 is expressed in the hypothalamus where it contributes to the formation of the mammilothoracic tract, in the midbrain where it promotes neuronal migration and GABAergic differentiation, in rhombomere 1 and in

the spinal cord. Our analysis was performed in Pitx2 cre homozygous animals. The cre recombinase cassette has been knocked-in in the homeodomain resulting in a Pitx2 allele that produces a truncated form of the Pitx2 protein and in homozygosis, a Pitx2-deficient animal. We have previously generated an antibody that targets an epitope preceding the homeodomain. This enables us to visualize the Pitx2 deficient cells, since they produce the truncated Pitx2. Pitx2 deficiency causes lethality around embryonic day e15.5 due to cardiac arrest, so we performed the analysis at e13.5 and e15.5. We have demonstrated that, during spinal cord development, Pitx2 expressing interneurons, which originate from the p0 domain, migrate from a dispersed ventrolateral position at e11.5 to a cluster around the central canal at e15.5. In Pitx2 deficient spinal interneurons the truncated protein displays diffuse cytoplasmic localization in contrast to the normal protein which is confined in the nucleus. These neurons are mislocalized in spinal cord sections of e13.5 and e15.5 embryos; a pattern that resembles an earlier developmental time point. We set out to analyze the phenotype of Pitx2 deficient neurons. We have shown before that half of the Pitx2 spinal interneurons are cholinergic. Here, we note that at e15.5 Pitx2 deficient neurons do not express the cholinergic markers vAChT and ChAT. Pitx2 deficient neurons seem to be lacking other subpopulation markers too. Moreover, we observed mislocalization of another migratory population of V0 origin, namely Evx1/2, in Pitx2 cre homozygotes when compared to heterozygotes. The aforementioned observations may be indicative of the involvement of the Pitx2 transcription factors in key developmental processes of spinal interneurons, like migration, specification and potentially, non-cell autonomous functions. However, more experiments are required to exclude general developmental arrest due to the overall lack of the Pitx2 transcription factor.

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Poster

030. Neuronal Differentiation

Location: Halls A-C

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Program#/Poster#: 30.08/A31

Topic: A.02. Neurogenesis and Gliogenesis

Support: NIH T32 1T32HD060600

NIH R01 MH066912-01

Title: Birthdate and SHH signaling differentially specify PV versus SST expressing cortical interneuron fates from mESCs

Authors: J. A. TYSON[1], A. M. MAROOF[2], *S. A. ANDERSON[3];
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[3]Psychiatry, Children's Hosp. of Philadelphia/Upenn Sch. Med., Philadelphia, PA

Abstract: Abstract: Medial ganglionic eminence (MGE)-derived GABAergic cortical interneurons consist of multiple subtypes that are involved in many cortical functions. In addition, they have a remarkable capacity to migrate, survive, and integrate into cortical circuitry after transplantation into postnatal cortex. These features have engendered considerable interest in generating distinct subgroups of interneurons from pluripotent stem cells for the study interneuron fate, function, and for the development of cell-based therapies. While advances in this effort have occurred, the capacity to generate highly enriched pools of subgroup fate-committed interneuron progenitors from PSCs has remained elusive. Previous studies have suggested that the two main MGE-derived interneuron subgroups, those that express somatostatin and those that express parvalbumin, are specified in the MGE from Nkx2.1-expressing progenitors at higher or lower levels of sonic hedgehog (Shh) signaling, respectively. To further explore the role of Shh and other factors in cortical interneuron fate determination, we generated a dual-reporter line such that Nkx2.1-expressing progenitors express mCherry and postmitotic Lhx6-expressing MGE-derived interneurons express GFP. We find that exposure to higher Shh levels and collecting GFP-expressing precursors after 12 days in culture results in a strong enrichment for SST interneurons over those expressing PV, while the reciprocal enrichment for PV interneurons is produced by lower Shh and collecting mCherry-expressing after 17 days in culture. These findings confirm that fate determination of cortical interneuron subgroups is critically influenced by Shh signaling, and provide a system for the further study of interneuron fate and function. This work is funded by R01 MH066912-01 and T32 1T32HD060600.

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Poster

030. Neuronal Differentiation

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 30.09/A32

Topic: A.02. Neurogenesis and Gliogenesis

Support: NIH Grant MH071679

Title: Non-canonical Wnt signaling regulates cortical interneuron subtype specification

Authors: *E. AU[1], M. MCKENZIE CHANG[2], G. J. FISHELL[2];
[1]NYU Med. Ctr., NEW YORK, NY; [2]Neurosci., NYU Langone Med. Ctr., New York, NY

Abstract: Cortical GABAergic interneurons exhibit remarkable diversity as a cell population with respect to intrinsic firing properties, subtype marker expression, layer organization, synaptic connectivity and morphology. The means by which this diversity is achieved is still largely unknown. We have identified a novel rostral-caudal Wnt gradient within the medial ganglionic eminence (MGE) that delineates the specification of the two main classes of cortical interneuron subtypes. Caudally- situated MGE progenitors receive high levels of Wnt signaling and give rise to somatostatin (SST)-expressing cortical interneurons Parvalbumin-expressing basket cells, by contrast, originate mostly from the rostral MGE where Wnt signaling is attenuated. Interestingly, canonical Wnt signaling through β-catenin is not required for this process. Wnt signals transmitted via cleavage of the intracellular domain of the non-canonical receptor Ryk, however, are sufficient to drive interneuron progenitors to a SST fate. Graded Ryk gain-of-function experiments performed in mouse ES-derived cortical interneurons reveal a dose-dependent effect, suggesting Ryk signaling acts in a gradient.

Disclosures: **E. Au:** None. **G.J. Fishell:** None. **M. McKenzie Chang:** None.

Poster

030. Neuronal Differentiation

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 30.10/A33

Topic: A.02. Neurogenesis and Gliogenesis

Title: Necdin regulates RanGAP1 sumoylation during neuronal differentiation

Authors: *K. FUJIWARA, K. HASEGAWA, K. YOSHIKAWA;
Lab. of Regulation of Neuronal Develop., Inst. For Protein Research, Osaka Univ., 3-2 Yamadaoka, Suita-Shi, Japan

Abstract: Sumoylation is a ubiquitin-like post-translational modification of proteins and modulates their biological function, subcellular localization and stability. Many sumoylated proteins have recently been identified in neurons. However, the physiological role of sumoylation in neuronal differentiation remains unclear. We analyzed the sumoylation profile of proteins during neurogenesis *in vivo* and *in vitro*. Expression levels of the Ran GTPase activating protein RanGAP1, the most abundant sumoylated protein involved in the nuclear transport, were

markedly reduced during embryonic development of the mouse brain. Furthermore, RanGAP1 was rapidly desumoylated and degraded during differentiation of primary neural progenitor cells (NPCs). Necdin, a pleiotropic protein expressed abundantly in postmitotic neurons, promotes neuronal differentiation and survival. We found that necdin interacted with sumoylated RanGAP1, and these proteins were co-localized at the nuclear envelop of NPCs. Although sumoylated RanGAP1 associated with RanBP2, a nuclear pore component, is intensively protected from SUMO protease (Senp), coexpression of necdin and Senp reduced the sumoylated RanGAP1 level even in the presence of RanBP2. Furthermore, the sumoylated RanGAP1 level was increased in necdin-deficient NPCs. To analyze the relation between neuronal differentiation and the nuclear transport, we stably expressed GFP-fused SV40 NLS (nuclear localization signal)-HIV Rev NES (nuclear export signal) shuttling reporter protein in primary NPCs by lentivirus-mediated gene transfer. The GFP-fused shuttling reporter localized to the nucleus of undifferentiated NPCs but was translocated to the cytoplasm when neuronal differentiation progressed. These results suggest that necdin promotes desumoylation and subsequent degradation of RanGAP1 to downregulate the nuclear transport during neuronal differentiation.

Disclosures: **K. Fujiwara:** None. **K. Hasegawa:** None. **K. Yoshikawa:** None.

Poster

030. Neuronal Differentiation

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Topic: A.02. Neurogenesis and Gliogenesis

Support: National Science Foundation, award # 11201839

Title: Neurog1 genetic inducible fate mapping (GIFM) reveals the existence of complex spatiotemporal cyto-architectures in the developing cerebellum

Authors: ***E. A. OBANA**[1], T. G. LUNDELL[1], K. J. YI[2], K. L. RADOMSKI[2], Q. ZHOU[2], M. L. DOUGHTY[2];
[1]USUHS Program in Neurosci., Bethesda, MD; [2]USUHS Dept. of Anatomy, Physiol. and Genet., Bethesda, MD

Abstract: Neurog1 is a pro-neural basic helix-loop-helix transcription factor (bHLH) expressed in progenitor cells located in the ventricular zone and subsequently the presumptive white matter tracts of the developing mouse cerebellum. We used genetic inducible fate mapping (GIFM) with

a transgenic Neurog1-CreER allele to characterize the contributions of Neurog1 lineages to cerebellar circuit formation in mice. Neurog1-CreER GIFM labeled Purkinje cells and all GABAergic interneuron cell types of the cerebellar cortex but no non-neuronal cells in the cerebellum. The spatio-temporal sequence of GIFM was unique to each neuronal cell type. GIFM on embryonic days (E) 10.5 to E12.5 labeled Purkinje cells with medial-lateral settling patterns dependent on the day of tamoxifen delivery. GIFM on E11.5 to P7 labeled interneurons and the temporal sequence of tamoxifen delivery correlated with the final inside-to-outside resting position of GABAergic interneurons in the cerebellar cortex. Proliferative status and long-term BrdU retention of GIFM lineages revealed Purkinje cells express Neurog1 around the time they become post-mitotic. In contrast, GIFM labeled mitotic and post-mitotic interneurons. Neurog1-CreER GIFM reveals a correlation between the timing of Neurog1 expression and the spatial organization of GABAergic neurons in the cerebellar cortex with possible implications for cerebellar circuit assembly.

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Poster

030. Neuronal Differentiation

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Topic: A.02. Neurogenesis and Gliogenesis

Support: PRESTO, JST

Title: Prdm8 regulates the guidance molecules during neocortical development

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Abstract: During neocortical development, a multitude of cellular events must all occur with exquisite spatiotemporal control, resulting in quite synchronized progress of neurogenesis and angiogenesis. Recent studies have led to the notion that neuronal and vascular networks appear to be guided by similar guidance molecules, which are mediated by ligand-receptor system. Extracellular guidance cues, including netrins, semaphorins and ephrins, act as a molecular signaling that are involved in establishment of these networks, although the importance of this

guidance molecules for the establishment of mature cortical cytoarchitecture and the precise genetic control of these molecules remain largely unknown. Members of the Prdm proto-oncogene transcription factor family are new candidates implicated in the control of the developing neocortex. This is because multiple genes in the Prdm family are expressed in the developing mouse neocortex in a spatially and temporally restricted manner. We established the Prdm8 reporter mouse line, Prdm8-mVenus, and observed that the expression of mVenus was mainly restricted to the intermediate zone in the developing neocortex. And, we confirmed that the mVenus expression was found to be gradually upregulated to the point at which the netrin receptor Unc5D expression was gradually downregulated. Also, the Prdm8 overexpression in neocortical cells significantly suppressed the expression of Unc5D. The microarray identified over 90 transcripts with two-fold higher expression in the mVenus-positive cells compared with that in the mVenes-negative cells at E15.5. Interestingly, these genes included those involved in the signaling of guidance molecules, such as semaphoring signaling (Plxnd, Ebf3, Nrp2, and Sema3c), ephrin signaling (Epha6), and slit signaling (Slit3). In addition, the genomic binding sites of Prdm8 in the dorsal telencephalon were mapped by chromatin immunoprecipitation followed by high-throughput sequencing (ChIP-seq), and we identified 6,622 specific Prdm8 binding sites, and found that some peaks contain upstream region of guidance molecules, such as Unc5D, Ebf3, and Slit3, suggesting the possible involvement of Prdm8 in the regulation of some guidance molecules during neocortical development. Furthermore, we established Prdm8 KO mice and observed that both neuronal migration and the vascular density were significantly affected adjacent to the intermediate zone. These results suggest that the Prdm8 is one of the important factors to control the precise neuronal and vascular development by the regulation of guidance molecules during neocortical development.

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Poster

030. Neuronal Differentiation

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 30.13/A36

Topic: A.02. Neurogenesis and Gliogenesis

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Title: The influence of chronic alpha1A-adrenergic receptor activation on cell survival and fate in the adult mouse dentate gyrus

Authors: *K. COLLETTE[1], S. SCHUCK[2], D. PETERSON[2], V. A. DOZE[1];
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Abstract: Norepinephrine (NE) is a catecholamine neurotransmitter implicated in a range of diverse functions from regulation of mood to epilepsy. Norepinephrine binds to adrenergic receptors (ARs) of which there are three families: α_1, α_2, and βARs, which were categorized pharmacologically and genetically. The α_1AR family is further divided into α_{1A}, α_{1B}, and α_{1D}ARs. The α_{1A} and α_{1B}AR subtypes are highly expressed in the brain, particularly the hippocampus, a region crucial for learning and memory. Chronic activation of the α_{1A}AR subtype increases proliferation in the subgranular zone of the adult mouse hippocampus. Previous work in our lab showed that stimulation of the α_{1A}AR also induces differentiation of neural progenitor cells in neurospheres cultured from the subventricular zone (SVZ) of the lateral ventricles. In corroboration with this, neurospheres cultured from α_{1A}AR knockout (α_{1A}AR-KO) mice reverted back to or maintained an undifferentiated state. However, the survival and differentiation of adult-born cells has not been evaluated *in vivo*. In the subgranular zone of normal mice, 60 percent of new cells do not survive longer than one week but of those that do most differentiate into excitatory granule cells. We treated mice with an α_{1A}AR-selective agonist, cirazoline, in drinking water for 8 wks to determine the effect of α_{1A}AR activation on survival and cell fate of the adult-generated cells. Control mice received untreated water. Animals were injected with BrdU after 4 wks of treatment to label dividing cells and perfused 4 wks later to allow time for the new cells to differentiate. Immunohistochemical markers combined with BrdU labeling were used to identify the surviving cells, including NeuN for mature neurons, Prox1 for granule cells, DCX for immature neurons, and GFAP for astrocytes. Fluorescence microscopy and stereological cell counting are being used to quantify the number and ratio of each type of cell that survived. We expect that cirazoline treatment will have increased the number of new cells differentiating into mature cell types. We also expect that the majority of these cells will be excitatory granule cells but preliminary work suggests the possibility that a very small number of adult-born cells may differentiate into interneurons. Future work may assess the integration and functionality of the newly integrated cells.

Disclosures: **K. Collette:** None. **S. Schuck:** None. **D. Peterson:** None. **V.A. Doze:** None.

Poster

030. Neuronal Differentiation

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Topic: A.02. Neurogenesis and Gliogenesis

Title: Cellular roles of Ebf1 in striatal Medium Spiny Neuron specification

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Abstract: Medium Spiny Neurons (MSN) control the initiation of voluntary movements, promoting the execution of certain motor actions while preventing unnecessary ones. This role is achieved by the parallel action of two MSN subtypes: D1 MSN promote movement initiation while D2 MSN inhibit motor activation. Achieving the correct specification of MSN subtypes is essential for brain functioning; however, it is still largely unknown how this process is regulated during development. FACS-sorting experiments and full-knockout analysis established that the transcription factor *Ebf1* (Early B-cell factor 1) is required for generating subsets of D1 MSN. However the cell-autonomous roles of Ebf1 remain largely to be deciphered. Here we have taken advantage of genetic targeting of MSN subtypes and Cre-Lox recombination to conditionally delete Ebf1 in all MSN or only D1 MSN and analyze how this deletion affects MSN developmental dynamics at both cellular and population scale. Our results provide novel insights on the way transcription factors act at different levels to determine the binary choice between neuronal subtypes and their integration in the brain circuitry.

Disclosures: **A. Tinterri:** None. **L. Lokmane:** None. **M. Diana:** None. **L. Danglot:** None. **I. Gyory:** None. **T. Galli:** None. **R. Grosschedl:** None. **S. Garel:** None.

Poster

030. Neuronal Differentiation

Location: Halls A-C

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Program#/Poster#: 30.15/A38

Topic: A.02. Neurogenesis and Gliogenesis

Support: CIHR Postdoctoral Fellowship

Ludmer Centre for Neuroinformatics and Mental Health

Title: Brain maturation and neuron activity dynamically regulate histone variant expression

Authors: *A. A. THAMBIRAJAH[1,2], J. C. DIORIO[1,2], M. J. MEANEY[1,2];
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Abstract: The formation of brain circuitry during early development relies upon the precise input of excitatory and inhibitory stimuli. While brain maturation is dependent upon coordinated cascades of gene expression, it will also alter the utilization of genetic information. Associated events, such as neuron activation, influence this modulation of gene expression. The molecular cues that govern these changes in gene expression are becoming better understood, but less is known of the epigenetic-mediated control. DNA, which encodes genetic information, is repeatedly wrapped around sets of histone proteins. This combination of DNA and histones produces the macromolecular structure of chromatin, which not only permits the spatial compaction of DNA, but controls gene expression. Specialized forms of histones, histone variants, have unique roles in regulating chromatin metabolism. Such roles are involved in transcription, replication, DNA damage repair and more. Histone variants of the H2A family (H2A.Z, H2A.X, and macroH2A) are significantly decreased during early postnatal development in the male rat hippocampus, particularly between postnatal day 4 (P4) and P15. Post-translationally modified forms of these H2A variants are similarly decreased during this time period. As P4 - P15 coincides with a period of enhanced postnatal synaptogenesis and neurogenesis, it was of interest to ascertain if these changes in the H2A histone variants are associated with neuron activation. Primary hippocampal neuron cultures were treated with KCl and the H2A variants showed differential changes in protein expression in response to calcium-dependent stimulation. Treatment of hippocampal primary cultures with the excitatory neurotransmitter glutamate recapitulated these results. The receptor-dependent activation of specific histone variants and their associated genomic locations is currently being interrogated. This work provides a deeper understanding of how neuron maturation and activity and the underlying epigenetic changes in histone variants reciprocally exert their effects on brain physiology and gene expression.

Disclosures: **A.A. Thambirajah:** None. **J.C. Diorio:** None. **M.J. Meaney:** None.

Poster

030. Neuronal Differentiation

Location: Halls A-C

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Topic: A.02. Neurogenesis and Gliogenesis

Support: NIH Grant R01HD042182

Title: TxnRd2-mediated metabolic redox dysregulation during neuronal differentiation results in changes in mitochondrial distribution and abnormal neurite arborization

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Abstract: Oxidative stress and metabolic redox imbalance may accompany altered cortical circuit organization and function in patients with neuropsychiatric disorders. However, the effects of abnormal redox regulation during development of cortical circuits are still unknown. 22q11 deletion syndrome (22q11DS) has been suggested as a model for studying the neurodevelopmental origins of neuropsychiatric disorders. Individuals with 22q11DS are more susceptible to these disorders than the rest of the population. About one quarter of the genes located in the commonly deleted region in the 22q11DS encode proteins that localize to mitochondria. Among those, mitochondrial thioredoxin reductase (TxnRd2) encodes a primary mitochondrial H2O2 scavenger that is maximally expressed during cortical circuit differentiation. Thus, we asked whether redox dysregulation due to changes in dosage of TxnRd2 alters cortical neuronal differentiation. Primary cortical neuronal cultures from E16.5 mouse embryos were electroporated with farnesylated-GPF and mitochondrial-flagged TxnRd2 siRNA or overexpression plasmids. TxnRd2 depleted neurons were also treated with ROS scavengers to reestablish intracellular redox balance and link TxnRd2 scavenging activity to cortical neuron differentiation. We also determined the role of TxnRd2-mediated redox regulation in arborization using a conditional knockout mouse line. We evaluated changes in arborization and mitochondrial distribution by Sholl analysis. Depletion of TxnRd2 results in decreased neurite outgrowth and complexity, which is reestablished upon treatment with ROS scavengers. TxnRd2 overexpression leads to no significant changes in arborization. Changes in neurite outgrowth and

mitochondrial distribution upon TxnRd2 imbalance suggest a key role of TxnRd2 in neuronal differentiation. Antioxidant rescue of neurite outgrowth in TxnRd2 depleted neurons implies an inverse correlation between ROS accumulation and TxnRd2 activity. These data suggest antioxidant defense as key for cortical neuron developmental capacity and provide insight into the role of redox imbalance in abnormal circuit formation in a wide range of behavioral disorders.

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Poster

030. Neuronal Differentiation

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 30.17/A40

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

Title: The tumor suppressor lkb1 regulates myelination through mitochondrial metabolism

Authors: *B. DASGUPTA;
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Abstract: Myelination of peripheral axons by Schwann cells (SC) is essential for proper transmission of action potentials. A prerequisite to myelination is SC differentiation, and compelling recent evidence indicates that metabolic reprograming from a glycolytic to oxidative metabolism occurs during cellular differentiation. It is unknown if this reprograming is required for SC differentiation. Little is also known about genes that regulate this metabolic transition. We have discovered that metabolic reprograming is required during SC differentiation and the tumor suppressor *Lkb1* regulates this critical step. *Lkb1* deletion in SCs causes hypomyelination, muscle atrophy, hindlimb dysfunction and peripheral neuropathy. *Lkb1* null SCs failed to optimally activate mitochondrial oxidative metabolism during differentiation. Mitochondrial deficits were reflected by diminished oxygen consumption and production of the TCA cycle substrate citrate, which is a precursor to cellular lipids. Consequently, myelin lipids were reduced in *Lkb1* mutant sciatic nerves. Citrate synthase (CS) activity was reduced in *Lkb1* mutant SCs and overexpression of CS or exogenous citrate rescued differentiation/myelination defects of *Lkb1* mutant SCs. We propose that *Lkb1*-mediated metabolic shift through regulation of CS activity during SC differentiation increases mitochondrial metabolism and lipogenesis, necessary for normal myelination.

Disclosures: **B. Dasgupta:** None.

Poster

030. Neuronal Differentiation

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 30.18/A41

Topic: A.02. Neurogenesis and Gliogenesis

Support: Swedish Research Council

ERC

Title: Dynamic myelination by mature oligodendrocytes in humans

Authors: ***M. YEUNG**[1], S. ZDUNEK[1], O. BERGMANN[1], S. BERNARD[2], M. SALEHPOUR[3], K. ALKASS[1], G. POSSNERT[3], L. BRUNDIN[4], H. DRUID[5], J. FRISEN[1];
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Abstract: Oligodendrocytes are the cells wrapping layers of specialized cell membrane around nerve fibers forming myelin, which provides electrical insulation to facilitate fast propagation of nerve impulses. This insulation increases also axonal conduction velocities and hence the speed of neural processing. Myelination is largely a postnatal process and it continues well into adulthood in humans. The myelination of axons is dynamically altered by experience throughout life and thought to mediate neural plasticity by optimizing the performance of the circuitry. Practicing a skill can increase the volume of white matter regions engaged in carrying out the task and, conversely, reducing external stimuli by social isolation, leads to hypomyelination and impaired cognitive functions. How myelin is reshaped during brain maturation and in response to experience is not fully understood. Myelination can in theory be modified by mature oligodendrocytes and/or by exchanging oligodendrocytes and their myelin sheaths. Recent observations support that oligodendrocyte generation contributes to myelin remodeling. It is less clear to what degree mature oligodendrocytes can modulate their myelination. However, how this appear in the human brain remains elusive as methods employed in experimental animals, such as paradigms with labeled nucleotide analogs are not possible to apply in humans. We have

assessed the dynamics of oligodendrocyte generation and myelination in the human brain. The final number of oligodendrocytes in the corpus callosum is established around five years of age, whereas the myelin volume increases well into adulthood. By analyzing the concentration of the integration of ^{14}C, derived from nuclear bomb testing during the Cold War, in genomic DNA of oligodendrocytes, we demonstrate that there is limited exchange of oligodendrocytes and in contrast a high turnover rate of myelin. We provide data that myelin remodeling is mainly carried out by mature oligodendrocytes in humans.

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Poster

030. Neuronal Differentiation

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Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

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Topic: A.02. Neurogenesis and Gliogenesis

Support: NIH intramural Grant

Title: Orphan GPR110 is a target receptor for synaptamide, a potent endogenous inducer of neuronal differentiation derived from omega-3 fatty acids

Authors: *J. LEE, G. KHAREBAVA, B. HUANG, M. RASHID, S. PATNAIK, J. MARUGAN, H.-Y. KIM;
NIH, Bethesda, MD

Abstract: Docosahexaenoic acid (DHA), a polyunsaturated fatty acid highly enriched in the brain, is known to be essential for proper brain development; however, underlying mechanisms are not clearly understood. N-Docsahexanoylethanolamine (synaptamide), an endogenous metabolite of DHA, potently induces neurogenic differentiation of neural stem cells (NSCs) and promotes neurite growth and synaptogenesis in developing neurons. Nevertheless, its target receptor has not been identified. In this study, we demonstrate that orphan G-protein coupled receptor 110 (GPR110) is the synaptamide receptor that mediates neurogenic differentiation and neurite outgrowth. Mass spectrometry-based proteomics approach using biotinylated synaptamide analogues followed by affinity purification revealed binding of synaptamide to GPR110 in brain cells. Among long chain N-acylethanolamines, synaptamide specifically

exhibited such binding, and induced cAMP-dependent neurogenic differentiation and neurite outgrowth which were effectively blocked by GPR110 knockdown or pretreatment with N-terminal targeting GPR110 antibody, indicating that GPR110 is a functional receptor for synaptamide. The GPR110 expression is high in NSCs and fetal brains but significantly decreases in the brain after birth. In adult brains, high localization of GPR110 was observed in the hippocampal dentate gyrus region, which is known to retain neurogenic capacity throughout life, suggesting a role of GPR110 in neurogenesis even after development. Identification of GPR110 as a functional synaptamide receptor provides new insight into the mechanism of DHA function in the brain and offers a potential novel target for controlling neurodevelopment and function.

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Poster

030. Neuronal Differentiation

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Topic: A.02. Neurogenesis and Gliogenesis

Support: CONACyT 2012-01-180178

INPer 21111

Title: Altered expression on the level of miR-125b in serum from gestational diabetic women a possible biomarker for fetal central nervous system development

Authors: M. LAMADRID[1,4], M. S. CRUZ-RESÉNDIZ[1], N. F. DÍAZ[1], H. FLORES-HERRERA[2], G. GARCÍA-LÓPEZ[1], *A. MOLINA[3];
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Abstract: MicroRNAs are a class of non-coding small RNAs of 18-25 nucleotides in length that regulated post-transcriptionally mRNAs targets by binding the 3' untranslated region. These molecules are involved in several cellular processes such as proliferation, differentiation, maturation, maintenance and death. Several microRNAs have been proposed as biomarkers since they have been detected in body fluids such as urine, serum or plasma. During central nervous

system development microRNAs are known to participate in neuron differentiation and several microRNAs are enriched during neurogenesis in embryo brain development, showing a particular profile and domains on its expression. MiR-125b is a homolog of the heterocronic microRNA lin-4. In murine its expression increases gradually from embryonic day twelve until birth, while its overexpression in human pluripotent stem cells impairs its self-renewal and induce differentiation into neurons. In zebra fish its loss lead to the accumulation of mitotic cells, an increase in cell death and a reduction in neural differentiation. Neuron differentiation by miR-125b is due to a repression of multiple targets involved in both proliferative and apoptotic processes. It has been reported that gestational diabetes impair offspring's cognitive, social and/or motor skills. By qRT-PCR we analyzed the expression of miR-125b in healthy and gestational diabetic women serum samples. To explore common targets between human and murine we use MicroCosm Targets Version 5 from The European Bioinformatics Institute, UK. Our results showed that miR-125b has a temporal regulation in healthy women that was not observed in gestational diabetic women. Relative expression levels in healthy samples were 1 ± 0.8, 903 ± 0.5 and 115.4 ± 0.8 at first, second and third trimester respectively. Significant increases were obtained in gestational diabetic samples per trimester when compared to healthy women, with 95 fold and 70 times at the second and third respectively. From our results we conclude that: 1.- miR-125b has a temporal regulation in healthy pregnant women serum samples, with its highest levels at the neurogenic period, similar of that observed in animal models, 2.- there is an up regulation in miR-125b level in gestational diabetic women and 3.- that miR-125b serum levels may be a reflect of a deregulation in embryo tissue during central nervous system development that could affect neurogenesis.

Disclosures: **M. Lamadrid:** None. **M.S. Cruz-Reséndiz:** None. **N.F. Díaz:** None. **H. Flores-Herrera:** None. **G. García-López:** None. **A. Molina:** None.

Poster

030. Neuronal Differentiation

Location: Halls A-C

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Topic: A.02. Neurogenesis and Gliogenesis

Support: USFDA Protocols E0736301, E075280, E0735901

Title: Reversal of ketamine-induced neurotoxicity and cardiotoxicity in zebrafish embryos: Direct involvement of the energy metabolism pathway

Authors: *J. KANUNGO[1], X. GUO[2], E. CUEVAS[2], M. DUMAS[2], S. ALI[2], M. PAULE[2], S. LANTZ-MCPEAK[2];
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Abstract: Ketamine, an antagonist of the N-methyl-d-aspartate (NMDA) receptors, is a pediatric anesthetic. We demonstrate that ketamine is cardiotoxic and neurotoxic in zebrafish embryos. The exact mechanism(s) of how ketamine causes similar effects in mammals remains unclear. Using image-based high content analysis, we also show that ketamine induces developmental toxicity in zebrafish embryos. We have characterized the pathways modulated by ketamine in causing these effects. First, we show that the amino acid, acetyl l-carnitine (ALCAR) rescues ketamine-induced attenuation of heart rate, neurotoxicity and developmental toxicity in the 28 hours post-fertilization embryos exposed to these drugs for 20h. In these embryos, ketamine reduced heart rate and caused both motor and sensory neuron toxicity. Our follow-up studies focused on the mode of action of ALCAR's counteractive effects on ketamine-induced toxicities. Since NMDA receptors are calcium permeable and ketamine as its antagonist could alter cardiac function and induce neurotoxicity in the zebrafish embryos, the effect was likely due to blockade of calcium entry in to the cardiomyocytes and neurons. ALCAR can open the L-type calcium channels and also generate ATP through fatty acid transport and metabolism. First, we tested whether in presence of calcium chelators and L-type calcium channel blockers (e.g., verapamil), ALCAR was ineffective in reversing ketamine's effect. Microarray studies also revealed that gene expression involved in ATP synthesis was altered in the embryos exposed to ketamine. Based on this genomic data, we interfered with the ATP synthesis pathway using specific inhibitors in order to monitor the effects of these drugs on the cardiac rate and the nervous system. These studies revealed that mitochondrial function is a target of ketamine. Verapamil and ketamine together were more toxic than each drug alone indicating that their effects were additive. ALCAR was effective in counteracting the effects of verapamil or verapamil and ketamine, but not in presence of an inhibitor of ATP synthesis. Our combined *in vivo* and *in vitro* studies suggest that the energy metabolic pathway plays a direct role in ketamine-induced effects on the zebrafish embryos.

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Poster

030. Neuronal Differentiation

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Topic: A.02. Neurogenesis and Gliogenesis

Support: the Pioneer Research Center Program through the National Research Foundation of Korea funded by the Ministry of Science, ICT & Future Planning grant NRF-2010 0019347

National Agenda Project(NAP) funded by the Korea Research Council of Fundamental Science&Technology grant NAP-09-04

KIST Institutional Program grant 2Z04000

Title: Development of neurospheroid using microwell for degenerative brain diseases

Authors: *J. YOU[1], J. JIN[1,2], Z. CHANG[1,4], J. JEONG[1], K. YANG[4], Y.-S. HWANG[5], J. PARK[3], S.-W. CHO[4], J. KIM[1];
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Abstract: Cell therapy has been studied a lot to apply to neurodegenerative diseases. Cell transplantation is one of the ways to recover the functionality of damaged neural tissue in these diseases. But overcoming limitation of low survival rate of transplanted cells is the most important thing for cell therapy. The purpose of this study is to develop a neurodegenerative disease treatment technology using Neurospheroid. Transplanted Neurospheroid could regenerate neuronal network and protect nerve by neurotropic factor and anti-inflammatory function. Through this study we aim to secure core medical technology and verify the utility of technology. We fabricated uniform sized Neurospheroids on the PEG microwell. Si master was fabricated using MEMS technology and PDMS mold was made of curing of PDMS solution on the Si master. PEG microwell with desired size was fabricated using PDMS mold. To observe advantage of culture method maintaining spheroid shape, we compared single cell (2D) culture and spheroid (3D) culture using primary neurons obtained from Sprague-Dawley rat embryo. Through Live and Dead assay, we identified viability of Neurospheroid in microwell and retrieved from microwell stage. We observed the neuronal properties of Neurospheroid with immunocytochemistry. The neuronal activity test was performed through multi-electrode arrays (MEAs). 2D cultured single cells showed signals 2 weeks after seeding on the MEAs. When reseeding spheroid, we observed electrical signal 1week after reseeding and identified strong and frequent electrical signals. This indicates spheroid shape cells may be helpful for recovery and regeneration. We proceeded with differentiation test which differentiates human neural stem cells into dopaminergic neurons using fabricated PEG microwell to maintain spheroid shape of stem cells. We observed differentiation states through immunocytochemistry and RT PCR.

Expression of stem cell markers, Nestin and Sox2, were decreased depending on the stage of differentiation. Expressions of MAP2, TuJ1, Kv1.1, Nfm and SCN5a, the neuronal markers, were increased. Especially, expression of TH, dopaminergic neuron marker, was increased and this indicates that neural stem cells differentiate into dopaminergic neuron. The result of MEA analysis demonstrates electrical function of differentiated Neurospheroid. Our findings suggest that this method could be basis for treating severe degenerative brain disease. This study provides possibility that 3D cultured Neurospheroids can be effective way in the future study of neurodegenerative diseases therapy.

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Poster

030. Neuronal Differentiation

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Topic: A.02. Neurogenesis and Gliogenesis

Support: Takeuchi Biohybrid Innovation Project, ERATO, JST, Japan

Title: Formation of complex 3D neural network by neural microfiber

Authors: ***M. KATO-NEGISHI**[1,2], H. ONOE[1,2], S. TAKEUCHI[1,2];
[1]The Univ. of Tokyo, Tokyo, Japan; [2]Takeuchi Biohybrid Innovation Project, ERATO, JST, Tokyo, Japan

Abstract: *In vitro* reconstruction of 3D neural network is important for the investigation of the mechanism of network formation and neuronal activity change in complex neural tissues *in vivo*. Many groups have reported 3D neuronal culture formed by hydrogel scaffolds, beads, and spheroids, but it is complicated to fabricate macroscopic neural networks using these methods without any scaffold materials. Here, we describe a method to fabricate 3D neural networks by neural microfiber assembly without using any scaffold material. We already developed a cell-encapsulating core shell hydrogel fiber, named the cell microfiber. Using this system, we fabricated mouse neural stem cell (NSC) microfibers by a coaxial laminar flow device. The formed microfiber provides a fiber-shaped 3D microenvironment (inner diameter: ~80 um, length: >several meters) constructed with sodium alginate shell. At 14 days after differentiation induction, we could observe that many neurons extend their dendrites and axons into the fiber-

shaped 3D microenvironment. We also observed many astrocytes. To fabricate complex 3D neural tissue, NSC microfibers were cut to the same lengths by a neural microfiber cutting system and cultured into a PDMS mold under differentiation-induction medium. After 24 hours, the edges of neural microfibers bounded to each other with cell-connection units. Using this process, we successfully made different angle connections of neural microfibers, and these architectures kept the angles for long term culture (> 1 month). Since alginate shell could be dissolved by alginate lyase treatment with the connection shape maintained, we can obtain 3D neural networks easily without any scaffold material. We believe that our neural microfiber assembly is a useful tool for fabrication of large complex 3D neural networks. Our 3D neural tissue is also a powerful approach for various fields including neural tissue engineering, analysis of 3D neural network, and pharmacological assay for drug screening.

Disclosures: **M. Kato-Negishi:** None. **H. Onoe:** None. **S. Takeuchi:** None.

Poster

031. Dendritic Development and Branching

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 31.01/A47

Topic: A.05. Axon and Dendrite Development

Support: NSF Graduate research fellowship DGE-0644492 (to L.C.)

NIH grant R01 DA018928 (to T.B.)

Title: Activity-dependent regulation of dendritic complexity by Semaphorin3A through Farp1

Authors: *L. CHEADLE[1,2], T. BIEDERER[3];
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Abstract: Dendritic arbors are complex neuronal structures that receive and process synaptic inputs. Investigation of mechanisms that govern the development of dendrites and their synapses is critical as disruption of these processes can lead to disorders such as Autism. One mechanism regulating dendrite differentiation is Semaphorin/Plexin signaling, specifically through binding of soluble Sema3A to Neuropilin/PlexinA co-receptors. We here show that the protein Farp1 (FERM, RhoGEF (ARHGEF), and pleckstrin domain protein 1), a Rac1 activator previously identified as a synaptogenic signaling protein that binds the adhesion molecule SynCAM 1, is

necessary to establish dendrite tip number and total dendritic branch length in maturing rat neurons, and is sufficient to promote dendrite complexity. Aiming to define its upstream partners, our results support that Farp1 interacts with the Neuropilin-1/PlexinA1 complex and co-localizes with PlexinA1 along dendritic shafts. Functionally, Farp1 is required by Sema3A to promote dendritic arborization of hippocampal neurons, and Sema3A regulates dendritic F-actin distribution via Farp1. Unexpectedly, Sema3A also requires neuronal activity to promote dendritic complexity, presumably because silencing neurons leads to a proteasome-dependent reduction of PlexinA1 in dendrites. These results provide new insights into how activity and soluble cues cooperate to refine dendritic morphology through intracellular signaling pathways.

Disclosures: **L. Cheadle:** None. **T. Biederer:** None.

Poster

031. Dendritic Development and Branching

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 31.02/A48

Topic: A.05. Axon and Dendrite Development

Support: Nagoya City University

Title: Effect of CSMD3, a candidate gene of neuropsychiatric disorders, on neuronal morphology

Authors: ***M. TOMOHARU**, T. KOHNO, M. HATTORI;
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Abstract: Schizophrenia and autism are characterized by difficulties with social interaction and communication. It is speculated that the onset of these neuropsychiatric disorders are greatly affected by hereditary factors. Recently, it was reported that CUB and sushi multiple domain 3 (CSMD3) is one of the genes that are mutated in a few schizophrenia and autism patients. CSMD3 encodes a huge putative transmembrane protein that is expressed in fatal and adult brains. However, its biochemical properties and functions remain largely uncharacterized. In this study, we tried to clarify the function of CSMD3 on neuronal morphology. We first confirmed that CSMD3 is a type I transmembrane protein that is mainly expressed on the plasma membrane. Myc-CSMD3 and Venus-CSMD were co-expressed in COS7 cells and immunoprecipitation with anti-GFP antibody that recognizes Venus was performed. Myc-CSMD3 was found in the precipitate, suggesting that CSMD3 can form a homomultimer. We

also found that co-expression of CSMD3 with constitutively active Fyn resulted in tyrosyl phosphorylation of CSMD3. When full length CSMD3 was overexpressed in cultured hippocampal neurons, the number of short branched dendrites increased. On the other hand, a mutant CSMD3 that lacked the extracellular and intracellular regions had no effect. A mutant CSMD3 without the intracellular region had a similar effect as the full length. It was thus suggested that extracellular region, but not intracellular region of CSMD3, is required for its effect on the dendritic morphology. We speculate that CSMD3 may function as an adhesion molecule or a co-receptor for unidentified membrane protein to regulate dendrite development and its malfunction may be one of the factors in the pathogenesis of neuropsychiatric disorders.

Disclosures: **M. Tomoharu:** None. **T. Kohno:** None. **M. Hattori:** None.

Poster

031. Dendritic Development and Branching

Location: Halls A-C

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Program#/Poster#: 31.03/A49

Topic: A.05. Axon and Dendrite Development

Support: MRC

Title: Acute and chronic effects of transient mutant DISC1 expression in layer 2/3 mouse barrel cortex

Authors: ***N. R. HARDINGHAM**[1], G. SEATON[2], K. FOX[2];
[2]Sch. of Biosci., [1]Cardiff Univ., Cardiff, United Kingdom

Abstract: DISC1 has been implicated in schizophrenia and has an important role in brain development. We used a transgenic mouse with a dominant negative fragment of the DISC1 c-terminal (DISC1-cc), activated for 24-48 hours by injection of tamoxifen. Activation of the mutant protein at P7 in mice results in schizophrenic like behaviour (Li *et al*, 2007) and a loss of experience dependent plasticity in layer 2/3 of adult barrel cortex, while activation of DISC1-cc at P28 has no effect on experience dependent plasticity (Greenhill and Fox, SfN 2012). We have already shown that mutant DISC1 retards dendritic growth and short term plasticity in layer 2/3 barrel cortex neurons at the P14 time point (Hardingham et al, SfN 2012). The transgene could thus have both short term and long term effects on layer 2/3 neurons. In order to temporally examine the effect of transient expression of mutant DISC1 on the neurons we made whole cell recordings from layer 2/3 neurons at P8, P14 and P28. To study excitatory synapses we recorded

mEPSPs and measured spine density from characterised dendrites. Deficits in spine density were observed in DISC1 mice at P8 but these had recovered by P28. We also pharmacologically measured NMDA to AMPA ratios at various stages of development and found there to be a difference between WT and DISC1 mutants at P28 ($p<0.05$) but no difference between the two at P14. To study the effects of transient expression of mutant DISC1 on inhibition we recorded mIPSCs from a population of neurons. By morphologically reconstructing a population of layer 2/3 neurons injected with biocytin we showed that dendritic development was attenuated in DISC1 mutants at both P11 and P14 but it had eventually recovered in adults and that in WTs most dendritic development had already taken place by P11. Furthermore, by detailed analysis of dendritic order and intermodal distance we demonstrated that DISC1 neurons had abnormal internodal distances in basal dendrites that were not compensated in adulthood and also enhanced higher order branching of the apical dendrite. We measured levels of LTP and LTD in adult mice (P50-P64) given transient expression of mutant DISC1 and in wild type littermates. We found that, similar to observations at P28, LTP was absent in the mutant DISC1 adult mice (comparison with WT, $p<0.05$) but that LTD was still present. Thus it would seem that LTP and certain aspects of dendritic branching are not compensated for in later life and that transient expression of mutant DISC1 can have both acute and chronic effects on the neurons.

Disclosures: **N.R. Hardingham:** None. **G. Seaton:** None. **K. Fox:** None.

Poster

031. Dendritic Development and Branching

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 31.04/A50

Topic: A.05. Axon and Dendrite Development

Title: Purkinje neuron developmental markers *in vivo* and *in vitro*

Authors: T. N. FAULL, L. M. ROBERTSON, A. R. DEMCHAK, *M. E. MORRISON; Biol., Lycoming Col., Williamsport, PA

Abstract: The cerebellum coordinates movement and balance. It is composed of only a few cell types including Purkinje neurons, whose dendrites receive inputs via synapses from granule cell parallel fibers and olivary climbing fibers. The Purkinje neurons integrate these inputs, calculate motor error, and send corrective signals through their axons which synapse onto the deep cerebellar nuclei. The early development of Purkinje neuron axons and dendrites is the subject of this study. The growth of Purkinje neurons in the mouse can be divided into several stereotyped

stages. In the prenatal stage, the Purkinje cells are arranged in masses within the cerebellar anlage. From postnatal day 0 to postnatal day 3 *in vivo*, the cells extend numerous, very simple processes. As the cell continues to develop during the first two postnatal weeks, these processes recede back into the cell, and apical dendrites begin to appear. By the second or third postnatal week, the Purkinje neurons have a highly branched dendrite studded with spines. This series of developmental changes is recapitulated in cell cultures made from neonatal mouse cerebella, with a slight time delay of a few days as the cells recover from the cell dissociation process. Knowing more about the nature of the early Purkinje cell processes could help in the design of treatments to support Purkinje cell regeneration after injury or in the face of cerebellar ataxias. Our goal is to characterize the primitive processes of Purkinje cells in early postnatal mice and in cerebellar cultures derived from these mice: are they axonal, dendritic, both, or neither? In this study, immunohistochemistry of cryostat sections and immunocytochemistry of cultured cerebellar cells was used to establish the locations of several proteins in developing Purkinje neurons, including calbindin D28k, MAP2 (a dendritic marker), and neurofilament H (an axonal marker).

Disclosures: **T.N. Faull:** None. **L.M. Robertson:** None. **A.R. Demchak:** None. **M.E. Morrison:** None.

Poster

031. Dendritic Development and Branching

Location: Halls A-C

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Program#/Poster#: 31.05/A51

Topic: A.05. Axon and Dendrite Development

Support: GNSF Grant 6/09

Title: Kainic acid induces alterations in dendritic spine number and motility in hippocampal neurons

Authors: *T. LORTKIPANIDZE[1], M. ZHVANIA[1,2], T. BIKASHVILI[2], N. JAPARIDZE[2]; [1]Inst. of Mol. Biochem., Ilia State Univ., Tbilisi, Georgia; [2]I.Beritashvili Ctr. of Exptl. Biomedicine, Tbilisi, Georgia

Abstract: Dendrites and spines undergo dynamic changes in physiological conditions, such as learning and memory, and in pathological conditions, such as epilepsy. Abnormalities in dendritic spines have commonly been observed in brain specimens from epilepsy patients and

animal models of epilepsy. However, the functional implications and clinical consequences of this dendritic pathology for epilepsy are uncertain. Motility of dendritic spines and axonal filopodia has been recently discovered by the advanced imaging techniques, and remains to a large degree an exciting phenomenology in search of function. Here we demonstrate the effect of Kainic Acid (KA), which is a structural analogue of glutamate, on dendritic spine number and motility in hippocampal CA1 area at the different stages of brain development. In order to reveal the changes that take place in spine and filopodial motility in the epileptic model of brain, time-lapse imaging of acute hippocampal slices treated with various concentrations of KA after different incubation time points was performed. The effects of KA exposure were tested on the slices from young (Postnatal day (P7-P10) and adolescent (P28-P30) Thy1-YFPH transgenic mice. Slices were treated with either 50 μM or 100 μM of KA, for either 30 or 100 min. The results obtained in our experiments show diverse effects of KA in 2 different age groups. According to our results, 100 μM/100 min KA treatment increases spine motility at early stage of brain development (P10) by 41.5%, while in P30 mice spine motility is increased only by 3%. As for spine number in was significantly increased in both age groups (P10 and P30) at 50μM /30 min KA treatment, and decreased at 50μM /100 min , but this reduction was more dramatic in P10 mice. Our findings also indicate that effect of KA on hippocampal dendritic spine motility is predominantly time - rather than concentration - dependent.

Disclosures: **T. Lortkipanidze:** None. **M. Zhvania:** None. **T. Bikashvili:** None. **N. Japaridze:** None.

Poster

031. Dendritic Development and Branching

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Topic: A.05. Axon and Dendrite Development

Support: German Research Foundation (SPP1365/KA3423/1-1)

Fritz Thyssen Foundation

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Onderzoekstoelage OT/12/089

Title: The ubiquitin E3 ligase Nedd4-1 promotes neurite growth and is regulated by PI3K/PTEN-mTORC1 signaling

Authors: *H.-E. HSIA[1], R. KUMAR[1], R. LUCA[3,4], M. TAKEDA[1], J. COURCHET[5], J. NAKASHIMA[6], S. GOEBBELS[2], S. WU[6], W. AN[7,8], B. EICKHOLT[7,8], F. POLLEUX[5], D. ROTIN[9], H. WU[6], M. ROSSNER[2], C. BAGNI[3,4,10], J.-S. RHEE[1], N. BROSE[1], H. KAWABE[1]; [1]Dept. of Mol. Neurobio., [2]Dept. of Neurogenetics, Max Planck Inst. of Exptl. Med., Goettingen, Germany; [3]Ctr. for Human Genet. and Leuven Inst. for Neurodegenerative Dis., Leuven, Belgium; [4]VIB Ctr. for the Biol. of Dis., Leuven, Belgium; [5]Dept. of Neurosci., Columbia Univ. Med. Ctr., New York, NY; [6]Dept. of Mol. and Med. Pharmacology,, UCLA Sch. of Med., Los Angeles, CA; [7]MRC Ctr. for Developmental Neurobiology, King's Col. London, London, United Kingdom; [8]Inst. for Biochemistry, Charité-Universitätsmedizin, Berlin, Germany; [9]The Hosp. for Sick Children, Toronto, ON, Canada; [10]Dept. of Biomedicine and Prevention, Univ. of Rome Tor Vergata, Rome, Italy

Abstract: Neurite growth is regulated by many intracellular signaling pathways, among which signaling via mammalian target of rapamycin complex 1 (mTORC1) is of particular relevance because it controls the translation of a substantial subset of mRNAs that encode proteins with roles in neurite growth. Phosphatase and tensin homolog (PTEN) is a lipid phosphatase that converts phosphatidylinositol-3,4,5-trisphosphate to phosphatidylinositol-4,5-bisphosphate, and thereby antagonizes the phosphoinositide-3-kinase-mTORC1 (PI3K-mTORC1) signaling. Therefore, the regulation of PTEN expression, subcellular localization, and activity in neurons is of particular interest in the context of neurite growth regulation. Previous studies indicated that the Nedd4 family E3 ubiquitin ligases Nedd4-1 and Nedd4-2 ubiquitinate PTEN and target PTEN for proteosomal degradation, thereby negatively regulates PTEN expression and subsequently activates the PI3K-mTORC1 signaling. However, other studies argued that PTEN is the physiological substrate of Nedd4-1. In the present project, we aim to resolve the conflict and address the relationship between PTEN and Nedd4-1/Nedd4-2 in the regulation of neurite growth in mammalian central nervous system neurons. Using brain specific conditional knock-out mice as models, we found that Nedd4-1 and Nedd4-2 are required for axonal growth in murine central nervous system neurons. However, account for the underlying molecular pathways, PTEN is not a substrate of Nedd4-1 and Nedd4-2. Rather, PTEN negatively regulates Nedd4-1, but not Nedd4-2 protein expression through modulating the activity of mTORC1. Our data indicate that Nedd4 family E3 ligases promote axonal growth in the developing mammalian brain, where PTEN is not a relevant substrate. Instead, PTEN controls neurite growth by regulating Nedd4-1 expression through mTORC1.

Disclosures: **H. Hsia:** None. **R. Kumar:** None. **R. Luca:** None. **M. Takeda:** None. **J. Courchet:** None. **J. Nakashima:** None. **S. Goebbels:** None. **S. Wu:** None. **W. An:** None. **B. Eickholt:** None. **F. Polleux:** None. **D. Rotin:** None. **H. Wu:** None. **M. Rossner:** None. **C. Bagni:** None. **J. Rhee:** None. **N. Brose:** None. **H. Kawabe:** None.

Poster

031. Dendritic Development and Branching

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 31.07/A53

Topic: A.05. Axon and Dendrite Development

Title: Carbamylated erythropoietin promotes neurite outgrowth and neuronal spine formation in association with CBP/p300

Authors: *M. CHOI[1], S. LEE[2], S. KO[2], S. WANG[2], H. SON[1,2];
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Abstract: Both erythropoietin (EPO) and carbamylated EPO (cEPO) have been shown to increase the length of neurites and spine density in neurons. However, the molecular mechanism underlying the EPO- and cEPO-induced neuronal differentiation has yet to be investigated. To address this issue, we investigated epigenetic modifications that regulate gene expression in neurons. Neurons treated with EPO or cEPO display an upregulation of E1A-binding protein (p300) and p300-mediated p53 acetylation, possibly increasing the transactivation activity of p53 on growth-associated protein 43 (GAP43). Treatment of cells with cEPO markedly increases spine formation and potentiates p300-mediated transactivation of PSD95, Shank2 and 3 compared to EPO. These results demonstrate that cEPO controls neuronal differentiation via acetylation of transcription factors and subsequent transactivation of target genes. These findings have important medical implications because cEPO is of interest in the development of therapeutic agents against neuropsychiatric disorders.

Disclosures: **M. Choi:** None. **S. Lee:** None. **S. Ko:** None. **S. Wang:** None. **H. Son:** None.

Poster

031. Dendritic Development and Branching

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Topic: A.05. Axon and Dendrite Development

Support: US Public Health Service NS060754

US Public Health Service NS052325

Title: The role of sap97 in asd and scz

Authors: *P. GUPTA[1], L. ZHANG[2], J. MOJSILOVIC-PETROVIC[2], R. G. KALB[2,3];
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Abstract: Aberrant brain circuitry is implicated in various neuropsychiatric disorders such as autism spectrum disorders (ASD), schizophrenia (SCZ), and intellectual disability. Both ASD and SCZ are classified as “synaptopathies,” as a majority of the genes mutated in these disorders play a critical role in synaptic development, function, and neuronal connectivity. Consequently, the genes regulating dendritic growth and arborization are imperative for the formation of functional neuronal networks. Synapse-associated protein of 97kDa (SAP97) is one such gene that is implicated in regulating glutamatergic synaptic transmission within the nervous system and is involved in mediating dendritic morphogenesis. Additionally, whole exome sequencing data suggests SAP97 may play a risk-determining role in the onset of ASD and SCZ. In order to determine whether SAP97 directly contributes to the behavioral phenotypes associated with ASD and SCZ, we will study mice that lack neuronal SAP97. We will subject these mice to a battery of behavioral paradigms to screen for a ASD or SCZ phenotype. Interestingly, signaling through the Wnt/β-catenin pathway is also involved in the pathophysiology of ASD and SCZ and is critical for dendritic growth. Integration of SAP97 and Wnt/β-catenin into a single pathway would bolster the role of SAP97 in ASD and SCZ, as well as clarify the mechanism by which SAP97 regulates dendritic growth and contributes to normal neuronal development. We are currently using rodent hippocampal cultures as an *in vitro* model to study dendritic growth after manipulation of the levels of SAP97 and β-catenin. In order to determine whether SAP97 functions downstream of β-catenin, we are stimulating β-catenin signaling by expressing a stabilized form of β-catenin along with a construct that knocks down SAP97. If SAP97 function is necessary for Wnt/β-catenin-mediated dendritic growth, we expect to see blunted dendrite and synapse growth upon knockdown of SAP97. Our findings will determine whether SAP97 plays a direct, causative role in the symptomology associated with ASD and SCZ, as well as elucidate the molecular pathways involved in the development of proper neuronal connectivity. Given that

aberrant brain circuitry is associated with myriad neuropsychiatric disorders, it is essential to establish the neurobiological mechanisms regulating dendritic morphogenesis.

Disclosures: **P. Gupta:** None. **L. Zhang:** None. **J. Mojsilovic-Petrovic:** None. **R.G. Kalb:** None.

Poster

031. Dendritic Development and Branching

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Topic: A.05. Axon and Dendrite Development

Support: NIMH (to YZ)

Swiss National Science Foundation (to MES and AZ)

Advanced ERC (to MES)

Title: Cellular constituents of Nogo-A contribution to structural plasticity in the mouse cerebral cortex

Authors: ***C.-C. CHEN**[1], A. ZEMMAR[2,3], F. VAJDA[2], G. CARSON[1], N. ISAAD[1], J. BOZEMAN[1], B. TEWS[2,3], M. SCHWAB[2,3], Y. ZUO[1];
[1]Mol. Cell and Dev Biol, Univ. of California, Santa Cruz, Santa Cruz, CA; [2]Brain Res. Institute, Univ. of Zurich, Zurich, Switzerland, Switzerland; [3]Biol., ETH Zurich, Zurich, Switzerland, Switzerland

Abstract: Nogo-A has been described as a myelin-associated inhibitor of neurite outgrowth and is important for functional neuroregeneration following central nervous system injury. Its physiological functions, however, remain less well defined. Aside from its main expression site in oligodendrocytes, Nogo-A is also expressed by subsets of cortical neurons. While Nogo-A is known to negatively regulate structural and functional synaptic plasticity, the specific roles played by neuronal vs. myelin Nogo-A for such structural alterations remain unclear. To address this question, we generated mouse lines in which the Nogo-A gene is specifically knocked out for (i) oligodendrocytes (glial Nogo-A KO) or (ii) neurons (neuronal Nogo-A KO). We analyzed neuronal morphology, dendritic branching pattern, and spine density in layer 2/3 pyramidal neurons from adult motor cortex under normal physiological conditions. We found that in the absence of Nogo-A, the complexity of dendritic features was increased, exemplified by an

overall elevation in dendritic length and the number of bifurcations in the animals lacking Nogo-A. In glial Nogo-A KO mice, these effects were larger than in neuronal Nogo-A KO mice. In addition, deletion of Nogo-A in neurons, but not oligodendrocytes, was associated with higher densities of perineuronal nets around cortical inhibitory neurons. Last, dendritic spine densities were elevated in apical dendrites but normal in basal dendrites, again with a more pronounced effect in glial Nogo-A KO than neuronal KOs. Our data indicate that oligodendrocytic and myelin Nogo-A influences complexity and plasticity of dendrites in the mouse motor cortex, but neuronal Nogo-A also participates in such regulation, although to a lesser extent. Understanding the different cellular constituents of Nogo-A contribution to the formation of dendrites and the limitation of adult plasticity provides a framework on how developmental critical period and maturation of cortical circuitries are related.

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Poster

031. Dendritic Development and Branching

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Topic: A.05. Axon and Dendrite Development

Support: JSPS KAKENHI 25430042

JSPS KAKENHI 25-7107

Title: CRMP4 contributes to formation of the olfactory bulb neural circuits

Authors: *A. TSUTIYA[1], H. WATANABE[1], M. NISHIHARA[2], Y. GOSHIMA[3], R. OHTANI-KANEKO[1];
[1]Toyo Univ., Itakura, Oura, Gunma, Japan; [2]The Univ. of Tokyo, Tokyo, Japan; [3]Yokohama City Univ., Kanagawa, Japan

Abstract: Although collapsin response mediator proteins (CRMPs, CRMP1-5) are indicated to play important roles in growth cone collapse and axon guidance induced by semaphorin 3A, the function of CRMP4 in the developing brain remains unclear. In our previous studies, we investigated the expression of *Crmp4* mRNA in brains of neonatal to adult mice and discovered prominent *Crmp4* mRNA expression in the olfactory bulb (OB) during the early postnatal period

(Tsutiya and Ohtani-Kaneko, 2012). This result promoted us to explore phenotypes of the OB in *Crmp4*-knockout (KO) pups to reveal roles of CRMP4 in the developing brain. We found altered thickness of layers in the OB and abnormal morphology of mitral cells (MCs) in *Crmp4*-KO pups. Moreover, we revealed that olfactory discrimination ability was impaired in *Crmp4*-KO pups, compared to that in wild-type (WT) pups (Tsutiya et al., Neuroscience 2013, San Diego). In this study, we aimed to elucidate mechanisms underlying the physiological alteration in *Crmp4*-KO pups. In order to examine the excitation of neural cells in the OB, we first compared c-Fos expression, an activity-dependent neuronal marker, in the OB between WT and *Crmp4*-KO pups after olfactory stimulation of ethyl acetate (EA), a commonly used non-biological odorant. Without stimulation, c-Fos-positive cells were slightly observed in both WT and *Crmp4*-KO pups. After EA-treatment, c-Fos-positive cells were significantly increased in a restricted small area of the MC layer and granule cell layer in WT pups, compared to those in WTs without stimulation. However, c-Fos-positive cells were broadly and dramatically increased in all layers of the OB in *Crmp4*-KO pups treated with EA. These results clearly showed altered neural activities in *Crmp4*-KO pups after olfactory stimulation. Next, we focused on tyrosine hydroxylase (TH) neurons, since they carry out an important task in regulating excitation of neurons in the OB. The number of TH neurons in the OB was significantly greater in *Crmp4*-KO mice than in WTs. Furthermore, we carried out primary culture of the OB cells to examine the morphological changes of developing TH neurons derived from *Crmp4*-KO mice. TH neurons derived from *Crmp4*-KO mice had more neurites than those from WT mice. In conclusion, these studies showing the altered excitation of neurons and changes in TH neurons revealed important roles of CRMP4 in the formation of the OB neural circuits.

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Poster

031. Dendritic Development and Branching

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Topic: A.05. Axon and Dendrite Development

Support: Deutsche Forschungsgemeinschaft (SPP1365/KA3423/1-1)

Fritz Thyssen Foundation

Title: The role of ubiquitin ligase E3B in neural development

Authors: *M. C. AMBROZKIEWICZ[1], B. ALTAS[1], S. RIPAMONTI[1], A. RONNENBERG[2], H. EHRENREICH[2], J. S. RHEE[1], N. BROSE[1], H. KAWABE[1];
[1]Mol. Neurobio., [2]Clin. Neurosci., Max Planck Inst. of Exptl. Med., Göttingen, Germany

Abstract: Protein ubiquitylation plays indispensable role in various aspects of neurodevelopment. Perturbations in the ubiquitin signaling follow with dramatic consequences for neuronal function, which ultimately leads to neurological disorders and intellectual disability. Recently, whole-exome sequencing of patients with blepharophimosis-mental retardation syndrome (BMR) revealed truncating mutations in Ube3b - gene encoding for ubiquitin ligase E3B. BMR is a heterogeneous group of disorders characterized by facial dimorphisms, failure to thrive and microcephaly. Neurological features of the BMR patients show abnormal brain image with Chiari type I malformation and hypoplasia of corpus callosum. Interestingly, in a similar study, recessive point mutation in Ube3b was identified in an individual suffering from autism - a neurodevelopmental disorder characterized by abnormalities in social behavior. To elucidate the role of this ubiquitin ligase in the development of the nerve cell, we generated UBE3B knock-out mouse line. Straight UBE3B KO animals recapitulate the phenotype of the BMR patients with most prominent features being severe facial dimorphisms, kyphosis and microcephaly. The analysis of primary hippocampal neurons prepared from UBE3B KO animals reveals a dramatic reduction of dendritic tree complexity, which seems to be controlled in a cell autonomous fashion. Using the UBE3B KO mice, we will further characterize the role of the ubiquitin ligase in the nerve cell using biochemical, electrophysiological and behavioral assays. This study will contribute to the present outlook on the pathology of neurodevelopmental disorders.

Disclosures: M.C. Ambrozkiewicz: None. **B. Altas:** None. **S. Ripamonti:** None. **A. Ronnenberg:** None. **H. Ehrenreich:** None. **J.S. Rhee:** None. **N. Brose:** None. **H. Kawabe:** None.

Poster

031. Dendritic Development and Branching

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 31.12/A58

Topic: A.05. Axon and Dendrite Development

Support: Japan Society for the Promotion of Science

Title: Activation of AhR signaling pathway regulates dendritic growth in the developing mice

Authors: *E. KIMURA[1], K.-I. KUBO[2], T. ENDO[1], W. LING[1], K. NAKAJIMA[2], M. KAKEYAMA[1,3], C. TOHYAMA[1];
[1]Lab. Environ. Hlth. Sci., CDBIM, Grad. Sch. of Med., Univ. of Tokyo., Tokyo, Japan; [2]Dept. of Anat., Keio Univ. Sch. of Med., Tokyo, Japan; [3]Grad. Sch. of Biomed. Sci., Nagasaki Univ., Nagasaki, Japan

Abstract: Normal growth of dendrites is required for the neural network formation that ensures higher brain function in humans and laboratory animals. Although abnormal activation of aryl hydrocarbon receptor (AhR) has been suggested to disrupt higher brain function, the physiological role of AhR remains elusive. In this study, we studied whether and how activation of AhR signaling affected dendritic growth in the developing brain from the fetal mice that were subjected to *in utero* electroporation (IUE) of constitutively active (CA)-AhR-expressing neurons. Pregnant C57BL/6 mice were deeply anesthetized on GD 14. Either of the following three kinds of plasmids, i.e., pCAG-tdTomato (Control), pCAG-tdTomato and pCAG-AhR (WT-AhR), or pCAG-tdTomato and pCAG-CA-AhR (CA-AhR), was injected into the lateral ventricle by IUE. On postnatal day 14, brains were collected and analyzed for dendritic morphology of tdTomato-expressing neurons using Neurolucida software. Dendritic length of apical tree of reconstructed pyramidal cells of hippocampal CA1 region was shown to be significantly decreased in CA-AhR-expressing cells compared with Control and WT-AhR-expressing cells. Dendritic complexity was estimated by Sholl analysis. CA-AhR-expressing cells were found to have a significantly smaller number of Sholl ring intersections of apical tree than Control and WT-AhR-expressing cells. No significant changes in both dendritic length and complexity were found between Control and WT-AhR-expressing cells. In conclusion, our present study demonstrates a possible role of activation of AhR signaling in dendritic growth and an involvement of AhR in dendritic growth in the developing brain. Further studies are needed to evaluate the significance of AhR signaling in the brain development such as cell migration and axonal growth.

Disclosures: **E. Kimura:** None. **K. Kubo:** None. **T. Endo:** None. **W. Ling:** None. **K. Nakajima:** None. **M. Kakeyama:** None. **C. Tohyama:** None.

Poster

031. Dendritic Development and Branching

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 31.13/A59

Topic: A.05. Axon and Dendrite Development

Support: KAKENHI 25430040

Title: Role of ryanodine receptor type 2 expressed by cerebellar granule cells in dendritic differentiation of Purkinje cells

Authors: *M. TANAKA, R. OHASHI, M. MIURA, N. HIRASHIMA;
Dept. Cell. Biophys., Grad. Sch. Pharmaceut. Sci., Nagoya City Univ., Nagoya, Japan

Abstract: Cerebellar Purkinje cells have the most elaborate dendritic trees among neurons in the brain. Purkinje cell dendrites receive numerous glutamatergic synaptic inputs from granule cells, forming a main component of cerebellar neuronal circuitry. Ryanodine receptor (RyR), an intracellular calcium release channel, mediates calcium-induced calcium release in many types of cells including cerebellar Purkinje and granule cells. In regard to the expression pattern of RyR subtypes in the cerebellum, Purkinje cells show high expression of RyR type 1 (RyR1) in addition to lower expression of RyR type 2 (RyR2), whereas granule cells highly express RyR2. The expression level of RyR type 3 is very low in both Purkinje and granule cells. Recently, we developed single-cell electroporation technique for introducing siRNA into specific neurons in primary neuronal cultures (J. Neurosci. Meth. 178:80-86, 2009; Neurochem. Res. 36:1482-1489, 2011; Neuromethods 65:129-139, 2012). Using this technique, we previously showed that Purkinje cell-specific knockdown of RyR1 inhibited dendritic differentiation of Purkinje cells in cerebellar cell cultures. In addition, knockdown of RyR2 expressed by granule cells using a transfection reagent also inhibited dendritic differentiation of Purkinje cells. In the present study, we analyzed the role of RyR2 expressed by granule cells in dendritic differentiation of Purkinje cells in detail. Ryanodine (10 μM), a blocker of RyR, reduced the elevation of intracellular calcium concentration in both Purkinje and granule cells in response to glutamate stimulation and markedly inhibited dendritic differentiation of Purkinje cells in cerebellar cell cultures. ELISA analysis showed that ryanodine reduced the levels of brain-derived neurotrophic factor (BDNF) in the culture medium. When we added BDNF in addition to ryanodine into cultures, the ryanodine-induced inhibition of dendritic differentiation of Purkinje cells was partially rescued. In contrast, nerve growth factor did not rescue the ryanodine-induced inhibition of dendritic differentiation of Purkinje cells. In addition, ryanodine reduced glutamate release from granule cells after depolarization. These results suggest that dendritic differentiation of Purkinje cells is promoted not only by RyR1 expressed by Purkinje cells themselves, but also by RyR2 expressed by granule cells, through secretion of BDNF and glutamate from granule cells.

Disclosures: **M. Tanaka:** None. **R. Ohashi:** None. **M. Miura:** None. **N. Hirashima:** None.

Poster

031. Dendritic Development and Branching

Location: Halls A-C

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Program#/Poster#: 31.14/A60

Topic: A.05. Axon and Dendrite Development

Support: VIEP- BUAP Grant FLAG/IND2014

CONACYT Grant 286535

Title: Neonatal blockade of glutamatergic transmission alters the dendritic spines types on dorsal hippocampus and the learning and memory processes in the rat

Authors: *C. A. PINZÓN;
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Abstract: The mechanisms by which the brain processes and retains information it receives from the environment has been a focus of interest for long. However several studies have shown the critical role of dendritic spines in the information process, being that the most of the excitatory synaptic inputs in the brain occur in dendritic spines (over 90 %), dendritic spines are also highly modifiable, and this process is called dendritic plasticity. Likewise is known that the glutamate receptors play an important role in modulating of synaptic strength, the growth of neurons and neuronal plasticity. The main objective of this study was to evaluate the effect of the neonatal blockade of different types of glutamate receptors on dendritic spines in the hippocampus and the memory and learning process. Chronic administration of specific antagonists of iGluRs on rats Sprague Dawley strain was made at an early stage (PD1 at PD15), to subsequently determine the changes, produced in the adult stage (PD60), in the hippocampus and behavior of the rat. This in order to assess learning and memory in animals treated with the antagonists and determine what type of spines formed by antagonizing iGluRs for behavioral changes ultimately bind to neuronal morphological alterations. The behavioral test was evaluated by NOR (Novel Object Recognition), the results shown a diminution in the treated groups of index of discrimination with respect to the control groups, indicating an alteration in the processes of learning and memory. In addition we evaluated the spines typification by using Golgi-Cox technique in dorsal hippocampus (CA1), finding reduction in the mature spines.

Disclosures: **C.A. Pinzón:** None.

Poster

031. Dendritic Development and Branching

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 31.15/A61

Topic: A.05. Axon and Dendrite Development

Support: NIH Grant NS045876

Title: Inhibition of Bcl-xL arrests neurite outgrowth

Authors: *H.-A. PARK, P. LICZNERSKI, K. N. ALAVIAN, M. SHANABROUGH, E. A. JONAS;
Yale Sch. of Med., New Haven, CT

Abstract: Bcl-xL, an anti-apoptotic member of the Bcl2 family, protects cell survival in rapidly dividing cells and developing neurons, but was not previously known to regulate neuronal growth. Neuronal outgrowth and synapse formation are necessary for cell survival in development, and it is apparent that growth and synapse formation go hand-in-hand. We have shown previously that Bcl-xL is necessary for changes in synapse number, size, activity, and for enhancement in mitochondrial metabolism. In this study we set out to determine if Bcl-xL were necessary for neurite outgrowth and if neurite outgrowth were in turn required for survival in the presence or absence of stress. We found that, in the absence of stress, depletion of Bcl-xL slowly impaired neurite outgrowth in hippocampal neurons followed by delayed cell death. The rapidity of decline in neurite outgrowth markedly increased upon exposure to hypoxia in neurons depleted of Bcl-xL. Hypoxic neurons depleted of Bcl-xL demonstrated a more striking loss of neuritic processes and an increased propensity to apoptotic and necrotic death compared to hypoxic controls. Normoxic hippocampal neurons lacking Bcl-xL had increased expression of death receptor 6 (DR6), a molecule that regulates axonal pruning; increased DR6 expression was also found in neurons after *in vivo* ischemia or *in vitro* hypoxia. Depletion of DR6 partially protected from loss of neurite outgrowth, suggesting that DR6 is an important downstream effector of Bcl-xL in neuronal outgrowth. We suggest that DR6 levels are normally suppressed by Bcl-xL expression, and that depletion of endogenous Bcl-xL leads to upregulation of DR6, failure of neuronal growth in non-stressed cells and exacerbation of hypoxia-induced neuronal injury. Taken together, this study suggests that Bcl-xL regulates neurite outgrowth during development and during hypoxic insult, opposed by DR6. Therapeutically, stimulation of factors implicated in neurite formation may protect neurons against acute hypoxic injury or chronic neurodegenerative stimuli.

Disclosures: **H. Park:** None. **P. Licznerski:** None. **K.N. Alavian:** None. **M. Shanabrough:** None. **E.A. Jonas:** None.

Poster

031. Dendritic Development and Branching

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 31.16/A62

Topic: A.05. Axon and Dendrite Development

Title: miR92a and 301a regulate human-specific changes in cortical development

Authors: *P. GUIJARRO[1], Z. QIU[2], P. KHAITOVICH[1];
[1]PICB, Shanghai, China; [2]Inst. of Neurosci., Shanghai, China

Abstract: Human cognition is considered to have evolved from the closest living relative the chimpanzee by specific changes in human brain developmental program, leading to an extended period of dendritogenesis, synaptogenesis and synaptic remodeling. This delay in acquiring the mature neural network is especially relevant in the prefrontal cortex, and it can possibly explain the unique capacity of human infants to learn cultural knowledge and cognitive skills. In previous studies we identified a group of mRNAs in the prefrontal cortex that showed different expression patterns between humans and chimpanzees. These genes were enriched in synaptic and neural connectivity functions, and were targets of microRNAs 92a and 301a. In the present study, plasmids containing miR92a or 301a were transfected in three different neuronal cell types (human SHSY5Y, mouse N2A and mouse primary neurons) using liposomes. 3 days after transfection, cells overexpressing miR92a or 301a had significantly shorter neurites, indicating that they might inhibit genes related to neuronal differentiation. Expression analysis of the mouse prefrontal cortex by qPCR showed high levels of miR92a and 301a at embryonic and early postnatal stages. Expression abruptly decreased during the second postnatal week, a period when dendritic and synaptic remodeling take place Overall, our preliminary data favor the hypothesis that miR92a and 301a are critical in the acquisition of the specific expression profile of the human prefrontal cortex by regulating genes involved in late stages of neuronal development like dendritogenesis and synaptogenesis.

Disclosures: **P. Guijarro:** None. **Z. Qiu:** None. **P. Khaitovich:** None.

Poster

031. Dendritic Development and Branching

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 31.17/A63

Topic: A.05. Axon and Dendrite Development

Support: NIH Grant NS055272

Title: Differential regulation of the Wnt signaling pathway by gamma-protocadherin family proteins

Authors: *K. MAH, J. A. WEINER;
Biol., Univ. of Iowa, Iowa City, IA

Abstract: The formation of neural circuits during brain development depends on a variety of growth factors (e.g., FGFs, Wnts, TGFβs) and their associated signaling pathways, as well as on specific cell-cell contacts mediated by a variety of adhesion molecules. How these distinct mechanisms might interact to regulate circuit formation is not clear. The protocadherin gamma (Pcdhg) gene cluster encodes a family of 22 cell adhesion molecules that we have implicated in the regulation of synaptogenesis, neuronal apoptosis, neuron-astrocyte interactions, and branching of axons and dendrites, primarily through mechanisms that remain unknown. Recently, it was reported that Pcdhg genes are epigenetically silenced in some cancer cell types, and that overexpression of Pcdhg genes in these cells could reduce tumor growth by inhibiting the canonical Wnt signaling pathway (Dallosso et al., 2009, 2012; PMIDs: 19956686, 22249255). As Wnt signaling regulates many aspects of neural development, we asked how the γ-Pcdh proteins might inhibit Wnt signaling, and whether this plays a role in the γ-Pcdhs' many neural functions. Using the TOP-FLASH assay and quantitative RT-PCR for Axin2 in HEK293 cells, we find that individual γ-Pcdhs differentially affect canonical Wnt3a signaling: γ-Pcdh-C3 and -A11 significantly attenuate Wnt signaling, while most other isoforms, surprisingly, significantly increase Wnt signaling. Focusing on the C3 isoform, we performed a structure-function analysis to determine which domains of the molecule (ectodomain with 6 extracellular cadherin repeats, variable cytoplasmic domain specific to this isoform, or constant C-terminal domain shared by all γ-Pcdhs) were important for its inhibition of Wnt signaling. We found that all constructs containing the variable cytoplasmic domain (VCD), but not one lacking it, were active; thus the differential effects of γ-Pcdh isoforms is consistent with the fact that the VCD is the domain with the most sequence diversity amongst the Pcdhg family. Though the VCD is normally juxta-membrane, the entire cytoplasmic domain (VCD+constant domain) of γ-Pcdh proteins has been reported to be cleaved by gamma-secretase and enter the nucleus. We are thus now utilizing new constructs, encoding the C3 VCD with or without palmitoylation or nuclear localization sequences to determine where in the cell the VCD acts to inhibit the Wnt pathway. We are also crossing a Wnt reporter line with a nuclear GFP read-out to both constitutive or floxed conditional Pcdhg mutant mice, and to mice harboring a Cre-inducible Pcdhg-A1 or -C3

transgene, to assess whether the γ-Pcdhs similarly regulate Wnt signaling in the nervous system *in vivo*.

Disclosures: **K. Mah:** None. **J.A. Weiner:** None.

Poster

031. Dendritic Development and Branching

Location: Halls A-C

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Topic: A.05. Axon and Dendrite Development

Support: NIH R01 NS39600 from NINDS

ONR MURI 14101-0198

Keck Nakfi to GAA

NIH R01 NS086082 from NINDS (CRCNS) to DNC and GAA

Title: Comparing stochastic growth models to explore the developmental mechanisms of dendritic arbors

Authors: *S. NANDA[1], R. ARMAÑANZAS[2], R. PAREKH[2], S. POLAVARAM[2], D. N. COX[2], G. A. ASCOLI[2];
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Abstract: Complex developmental processes produce mature dendritic morphologies, which in turn affect the computational properties of neurons and networks. Stochastic algorithms to generate artificial dendritic arbors provide a powerful method for exploring the mechanisms of dendrite development. Furthermore, these algorithms allow for a complete quantitative characterization of dendritic morphology. Here we focus on distinct morphological classes of dendritic arborization (da) neurons from *Drosophila* larvae. Computer simulations are both constrained and validated directly with experimental data using digital reconstructions of dendritic morphology of class I-IV da neurons from NeuroMorpho.Org. First, the statistical distributions of the (basic) model parameters controlling growth are extracted from real neurons; then the resulting synthetic morphologies are compared to the real ones using different (emergent) morphological measurements. In one model, growth rules are expressed in terms of

local morphological properties (e.g. tapering, branch elongation, and termination probability) that depend on “fundamental determinants”, such as radius, branch order, and path distance from the soma. A complementary model controls dendritic structure by the density profile of the (desired) spanning field and a balancing factor, which weighs the costs of wiring length and conduction time. In a third (intermediate) alternative, branching is constrained by the expected number of terminal tips in a specific sub-tree and the path distance from the soma. Comparing the outcomes of these models reveals the relative importance of various morphological and functional parameters in producing the observed structural variations found in real neurons. The resulting quantitative hypotheses are being tested with multi-channel time-lapse imaging of da neuron development tagging the putative molecular substrates, including microtubules and neurofilaments.

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Poster

032. Mechanisms of Dendritic Growth and Branching

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 32.01/A65

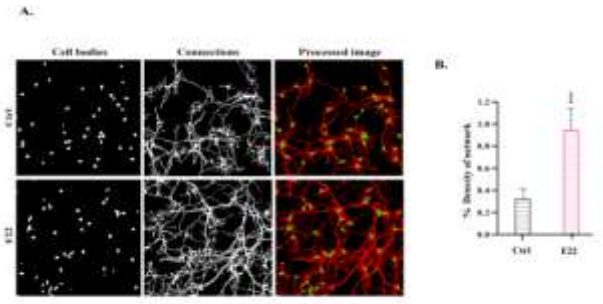
Topic: A.05. Axon and Dendrite Development

Title: A novel bioinspired nanoparticle promotes neurite outgrowth and increases neural network activity in primary neuronal cultures

Authors: *S. LATIFI[1], F. CESCA[1], A. TAMAYOL[2], R. HABIBI[1], R. SABZEVARI[3], A. BLAU[1], M. LINDER[4], E. ARAB-TEHRANY[4];
[1]NBT, IIT, Genova, Italy; [2]Biomed. Engin. Dept. and McGill Univ., Genome Quebec Innovation Ctr., Montreal, QC, Canada; [3]Artificial Intelligence Lab, Univ. of Zurich, Zurich, Switzerland; [4]Lab. d’Ingénierie des Biomolécules, Univ. de Lorraine, Vandoeuvre-Lès-Nancy, France

Abstract: The phospholipids in brain membranes contain different polyunsaturated fatty acids (PUFAs), which are critical to the nervous system function and structure. Particularly, brain function is critically dependent on the intake of the so-called ’essential’ fatty acids that cannot be readily synthesized by the human body. Among these are omega-3 (n-3) and omega-6 (n-6) PUFAs, which are required for brain development and maintaining optimal brain function. Here, we developed a novel phospholipid liposome formulation (F22) containing a variety of fatty acids as nanocarriers. They were derived from natural lecithins by enzymatic processing and

enhance metabolic activity and support neural network formation in 2D and 3D cultures of primary cortical neurons. Furthermore, extracellular recordings with microelectrode arrays (MEA) revealed a significant increase in spontaneous activity in cultures treated with these bio-inspired materials.



A & B. Network formation analysis in cortical neurons after treatment with F22 for 3 days and in control condition as indicated. Different channels of cell bodies, connection and processed images are represented. Values are presented as mean ± SD, (for B, n=80 images for each condition, from 4 independent experiments).

Disclosures: **S. Latifi:** None. **F. Cesca:** None. **A. Tamayol:** None. **R. Habibi:** None. **R. Sabzevari:** None. **A. Blau:** None. **M. Linder:** None. **E. Arab-Tehrany:** None.

Poster

032. Mechanisms of Dendritic Growth and Branching

Location: Halls A-C

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Topic: A.05. Axon and Dendrite Development

Support: NSF IGERT 0965918

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NIH R21 MH101655

Title: High-resolution analysis of Semaphorin 3A effects on filopodia of developing dendrites using SLIM imaging and microfluidic environments

Authors: *A. JAIN[1], T. KIM[2], G. POPESCU[2], M. U. GILLETTE[1];
[1]Dept. of Cell and Develop. Biol., [2]Electrical & Computer Engin., Univ. of Illinois at Urbana-Champaign, Urbana, IL

Abstract: The intricate wiring of the nervous system relies on filopodial navigation to form complex interconnections between neurons through their axons, dendrites, and the cell soma itself. Until recently, cellular investigations into filopodial dynamics had focused primarily on axonal growth cone filopodia. Spurred by technological advances, scientists have now begun to explore the structural and functional landscape of dendritic filopodia. These are dynamic 200-300 nm wide, 2-20 μm long protrusions that occur predominantly during early postnatal development and mediate crucial developmental processes. Even so, several structural and chemical cues guiding filopodial navigations remain obscure. Semaphorin 3A (Sema3A) is one such cue that, in particular, needs to be investigated, since, being a signaling protein that selectively promotes dendrite survival and growth, its effects on the dendritic filopodia are all the more relevant. Here we investigate the role of Sema3A in guiding dendritic morphogenesis, spinogenesis, and synaptogenesis. We show that it acts not only at the level of the dendrites, promoting neurite survival and growth, but also at the level of the filopodia. Since there has been some evidence indicating a difference in filopodia borne along dendrite tips vs. those borne along dendrite shafts, we treat the two populations as distinct and tease apart their different responses. Structural analyses of numbers, lengths, and locations are complemented by studies of dynamic functional aspects, such as motility, and extension-retraction rates. This is made possible through Spatial Light Interference Microscopy (SLIM), an innovative quantitative phase imaging method that makes possible high-resolution label-free imaging of live cells through interferometry (Wang et al, Opt Express, 2011) and allows measurements of the dry mass of live neurons at femtogram levels (Mir et al, Sci Rep, 2014). This convergence of filopodial investigations and the technology for engineering micro-environments, when coupled with high resolution imaging and analysis, enabled new insights on local signals, including Sema3A, that initiate and establish neuron-neuron interactions at the filopodial level. A greater comprehension of such processes that shape the development of neuronal networks is helping unravel the mechanistic bases of developmental disorders and diseases.

Disclosures: **A. Jain:** None. **T. Kim:** None. **G. Popescu:** None. **M.U. Gillette:** None.

Poster

032. Mechanisms of Dendritic Growth and Branching

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Topic: A.05. Axon and Dendrite Development

Support: NIH Grant EY021855

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NIH Grant EY013360

Title: Mitochondrial motility and function in developing neural dendrites

Authors: *M. FAITS, D. KERSCHENSTEINER;
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Abstract: Mitochondria are essential for neuronal function; they provide energy (ATP) to maintain ionic gradients and support synaptic function, and participate in Ca^{2+} buffering. The complex morphology of neurons presents a unique challenge: mitochondria must traffic from the soma throughout long and highly branched axons and dendrites to reach sites of demand. While previous studies have focused on mitochondrial movement in axons, much less is known about the signals and transport steps that control mitochondrial movement and localization in dendrites. In particular, how mitochondrial distribution is regulated during dendritic development, when sites of energy consumption and Ca^{2+} entry in neurons are in flux, has not been explored. Here, we analyze the trafficking of mitochondria in dendrites of ganglion cells (GCs) in the intact developing mouse retina combining biolistic and viral gene delivery with confocal and multiphoton live imaging. Fast time-lapse imaging revealed that dendritic mitochondria can be grouped into two pools: motile and stationary. Whereas stationary dendritic mitochondria stay in place for long periods, motile mitochondria are highly dynamic and engage in runs of movement and pauses of various durations. Interestingly, the ratio of stationary/motile mitochondrial pools increases with developmental age. Pharmacologic manipulations that artificially increase or decrease neuronal activity in mature cultured neurons had been found to decrease and increase, respectively, mitochondrial motility in a Ca^{2+}-dependent manner, but how physiologically occurring activity patterns affect mitochondrial movement in dendrites has not been examined. To test whether physiologic neural activity affects mitochondrial movement during development, we simultaneously imaged mitochondrial movements and intracellular Ca^{2+} in the intact developing retina. We find that GCs exhibit both global increases in dendritic Ca^{2+} associated with action potential firing as well as localized increases limited to small regions of dendrite. Surprisingly, neither local nor global Ca^{2+} signals affect mitochondrial movements, suggesting that physiologic patterns of activity during development play only a minor role in regulating mitochondrial transport and localization.

Disclosures: **M. Faits:** None. **D. Kerschensteiner:** None.

Poster

032. Mechanisms of Dendritic Growth and Branching

Location: Halls A-C

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Program#/Poster#: 32.04/A68

Topic: A.05. Axon and Dendrite Development

Support: TWU Department of Biology

Research enhancement

Closing the GAPS

TWU Undergraduate Microgrant

Title: Interplay of non-prenylatable Rac1 and RhoA in B35 neuroblastoma cells

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

Abstract: Rho family guanine nucleotide triphosphatases (GTPases) are molecular switches that play a fundamental role in axon growth and guidance by directing growth cone cytoskeletal changes through interaction with downstream effectors. Small GTPases are considered active when bound to guanosine triphosphate (GTP), a process promoted by plasma membrane-associated guanine exchange factors (GEFs), and inactive when bound to guanosine diphosphate (GDP). RhoA, a member of the Rho family GTPases, participates in the formation of focal adhesions, retraction of neuron processes in response to injury or insult and growth cone collapse. Rac1, thought to be a competitor of RhoA, is responsible for cell proliferation, cell cycle entry, and extension of neuronal processes. The interplay between Rac1 and RhoA dynamics is not well elucidated, though they are thought to be direct competitors with opposing functions. Both require prenylation for membrane localization, though active forms of both have been found in other cellular compartments (GTP bound Rac1 in the cytosol and GTP RhoA primarily in the cytosol and nucleus). We have created non-prenylatable RhoA and Rac1 constructs to inhibit membrane attachment, and after transfection, we determined overexpression of wild-type RhoA decreases Rac1 GTP loading in the cytosol, and overexpressing the mutant form of RhoA demonstrated near normal levels of Rac1 GTP loading. Overtransfection of wild-type Rac1 inhibited RhoA GTP loading in all cell fractions while overexpressing the Rac1 mutant did not affect RhoA loading. With emerging evidence of differential activation of these Rho GTPases based on their subcellular localization, determining how activation of these GTPases are regulated is next step in understanding Rho GTPase dynamics. Understanding how

prenylation of RhoA and Rac1, and how their competition impacts function in neurite outgrowth, may identify novel targets to facilitate axon regeneration in traumatic or degenerative neurological conditions.

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

Poster

032. Mechanisms of Dendritic Growth and Branching

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Topic: A.05. Axon and Dendrite Development

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NIH Grant RO1EY020857

Title: Dscams promote self-avoidance by masking adhesion through both MAGI-dependent and -independent mechanisms

Authors: ***R. W. BURGESS**[1], A. M. GARRETT[1], A. L. D. TADENEV[1], A. KHALIL[2], P. G. FUERST[3];
[1]The Jackson Lab., BAR HARBOR, ME; [2]The Univ. of Maine, Orono, ME; [3]Univ. of Idaho, Moscow, ID

Abstract: During development, neurons balance attractive and repulsive signals to properly position cell bodies and processes and to form synapses with appropriate partners. The Dscams (from Down syndrome cell adhesion molecule) are Ig-superfamily members important for self-avoidance in developing neurons. *Drosophila* Dscam1 promotes self-avoidance by generating up to 19,008 strongly homophilic isoforms, of which a given neuron expresses 10-50 chosen stochastically. This allows neurites to recognize and actively repel those from the same cell, while still interacting with neighboring cells. Dendritic arbors from Dscam1 mutant neurons exhibit increased self-crossings and self-fasciculation. Similar phenotypes are observed in mice mutant for the orthologous Dscam or Dscaml1 genes, despite the absence of extensive alternative splicing. In the retina, mutant neurons of a given subtype form tight dendritic fascicles and lose

their non-random mosaic spacing as their cell bodies pull into clumps. These clumps are homotypic, indicating that a shared cell-type identity promotes this interaction. In the wild type retina, processes from neurons expressing a single Dscam (either Dscam or Dscaml1) overlap extensively with other cells expressing the same gene, suggesting that normal homotypic avoidance does not involve a strong repulsive mechanism. The C-termini of both DSCAM and DSCAML1 comprise canonical PDZ-interacting domains that interact with members of the MAGI family (membrane associated guanylate kinase inverted). To investigate the functional significance of this interaction, we generated knock-in mice in which the sequences encoding the final ten amino acids were replaced with epitope tags. We found that the C-termini are required for homotypic avoidance in some cell types, but not others. Furthermore, in one cell type DSCAM's C-terminus was required to promote cell body self-avoidance, but not dendrite self-avoidance. We hypothesize that Dscams do not mediate active repulsion of homotypic neurons, but instead mask a cell-type-specific repertoire of cell adhesion molecules (CAMs) that cause fasciculation and cell body clumping when unopposed. In this hypothesis, some CAMs are masked by a DSCAM/MAGI dependent mechanism, and other CAMs can be masked by DSCAM independent of MAGIs. This hypothesis predicts that loss of function of key CAMs along with Dscams will partially rescue the fasciculation. We are testing this masking hypothesis by generating double-mutant mice with Dscam and known CAMs. We are also examining protein-protein interactions and cell-type-specific transcriptomes to identify other candidate members of the proposed adhesion code.

Disclosures: R.W. Burgess: None. **A.M. Garrett:** None. **A.L.D. Tadenev:** None. **A. Khalil:** None. **P.G. Fuerst:** None.

Poster

032. Mechanisms of Dendritic Growth and Branching

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 32.06/B2

Topic: A.05. Axon and Dendrite Development

Support: GCOE

KAKENHI

Title: The role of Sox11 in neuronal maturation in the mouse cerebral cortex

Authors: *Y. HOSHIBA[1], T. TODA[1], M. WEGNER[2], S. YANAGI[3], H. TANAKA[3], H. KAWASAKI[1];
[1]Dept. of Med. Neurosci., Kanazawa Univ., Ishikawa, Japan; [2]Inst. for Biochemistry, Univ. of Erlangen, Erlangen, Germany; [3]Sch. of Life Sciences, Tokyo Univ. of Pharm. and Life Sci., Tokyo, Japan

Abstract: Although the maturation of neurons during development is important for neuronal functions, the molecular mechanisms underlying the maturation of neurons are not fully understood. In this study, we examined the role of the transcription factor Sox11 in the maturation of excitatory neurons in the cerebral cortex during development. We found that Sox11 expression was decreased in maturating neurons. Overexpression of Sox11 using *in utero* electroporation suppressed the expression of mature neuron markers and increased the expression of the immature neuron markers. *Sox11*-knockout mice showed enhanced expression of immature neuron markers. We also found that Sox11 overexpression led to the retardation of morphological maturation of neurons. These results suggest that Sox11 inhibits the maturation of post-mitotic neurons in the cerebral cortex during development.

Disclosures: Y. Hoshiba: None. **T. Toda:** None. **M. Wegner:** None. **S. Yanagi:** None. **H. Tanaka:** None. **H. Kawasaki:** None.

Poster

032. Mechanisms of Dendritic Growth and Branching

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 32.07/B3

Topic: A.05. Axon and Dendrite Development

Support: R01 MH086629

Title: Modulation of dendritic differentiation by dopamine D1 receptor agonists

Authors: K. M. MONEY[1], *G. D. STANWOOD[2];
[1]Neurosci. Grad. Program, [2]Pharmacol., Vanderbilt Univ. Sch. of Med., NASHVILLE, TN

Abstract: Dopamine (DA) receptor signaling modulates the development of normal brain structure and function and is implicated in the pathophysiology of psychiatric diseases including schizophrenia. The striatum and frontal cortex receive significant dopaminergic innervations even prior to synaptogenesis, express high levels of DA receptors, and appear to be involved in

the developmental origins of psychiatric disease. DA receptor activation affects developmental processes in these regions, altering dendritic trajectories *in vivo*, and neurite outgrowth *in vitro*. This regulation appears to depend both on the brain region and receptor subtype activated, suggesting complex neuronal heterogeneity in responses. Using a DA D1 receptor reporter line (Drd1a-tdtomato line 6), we have observed widespread late embryonic [embryonic day 16.5 (E16.5)] expression of the D1 receptor in both frontal cortex and striatum. We therefore designed a series of experiments to assess the effects of D1 receptor stimulation on neuronal differentiation. We prepared neuron-enriched dissociated cell cultures derived from the medial frontal cortex and striatum from E16.5 mice (C57Bl/6). Cultures were grown for several days in the presence and absence of the D1-like receptor agonist, SKF 38393. Neurite outgrowth, branching, and neuronal density were measured with HCA Vision software following immunostaining for specific neuronal markers. Dose-responsive curves were conducted in wildtype cultures, and Drd1a knockout mice were used to confirm D1 receptor-dependence. SKF 38393 induced dose-dependent increases in neurite outgrowth, branching, and cell density. Preliminary data suggests that at least some of these effects are not mediated exclusively by DA D1 receptors. Current studies are evaluating potential roles for "off-target" receptors in mediating the effects, and are testing additional D1-like receptor agonists. Our studies will define the mechanisms through which D1 receptor agonists alter neurite outgrowth and contribute to understanding how the D1 receptor contributes to the development of psychiatric disorders and brain DA homeostasis.

Disclosures: **K.M. Money:** None. **G.D. Stanwood:** None.

Poster

032. Mechanisms of Dendritic Growth and Branching

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 32.08/B4

Topic: A.05. Axon and Dendrite Development

Support: MRC

Title: Regulation of dendritic morphogenesis by Miro1-dependent mitochondrial transport

Authors: ***N. F. HIGGS**, A. F. MACASKILL, G. LOPEZ-DOMENECH, J. T. KITTLER; Univ. Col. London, London, United Kingdom

Abstract: The proper intracellular distribution of mitochondria is thought to be critical for normal brain development and function. Correct dendritic development is important for the formation of neuronal circuits. Dendritic arbors form a highly extensive, complex network that cannot be sustained by diffusion of energy from the soma alone. Mitochondria are highly dynamic organelles, important for providing energy, buffering intracellular calcium and playing a role in apoptosis. These organelles must be actively transported and localised within cells to match their energy and calcium buffering requirements, however the role of mitochondrial trafficking for correct neuronal development, and dendritic morphogenesis remain poorly understood. The mitochondrial GTPase Miro1 acts as a critical adaptor protein linking mitochondria to the kinesin and dynein motor proteins, for their microtubule dependent transport along axons and dendrites. We have investigated the role played by Miro1, for regulating mitochondrial distribution and neuronal morphology, in the dendrites of developing hippocampal neurons. Here we show using loss and gain of function approaches that Miro1 is important for correctly distributing mitochondria throughout dendritic processes. We also investigate how this trafficking affects dendritic morphogenesis and the architecture of the dendritic tree and show that the fine tuning of mitochondrial distribution by Miro1 is reciprocally important for correct dendritic development.

Disclosures: **N.F. Higgs:** None. **A.F. MacAskill:** None. **G. Lopez-Domenech:** None. **J.T. Kittler:** None.

Poster

032. Mechanisms of Dendritic Growth and Branching

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 32.09/B5

Topic: A.05. Axon and Dendrite Development

Support: NIH Grant 1P20GM103653 - 01A1

Title: Dynamic effects of CXCR4/SDF-1 in neuroblastoma neurite outgrowth

Authors: Y. S. SHAN[1], *C. M. VAN GOLEN[2];
[1]Dept Biol., [2]Asst Professor, Dept Biol, Delaware State Univ., DOVER, DE

Abstract: Neurite formation counts as one of the most important progressions in neuronal development and differentiation. The N-like character of neuroblastoma cells makes them ideal as models for neuritogenesis research. Studies in neuroblastoma suggest CXCR4 has a potential

role in regulating the neurite outgrowth, which is also well involved in neuroblastoma metastasis. Our recent data demonstrates CXCR4 is co-localized with IGF-1R in neuroblastoma cells and modulates neurite extension through actin reorganization, although the underlining mechanism has yet to be clarified. We hypothesize that at low level of SDF-1(ligand of CXCR4), migration is induced for neuroblastoma cells; upon increased activation of the receptor (higher level of SDF-1), CXCR4 is internalized, reducing the amount of receptor on the cell membrane, and the internalized receptor will then interact with actin to increase neurite extension. This will promote neuroblastoma cells to migrate toward favorable conditions, such as higher SDF-1 levels in bone marrow. Once the cells have reached the favorable conditions, they will remain in that environment because of the reduction of CXCR4 on membrane surface. Same progress can take place in neuronal migration in development. By testing the expression level of AKT/p-AKT and ERK/p-ERK using western blot, we were able to test if any downstream signaling pathways were involved during this internalization. Combine treatment of AMD3100 (inhibitor of CXCR4) and SDF-1, we were able to see how neuritogenesis was impact using neurite outgrowth kit and immunofluorescent microscopy. On the other hand, flow cytometry and immunoprecipitation were as well used to examine the CXCR4 expression/localization on the cell surface and if the formation of CXCR4/F-actin complex took place in cytoplasm.

Disclosures: **Y.S. Shan:** None. **C.M. Van Golen:** None.

Poster

032. Mechanisms of Dendritic Growth and Branching

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 32.10/B6

Topic: A.05. Axon and Dendrite Development

Support: Progetto Giovani Ricercatori GR-2010-2318138 from Ministero della Salute

Title: Na+/Ca2+ exchanger 1 (NCX1)Involvement in neuronal differentiation through ca2+-dependent Akt phosphorylation

Authors: A. SECONDO[1], A. ESPOSITO[1], R. SIRABELLA[2], F. BOSCIA[2], A. PANNACCIONE[2], P. MOLINARO[2], M. CANTILE[2], M. SISALLI[2], R. CICCONE[2], C. FRANCO[2], *A. SCORZIELLO[3], G. DI RENZO[2], L. ANNUNZIATO[2];
[1]Neurosci., [2]Federico II Univ. of Naples, Naples, Italy; [3]Dept Neurosci Unit Pharmacol, Univ. Naples, Naples, Italy

Abstract: Neuronal growth factor (NGF) induces differentiation of neurons by modulating intracellular Ca2+ ions. However, the role of the three isoforms of the main Ca2+-extruding system, the Na+/Ca2+ exchanger (NCX), in neurite outgrowth remains unexplored. We investigated whether NCX1, NCX2 and NCX3 isoforms could play a relevant role in neuronal differentiation through the modulation of intracellular Ca2+ concentration ([Ca2+]i) and Akt pathway. Here we show that during NGF-induced differentiation (a) NCX1 protein level increased, NCX3 decreased, whereas NCX2 remained unaffected. Moreover, NGF caused a progressive neurite elongation, a significant increase of the well known marker of growth cones GAP-43, an enhancement of endoplasmic reticulum (ER) Ca2+ content, and of Akt phosphorylation; (b) at the same time, NCX total activity increased as measured by Fura 2AM single-cells microfluorimetry, 45Ca2+ uptake and patch-clamp; (c) NCX1 significantly colocalized with Gap-43 in NGF-treated cells; (d) the knocking-down of NCX1 prevented NGF-induced GAP-43 expression and neurite outgrowth; (e) the overexpression of the neuronal splicing isoform of NCX1 (NCX1.4), even in the absence of NGF, induced an increase in Akt phosphorylation and in GAP-43 protein expression; (f) the overexpression of NCX1.4 induced a significant increase in endoplasmic reticulum Ca2+ content measured as ATP- and thapsigargin-induced Ca2+ release; and finally (g) either the [Ca2+]i chelator BAPTA-AM or the PI3'K inhibitor LY 294002 prevented Akt phosphorylation and GAP-43 protein expression rise in neuronal cells overexpressing NCX1. Interestingly, the knocking-down of NCX1 in primary cortical neurons prevented Akt phosphorylation and protein expression of the neuronal markers of differentiation GAP-43 and MAP-2. Collectively, these data showed that NCX1 participates to neuronal differentiation by Akt phosphorylation through the modulation of ER Ca2+ content and PI'3K signaling.

Disclosures: **A. Secondo:** None. **A. Esposito:** None. **R. Sirabella:** None. **F. Boscia:** None. **A. Pannaccione:** None. **P. Molinaro:** None. **M. Cantile:** None. **M. Sisalli:** None. **R. Ciccone:** None. **C. Franco:** None. **A. Scorziello:** None. **G. Di Renzo:** None. **L. Annunziato:** None.

Poster

032. Mechanisms of Dendritic Growth and Branching

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 32.11/B7

Topic: A.05. Axon and Dendrite Development

Title: EphA7 guides dendritic morphology and synaptogenesis in cortical neurons

Authors: *C. LEONARD;
Georgetown Univ., Washington, DC

Abstract: Intercellular communication is a critical process that regulates the development of neurons, from migration to synaptogenesis. In order to achieve the proper shape and connections, neurons must interact in a variety of ways to determine their fate. One group of molecules, the Eph receptors and their ephrin ligands, play versatile roles in development. EphA7, in particular, has been shown to influence parcellation of brain structures, retinotectal axon guidance, and topographic mapping between the cortex and thalamus. The role of EphA7 in dendritic arborization and synaptogenesis, however, is only beginning to be understood. Our lab demonstrates that EphA7 is required for multiple stages of cortical neuronal maturation. Analysis of EphA7 mRNA and protein expression show EphA7 is present in the cortical plate during embryonic development. At this time, the ligand ephrin-A5 is also expressed in the cortical plate, indicating potential interactions between EphA7 and ephrin-A5. When EphA7 is absent from cortical neurons *in vitro*, dendrites can no longer avoid ligand ephrinA5, resulting in altered cell shape. *In vitro* experiments reveal that when EphA7 is ectopically expressed in cultured neurons, dendrites are shorter and less complex compared to controls, while mice mutant for EphA7 have neurons with longer and more complex dendrites *in vivo*. These results suggest EphA7 and ephrin-A5 interactions regulate dendritic arborization in the cerebral cortex. Furthermore, EphA7 is necessary for the formation and function of excitatory synapses in the cortex. *In vitro* and *in vivo* analyses reveal that neurons lacking EphA7 have fewer mature postsynaptic markers and fewer dendritic spines compared to wildtype neurons. Additionally, electrophysiological recording from cultured cortical neurons demonstrates that neurons lacking EphA7 exhibit delayed maturation of synaptic function compared to wildtype neurons. Together these data suggest that EphA7 is necessary early for proper restriction of dendritic arbors and later for formation and function of excitatory postsynaptic sites. To our knowledge, this is the first evidence of dendrite-specific roles for EphA7 during neuronal maturation.

Disclosures: **C. Leonard:** None.

Poster

032. Mechanisms of Dendritic Growth and Branching

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 32.12/B8

Topic: A.05. Axon and Dendrite Development

Support: Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology (MEXT) of Japan

Title: A chaperone protein controls the morphology of neuronal spines via filamin -A interacting protein

Authors: ***H. YAGI**[1], M. SATO[2,3,4], K. NOGUCHI[1];
[1]Dept. of Anat. and Neurosci., Hyogo Col. of Med., Nishinomiya, Japan; [2]Dept. of Anat. and Neuroscience, Grad. Sch. of Medicine, Osaka Univ., Osaka, Japan; [3]United Grad. Sch. of Child Development, Osaka University, Kanazawa University, Hamamatsu Univ. Sch. of Medicine, Chiba Univ. and Univ. of Fukui, Osaka, Japan; [4]Res. Ctr. for Child Mental Development, Univ. of Fukui, Fukui, Japan

Abstract: The cytoskeleton and its binding partners such as nonmuscle myosin IIB are essential components in the control of neuronal spine morphology. We previously reported that Filamin A-interacting protein (FILIP) binds to nonmuscle myosin IIB and changes its subcellular localization, from actin fibers to the cytosol, and the presence/absence of FILIP affects spine morphology. We studied how and what region of FILIP involved in these activities. We then identified binding partners of FILIP by using COS-7 cells and studied the effects of the binding partners on FILIP function in primary cultured hippocampal and piriform cortex neurons. We found that one chaperone protein bound to the FILIP C-terminal region, and was responsible for the FILIP-mediated change in intracellular localization of nonmuscle myosin IIB. Indeed, an inhibitor of this chaperone protein influenced FILIP-induced morphological changes in neuronal spines. Therefore, it is likely that this chaperone protein promotes FILIP-myosin IIB activity over FILIP-mediated morphological changes to spines. All experiments were conducted in accordance with the Regulations for Animal Experimentation of Hyogo College of Medicine.

Disclosures: **H. Yagi:** None. **M. Sato:** None. **K. Noguchi:** None.

Poster

032. Mechanisms of Dendritic Growth and Branching

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 32.13/B9

Topic: A.05. Axon and Dendrite Development

Support: Department of Defense (Idea Development Award W81XWH-10-1-0041 (A.B.)

McKnight Endowment Fund for Neuroscience award (AB)

Training grant from the China Scholarship Council (LZ)

NINDS MSTP T32 NS07224 (C.M.B)

Title: FLNA overexpression contributes to abnormal dendritic patterning in tuberous sclerosis complex

Authors: *L. ZHANG[1], C. M. BARTLEY[2], X. GONG[1], L. S. HSIEH[1], T. V. LIN[1], A. BORDEY[1];
[1]Neurosurgery, and Cell. & Mol. Physiol., [2]Neurobio., Yale Univ. Sch. of Med., New Haven, CT

Abstract: Abnormal dendritic complexity is a shared feature of many neurodevelopmental disorders associated with neurological defects. Here, we identified a novel role for the actin-crosslinking protein filamin A (FLNA) in normal dendritogenesis and its contribution to dendritic dysmorphogenesis in tuberous sclerosis complex (TSC), a disorder of the PI3K-mTOR pathway. Both knockdown and overexpression of FLNA in wild type neurons *in vivo* led to a more complex dendritic arbor, suggesting that an optimal FLNA level is required for normal dendritogenesis. In TSC, loss-of-function mutations in Tsc1 or Tsc2 leads to neuron enlargement with increased dendritic complexity. In Tsc1null neurons short-hairpin RNA against FLNA normalized FNA levels and prevented abnormal dendritic arborization. In addition, decreasing FLNA levels in Tsc1null neurons restored the frequency of their synaptic currents consistent with dendritic normalization although resting membrane potentials and membrane resistance were not normalized. These data demonstrate that altered FLNA expression increases dendritic complexity and contributes to pathologic dendritic patterning in TSC.

Disclosures: **L. Zhang:** None. **C.M. Bartley:** None. **X. Gong:** None. **L.S. Hsieh:** None. **T.V. Lin:** None. **A. Bordey:** None.

Poster

032. Mechanisms of Dendritic Growth and Branching

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 32.14/B10

Topic: A.05. Axon and Dendrite Development

Support: Intramural research program, National Institue of Neurological Disorders and Stroke

Title: Activity-Dependent regulation of dendrite dynamics in developing *Drosophila* larval visual circuit

Authors: C. SHENG, J. YIN, C. LONG, D. TANG, *Q. YUAN;
NIH/NINDS, Bethesda, MD

Abstract: Neuronal plasticity accompanying development and experience-dependent processes facilitates the establishment and refinement of the nervous system, while presenting significant challenges to the functional stability of the neural networks. The nervous system uses a variety of compensatory mechanisms to cope with perturbations. Recent studies suggested that structural plasticity serves as a major component for neuronal homeostasis. Our previous studies have demonstrated experience-dependent plasticity in the developing *Drosophila* larval visual circuit, in which ventral lateral neurons (LNvs), the postsynaptic targets of larval photoreceptors, exhibit robust structural plasticity of their dendritic arbors when animals are subjected to different visual experience. These observations also established a genetically tractable model system for mechanistic studies on the activity-dependent regulation of developmental plasticity. To analyze the change of neuronal morphology with high spatial and temporal resolutions, we carried out two-photon live imaging of LNvs in the developing larval brain. We observed fast structural dynamics of dendritic arbors, which was strongly influenced by the visual experience of the animals and their developmental stages. In addition, to identify genetic factors involved in the activity-dependent regulation of dendrite morphology, we carried out non-biased genetic screens and cell type specific manipulations. Our study identified activity-dependent distribution of a synaptic cell adhesion molecule babos/CG3624 as a critical component involved in the regulation of dendrite dynamics. These findings provided evidences for novel cellular and molecular mechanisms underlying homeostatic structural plasticity in the developing central nervous systems.

Disclosures: **C. Sheng:** None. **J. Yin:** None. **C. Long:** None. **D. Tang:** None. **Q. Yuan:** None.

Poster

032. Mechanisms of Dendritic Growth and Branching

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 32.15/B11

Topic: A.05. Axon and Dendrite Development

Support: National Honor Scientist Program of Korea

Title: Regulation of dendrite and spine growth by a novel transducible protein mLLP

Authors: *N.-K. YU, B.-K. KAANG, H. KIM, J. SHIM, S. KIM;
Dept. of Biol. Sci., Seoul Natl. Univ., Seoul, Korea, Republic of

Abstract: Various transcriptional regulators are involved in neuronal morphogenesis and development. We report here that mLLP, a mouse homolog of ApLLP which was previously shown to be a transcription factor facilitating synaptic plasticity in *Aplysia* neurons, is a nuclear protein interacting with transcriptional regulators and can promote dendritic growth, spinogenesis, and synaptic transmission. mLLP protein can be internalized into cells when extracellularly applied and can affect the dendritic arborization. These data suggest a regulatory role for neural development by a novel transducible nuclear protein mLLP.

Disclosures: **N. Yu:** None. **B. Kaang:** None. **H. Kim:** None. **J. Shim:** None. **S. Kim:** None.

Poster

032. Mechanisms of Dendritic Growth and Branching

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 32.16/B12

Topic: A.05. Axon and Dendrite Development

Support: NIH / NINDS / R01 NS072446-01

Title: Mutant SPT-dependent activation of ERM proteins mediates altered growth dynamics in sensory neurons: Implications for HSAN1

Authors: *B. JUN, A. CHANDRA, F. EICHLER;
Neurol., MGH, Boston, MA

Abstract: The enzyme serine palmitoyltransferase (SPT) is responsible for the first and rate-limiting step in the de novo synthesis of sphingolipids, namely the condensation of L-serine and palmitoyl-CoA. Mutations in the SPTLC1 gene encoding a subunit of SPT have been shown to cause hereditary sensory and autonomic neuropathy type I (HSAN1). Mutant SPT exhibit a shift in enzymatic specificity from the canonical amino acid substrate L-serine, to the alternative L-alanine. The effects of altered sphingolipid metabolism due to SPT mutation, and availability of SPT substrates upon the development of sensory neurons remain unknown. Recently, ERM (ezrin/radixin/moesin) cytoplasmic scaffolding proteins have been implicated in neurite guidance and cytoskeletal reorganization (Marsick et al., 2012a,b). Here, we show that dorsal root ganglia (DRG) isolated from transgenic SPTLC1C133W mice have increased neurite length and branching compared to wild-type. In addition, wild-type DRG displayed a similar increase in branching following L-alanine supplementation. The aberrant morphology observed in both the mutant DRG and L-alanine supplemented wild-type DRG occurred in conjunction with increased expression of phosphorylated-ERM proteins. However, as inhibition of SPT and sphingosine kinase (SphK), key enzymes in sphingolipid metabolism, diminished the aberrant morphology and pERM expression in those conditions, we cannot rule out that metabolites downstream of SPT could mediate its effects upon neuronal development.

Disclosures: **B. Jun:** None. **A. Chandra:** None. **F. Eichler:** None.

Poster

032. Mechanisms of Dendritic Growth and Branching

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 32.17/B13

Topic: A.05. Axon and Dendrite Development

Title: βPix heterozygote mice ($\beta Pix^{+/-}$) exhibit reduced dendritic spine formation and also defects in social behavior

Authors: Y. KWON[1], T. KANG[1], D. KIM[2], *D.-E. PARK[1];
[1]Sch. of Biol. Sci., Seoul Natl. Univ., Seoul, Korea, Republic of; [2]Dept. of Biol. Sci., KAIST, Daejeon, Korea, Republic of

Abstract: βPix (Pak interacting exchange factor) is a guanine nucleotide exchange factor (GEF) for Rho family small GTPases, Rac1 and Cdc42. It has been implicated in the modulation of diverse cellular events such as remodeling of actin cytoskeleton, regulation of focal adhesion dynamics, and cell migration. To investigate the role of βPix *in vivo,* we have generated βPix knock-out mouse. βPix null ($\beta Pix^{-/-}$) mouse was early embryonic lethal at E8.5. At E8.5, $\beta Pix^{-/-}$ embryos exhibited a significant developmental delay and were smaller in size compared to wild-type littermates. $\beta Pix^{-/-}$ embryos have several defects, lack of chorion-allantoic fusion, deficient axial rotation, and impaired closure of the cephalic neural folds. In contrast, the heterozygote mice ($\beta Pix^{+/-}$) that exhibit a reduced level of βPix expression were viable and apparently normal. However, Golgi staining of brain sections revealed that the spine density of basal dendrites in hippocampal CA1 region was decreased and the extent of dendritic branching was also somewhat reduced compared to those of wild-type littermates. In behavioral analysis, the $\beta Pix^{+/-}$ mice showed a defect in social preference test. These results indicate that βPix plays essential roles in early embryonic development, thus resulting in embryonic lethality of $\beta Pix^{-/-}$ mice. In addition, the expression level of βPix is also important for normal brain development, thus resulting in the reduced spine density and dendritic branching that may cause defects in social behavior of $\beta Pix^{+/-}$ mice.

Disclosures: **Y. Kwon:** None. **T. Kang:** None. **D. Kim:** None. **D. Park:** None.

Poster

032. Mechanisms of Dendritic Growth and Branching

Location: Halls A-C

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Topic: A.05. Axon and Dendrite Development

Support: NIH Grant 5P20RR016463-12

NIGMS grant 8 P20 GM103423-12

Maine Space Grant from NASA

Title: Exploration of two different compensatory strategies for recovery from injury in the adult cricket CNS

Authors: *H. W. HORCH[1], L. SAIDENBERG[1], M. CHONG[1], B. FIORILLO[1], J. VERGARA-BENITEZ[1], A. ZHANG[1], A. PFISTER[2], O. ELLERS[1], A. JOHNSON[1];
[1]Bowdoin Col., BRUNSWICK, ME; [2]American Museum of Natural History, New York, NY

Abstract: The consequences of injury in adult central nervous systems (CNS) are often devastating and irreversible. The CNS of the cricket is unusual in that it is capable of compensatory plasticity after injury as demonstrated by deafferentation-induced compensation in two different sensory systems. Unilateral deafferentation of the auditory neurons of the prothoracic ganglia induces these cells to send dendrites across the midline, a boundary they typically respect, to form synapses with contralateral auditory afferents. In contrast, injury to the cercal escape system shows an entirely different type of recovery, consisting mainly of physiological instead of structural reorganization. Past experiments have shown that the morphological compensation of the adult auditory system is remarkably precise, reinstating interneuron-specific tuning curves within several days. Careful anatomical analysis indicates that female dendrites branch rapidly once across the midline but then stop adding branches by about 5 days. Male dendrites, in contrast, are slower to add branches once across the midline, but extend, on average, twice as many branches as female dendrites after several weeks. Auditory axons from the contralateral, intact side, also extend additional branches from processes that have crossed the midline, though there is no sexual dimorphism. Are there specific protein candidates that correlate with recovery in either of these two systems? Initial results indicate that semaphorins 1 and 2a may differentially correlate with the different strategies employed in these two systems. Expression of sema1 and sema2a were examined using *in situ* hybridization, Q-PCR , and immunohisotochemistry. In addition, we explore a causative role for these semaphorins by blocking this deafferentation-induced upregulation in sema levels, via dsRNA injections in adult animals, and examining recovery. Our findings indicate that changes in the expression levels of semaphorins after deafferentation may play important differential roles in the deafferentation-induced plasticity seen in these two systems.

Disclosures: H.W. Horch: None. **L. Saidenberg:** None. **M. Chong:** None. **B. Fiorillo:** None. **J. Vergara-Benitez:** None. **A. Zhang:** None. **A. Pfister:** None. **O. Ellers:** None. **A. Johnson:** None.

Poster

032. Mechanisms of Dendritic Growth and Branching

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Topic: A.05. Axon and Dendrite Development

Support: NIH R21 MH101655

NSF EBICS 0939511

Title: miR-125b mediated filopodial dynamics in developing dendrites

Authors: *R. IYER[1], T. KIM[2], G. POPESCU[2], M. U. GILLETTE[1];
[1]Cell and Developmental Biol., [2]Electrical & Computer Engin., Univ. of Illinois At Urbana-Champaign, Urbana, IL

Abstract: Neuronal dendrites possess a complex dendritic arbor, which in large part gives rise to the intricate circuitry found in the nervous system. The establishment of this arbor is a result of the interplay between intrinsic cellular machinery of neurons and external stimuli, allowing dendrites to find their intended axonal partners. However, the identity, localization, and function of this intrinsic cellular machinery is not well understood. Small noncoding RNAs around 22 nucleotides long, called microRNAs (miRNAs), serve as post-transcriptional regulators of gene expression. Recent studies have pointed to localized populations of miRNAs as putative computational integrators that help translate stimuli sensed by dendritic filopodia into appropriate decisions on protein expression. This enables local, rapid decision-making in dendrites, a key requirement to navigate the spatio-temporally variant signals they receive. Here we investigate the brain-abundant miRNA, miR125b, for its role in dendrite development. miR125b has been shown to be maximally expressed at the developmental period corresponding to filopodial outgrowth in dendrites. Overexpression of this miRNA in cultured neurons leads to longer, more filopodia-like spines along dendrites. To study the role of miR125b during development, we inhibit its activity in cultured hippocampal neurons from rat during various developmental milestones as dendritic filopodia explore their microenvironment and give rise to dendrite branches and spines. Using a combination of confocal microscopy and high-resolution image analysis, we study the effect of miR125b inhibition on filopodial structure and density as well as dendritic outgrowth. To understand the effect of miR125b in the dynamics of filopodia, we use an innovative imaging technique, Spatial Light Interference Microscopy (SLIM), an interferometry-based label-free, live imaging system that has topographic accuracy comparable to atomic force microscopy (Wang et al, Opt Express, 2011). Using SLIM, we characterize the rate of filopodial extension and retraction compared with stability in response to miR125b inhibition. These high-resolution analyses reveal fresh insights into the process by which neurons integrate multiple external signals to establish the appropriate connections. Such insights are critical to understanding the implicated role of miR125b in neurological disorders such as Fragile X Syndrome and Alzheimer Disease.

Disclosures: **R. Iyer:** None. **T. Kim:** None. **G. Popescu:** None. **M.U. Gillette:** None.

Poster

032. Mechanisms of Dendritic Growth and Branching

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 32.20/B16

Topic: A.05. Axon and Dendrite Development

Support: CNPQ

Title: CREB signaling affects survival and differentiation of immature neurons

Authors: ***T. SANTANA**, M. *COSTA;
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Abstract: Introduction Cortical development demands appropriate proliferation, migration and differentiation of newly formed neurons in order to achieve the formation of a functional network. Dendritic branching defines the functionality of the cell and the efficiency of information transmission. CREB mediated transcription is an important mechanisms to control activity-dependent dendritic morphology development and neuronal. Here, we show that CREB also plays a role in these phenomena in early post-mitotic neurons, likely prior establishment of synaptic contacts. **Materials and Methods** Primary cerebral cortex cultures were derived from dorsolateral telencephalon of E14 embryos. Dissociated cells were plated at a density of 5×10^5 cells per well in a 24-well plate onto poly-D-lysine-coated glass coverslips in DMEM (10% FCS, 1% penicillin/streptomycin). Infection was performed 2 h after plating using retroviruses containing either the GFP, A-CREB (negative dominant of CREB) or CREB-FY (constitutively activated form of CREB) plasmid. 24 h after plating serum was decreased to 5 % by adding DMEM/B27. Phase contrast images were acquired every 2 minutes for 84 h (37°C, 5% CO2) for time-lapse video microscopy and single-cell tracking was performed using a computer program (TTT). After 7 div, cells were fixed in 4% PFA and processed for immunohistochemistry using antibodies against GFP and MAP2. Morphological analysis were done using ImageJ (v. 1.46e) and NeuronJ (v. 1.4.2) plugin. **Results** A-CREB significantly decreased (41%) the total length of neuronal processes. A-CREB also decreased significantly the total number of neuronal processes, probably due to reduction in the number of ramifications, once the number of primary neurites remains similar between groups. A-CREB decreased the number of bifurcations in 36%, although CREB-FY didn't increase values over control level. Expression of A-CREB leaded to a significant decrease in neuronal survival from 60 h on. Our

data indicate that signaling through CREB influences the morphology and survival of early post-mitotic cortical neurons prior establishment of fully functional synaptic contacts.

Disclosures: **T. Santana:** None. **M. *Costa:** 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.; UFRN.

Poster

033. Adolescent Drug Exposure: Alcohol and Nicotine

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 33.01/B17

Topic: A.10. Adolescent Development

Support: NIH grant P50 AA017823

Title: Social anxiety and the dynorphin/kappa opioid system during withdrawal from repeated ethanol: Impact of age and sex

Authors: ***T. L. DOREMUS-FITZWATER**, E. M. TRUXELL, E. I. VARLINSKAYA; Psychology, Binghamton Univ., Binghamton, NY

Abstract: Although adolescents are primarily insensitive to a number of acute alcohol effects, accumulating evidence suggests that they may be unusually vulnerable to adverse consequence of repeated alcohol exposure later in life, with the emergence of anxiety disorders being one of these consequences. However, less is known regarding the more immediate impact of repeated ethanol exposure during adolescence. Thus, the present study was designed to assess social anxiety-like behavioral alterations in adolescent and adult male and female Sprague-Dawley rats shortly after termination of repeated ethanol exposure. Adolescent (P28-34) and adult (P63-69) rats were given ethanol (3.5 g/kg, 25% v/v in tap water; intragastrically) once-daily for 7 days, with controls receiving an isovolumetric amount of water. Animals were then tested in a modified social interaction test 48 hr after the final exposure, and social anxiety-like behaviors were indexed via decreased social investigation and/or social preference. Immediately after testing, trunk blood and brains were collected. Behavioral results demonstrated that adolescent males, but not their female counterparts, exposed to repeated ethanol exhibited pronounced social anxiety, as evident by significant decreases in both social investigation and social preference. Adult females exposed to ethanol also demonstrated social anxiety, but only when

indexed via social preference. In contrast, adult males were resistant to the social anxiogenic effects of ethanol withdrawal under these test circumstances. As age- and sex-related differences in the dynorphin/kappa opioid system are likely contributors to ethanol's effects on social anxiety-like behavior, a preliminary assessment of opioid-related gene expression in the anterior cingulated cortex (ACC) was conducted. While previous results demonstrated that adolescent animals non-manipulated prior to the social interaction test (or exposure to the test context) exhibited significantly lower preprodynorphin (PPD) gene expression than adults, in the current study, PPD gene expression was substantially higher in adolescents relative to adults. Furthermore, ethanol-exposed adolescent males demonstrated a trend for higher PPD gene expression than their water-exposed counterparts. Taken together the present results suggest that adolescent males are most sensitive to the detrimental effects of withdrawal from repeated ethanol under social test circumstances, with future studies needed to further investigate the role of the dynorphin/kappa opioid system in this vulnerability.

Disclosures: **T.L. Doremus-Fitzwater:** None. **E.M. Truxell:** None. **E.I. Varlinskaya:** None.

Poster

033. Adolescent Drug Exposure: Alcohol and Nicotine

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 33.02/B18

Topic: A.10. Adolescent Development

Support: Rosalind Franklin University (KYT)

NIH Grant MH086507 (KYT)

NIH Grant MH083729

Title: Contribution of alpha-7 nAChR tone in sustaining the gain of GABAergic transmission in the adult prefrontal cortex

Authors: D. R. THOMASES[1], E. FLORES-BARRERA[1], J. P. BRUNO[2], R. SCHWARCZ[3], *K.-Y. TSENG[1];
[1]Cell & Molec Pharmacol, RFUMS/Chicago Med. Schl, NORTH CHICAGO, IL; [2]2Departments of Psychology & Neurosci., The Ohio State Univ., Columbus, OH; [3]Dept. of Psychiatry, Maryland Psychiatric Res. Center, Univ. of Maryland Sch. of Med., Baltimore, MD

Abstract: The functional maturation of GABAergic circuits in the prefrontal cortex (PFC) during the adolescent transition to adulthood is one key mechanism for enabling proper prefrontal processing of ventral hippocampal afferent information and for supporting PFC-dependent cognitive functions. It is thought that a dysregulation of such GABAergic control of PFC response underlies the adolescent onset of cognitive deficits in a variety of psychiatric disorders including schizophrenia (SZ). Thus, further understanding of the mechanisms contributing to the initiation and maintenance of proper GABAergic inhibition in the PFC is a critical step towards restoring normal cognition in SZ and related psychiatric syndromes. Here the role of alpha-7 nicotinic acetylcholine receptor (nAChR) regulation of PFC response to ventral hippocampal train stimulation (10, 20 & 40Hz) was assessed by means of local field potential (LFP) recordings combined with local infusions of selective antagonists *in vivo*. Results showed that single prefrontal infusion of 300nM kynurenic acid (KYNA; a tryptophan metabolite known to preferentially block alpha-7 nAChR activity) was sufficient to induce a state of frequency-dependent disinhibition in the adult PFC that resembles the pattern of response resulting from local GABAergic blockade. A similar pattern of LFP disinhibition was found following local prefrontal infusion of the alpha-7 nAChR antagonist MLA (300nM), suggesting that alpha-7 nAChR tone in the PFC is required to enable the normal prefrontal GABAergic response to ventral hippocampal drive. Patch-clamp recordings in adult PFC brain slices further support this view, as the frequency of GABAergic synaptic activity onto layer V pyramidal neurons is positively regulated by local alpha-7 nAChR tone. Accordingly, the frequency-dependent LFP deficit induced by KYNA or MLA was not longer observed when the GABA-A alpha-1 positive allosteric modulator Indiplon (10uM) was co-administered into the PFC *in vivo*. Together, these results indicate that an upregulation of prefrontal alpha-7 nAChR tone could play an essential role in sustaining the increasing functional capacity of GABAergic transmission in the PFC during the adolescent transition to adulthood.

Disclosures: **D.R. Thomases:** None. **K. Tseng:** None. **E. Flores-Barrera:** None. **J.P. Bruno:** None. **R. Schwarcz:** None.

Poster

033. Adolescent Drug Exposure: Alcohol and Nicotine

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 33.03/B19

Topic: C.17. Drugs of Abuse and Addiction

Support: NIAAA AA019767

NIAAA AA11605

NIAAA AA007573

NIAAA AA021040

NIAAA AA020023

NIAAA AA020024

NIAAA AA020022

Title: Adolescent, but not adult, binge ethanol exposure leads to persistent global reductions of choline acetyltransferase expressing neurons in brain

Authors: *R. P. VETRENO[1], M. BROADWATER[2], W. LIU[3], L. SPEAR[2], F. CREWS[3]; [1]Sch. of Med., Univ. of North Carolina At Chapel Hill, Chapel Hill, NC; [2]Ctr. for Developmental and Behavioral Neurosci., Binghamton Univ., Binghamton, NY; [3]Univ. of North Carolina at Chapel Hill, Chapel Hill, NC

Abstract: During the adolescent transition from childhood to adulthood, notable maturational changes occur in brain neurotransmitter systems. The cholinergic system is composed of several distinct nuclei that exert neuromodulatory control over cognition, arousal, and reward. Binge drinking and alcohol abuse are common during this stage, which might alter the developmental trajectory of this system leading to long-term changes in adult neurobiology. Experiment 1 tested the hypothesis that adolescent intermittent ethanol (AIE; 5.0 g/kg, i.g., 2-day on/2-day off from postnatal day [P] 25 to P55) leads to persistent, global reductions of choline acetyltransferase (ChAT) expression, an enzyme critical for acetylcholine synthesis. Immunohistochemical analysis found that AIE reduced ChAT-immunoreactive (+IR) cells in the basal forebrain that persisted from late adolescence (P56) into later adulthood (P220). In addition to the basal forebrain, ChAT+IR was decreased in cholinergic nuclei of the hindbrain and striatum of young adult (P80) rats exposed to AIE. To determine if the binge ethanol-induced ChAT decline was unique to the adolescent, Experiment 2 examined ChAT+IR in the basal forebrain following adolescent (P28 - P48) and adult (P70 - P90) binge ethanol exposure. Twenty-five days later, ChAT expression was reduced in adolescent, but not adult, binge ethanol-exposed animals. Administration of the Toll-like receptor 4 agonist lipopolysaccharide to young adult rats (P70) produces a similar reduction in ChAT+IR that mimicked AIE, but did not further reduce ChAT+IR after AIE exposure. Together, these data suggest that adolescent binge ethanol decreases adult ChAT expression, possibly through neuroimmune mechanisms, which might impact adult cognition, arousal, or reward sensitivity.

Disclosures: **R.P. Vetreno:** None. **M. Broadwater:** None. **W. Liu:** None. **L. Spear:** None. **F. Crews:** None.

Poster

033. Adolescent Drug Exposure: Alcohol and Nicotine

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 33.04/B20

Topic: A.10. Adolescent Development

Support: NIH Grant F31 AA022842

NIH Grant U01 AA019967

NIH Grant T32 AA007474

Title: Adolescent alcohol exposure decreases gabaa receptor mediated currents in the adult medial prefrontal cortex

Authors: *S. CENTANNI[1], H. TRANTHAM-DAVIDSON[2], J. CHANDLER[2];
[1]Neurosci., [2]Med. Univ. of South Carolina, Charleston, SC

Abstract: During adolescence, the prefrontal cortex (PFC) undergoes a critical period of cortical development and refinement of neuronal circuits that occurs in conjunction with the maturation of complex cognitive behaviors. Although accumulating evidence suggests that binge-like alcohol consumption during adolescence adversely affects the development of the PFC, little is known about the long-term consequences of this on PFC-dependent behaviors in adulthood. The goal of the current study was to investigate the effect of adolescent alcohol exposure (AIE) on the adult GABAergic system in the PFC. Whole-cell patch clamp recordings from prelimbic PFC (PrL-PFC) layer V pyramidal neurons was performed in slices obtained from adult rats (PD90-120) that had been subjected to 4 cycles of AIE exposure by vapor inhalation between PD28-42. Litter-matched controls received sham exposure. We observed no differences in paired-pulse ratio or sIPSC frequency in slices from AIE treated rats relative to controls. In contrast, AIE exposure resulted in a significant decrease in spontaneous IPSC (sIPSC) amplitude suggesting AIE alterations in postsynaptic $GABA_AR$. Miniature IPSC amplitude and frequency were unaffected suggesting that the observed reduction in sIPSC amplitude may not be a result of synaptic $GABA_AR$, but rather those $GABA_AR$ located outside of the synapse. Next, we isolated the tonic $GABA_A$ current by measuring the holding current at -70mV in the presence and absence of the $GABA_AR$ antagonist picrotoxin (100 μM). Tonic current amplitude was significantly attenuated in AIE-exposed rats relative to controls in adulthood and at PD45 and PD60. Furthermore, pharmacological stimulation of the exclusively extrasynaptic ∂-$GABA_AR$ using the selective agonist THIP (1μM) revealed that AIE exposure significantly reduced the currents specifically mediated by $GABA_ARs$ containing the ∂ subunit. Preliminary data suggests

that the effect of AIE on the ∂-$GABA_A$R-mediated currents evident in adulthood is not present at PD45 or PD60. Additionally, AIE had no effect on total ∂-$GABA_A$R expression or surface expression of ∂-$GABA_A$R in the adult mPFC. These findings suggest that AIE significantly compromises $GABA_A$R mediated tonic currents immediately after AIE exposure and continuing on into adulthood. Moreover, preliminary evidence suggests that the major contribution of specific $GABA_A$R subunits underlying the reduction in tonic current may differ between adolescence and adulthood. The net consequence of AIE on the GABAergic system in the mPFC may negatively impact cognitive functioning in the adult by disrupting the balance of excitatory-inhibitory neurotransmission in the mPFC.

Disclosures: **S. Centanni:** None. **H. Trantham-Davidson:** None. **J. Chandler:** None.

Poster

033. Adolescent Drug Exposure: Alcohol and Nicotine

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 33.05/B21

Topic: A.10. Adolescent Development

Support: NIH Grant U01 AA019973

Title: Adolescent intermittent alcohol exposure (AIE) alters adult HPA axis responses to an alcohol challenge

Authors: *S. O. LEE[1], S. IM[2], S. BAE[2], J. HONG[2], J. VAUGHAN[2], C. RIVIER[2], E. VUONG[2], M. L. LOGRIP[2,3];
[2]The Clayton Fndn. Labs. for Peptide Biol., [1]Salk Inst., La Jolla, CA; [3]Committee on the Neurobio. of Addictive Disorders, The Scripps Res. Inst., La Jolla, CA

Abstract: The hypothalamic-pituitary-adrenal (HPA) axis undergoes critical developments during adolescence. As a result, stressors experienced during this period potentially have long-lasting effects on the adult HPA axis function. We investigated the effects of adolescent intermittent ethanol (AIE) exposure in rats on the response to acute stress challenges in adulthood. We hypothesized that AIE would have long-lasting effects on the adult HPA axis, resulting in altered responses to an alcohol challenge or physico-emotional stressors, such as shocks or restraint. To test these hypotheses, male rats were exposed to alcohol vapor for 6 h per day from PND 28-42 (AIE), then acutely challenged with alcohol intragastrically (4.5 g/kg, ig), shocks (0.3mA, 1 sec, 2 shocks/min, 30 min) or restraint (15 min) on PND 70-71 or PND 90-91.

As previously reported, we observed blunted HPA axis responses to alcohol challenge due to AIE. Specifically, AIE tended to inhibit the alcohol challenge-induced plasma corticosterone (cort) levels and expression of CRF mRNA in the paraventricular nucleus (PVN) of PND 70-71 male rats, while AIE significantly blunted the alcohol challenge-induced plasma cort levels and elevation of AVP mRNA in the PVN of PND 90-91 male rats. Previously we have shown that these changes in PVN responsiveness may result from AIE-induced alterations in adrenergic neurons in brainstem regions C1-C3 known to project to the PVN. AIE elevated the numbers of PNMT-positive cell bodies in the C2 region of males on PND 70-71, while AIE increased PNMT-positive cell numbers in the C1 regions of males on PND 90-91. Unlike the alcohol challenge responses, no differential modulation of plasma cort or CRF/AVP mRNA levels was observed after shock or restraint challenges in AIE vs. control rats. Brainstem regions C1-C3 were also unchanged in response to the shock challenge at PND 70-71 and PND 90-91. Together, this data suggests that adolescent alcohol exposure produces alterations in HPA axis responsiveness to administration of an acute alcohol challenge that may be very long-lasting and stress-specific.

Disclosures: **S.O. Lee:** None. **S. Im:** None. **S. Bae:** None. **J. Hong:** None. **J. Vaughan:** None. **C. Rivier:** None. **E. Vuong:** None. **M.L. Logrip:** None.

Poster

033. Adolescent Drug Exposure: Alcohol and Nicotine

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 33.06/B22

Topic: A.10. Adolescent Development

Support: Penn State Social Science Research Institute through a SSRI/CBBC Level 1 Funding grant

R01 DA003194

Title: Adolescent nicotine exposure induces binge ethanol consumption and nAChR density increases in the reward pathway

Authors: *A. R. REVITSKY[1], M. J. MARKS[2], L. C. KLEIN[1];
[1]Neurosci., The Pennsylvania State Univ., State College, PA; [2]Inst. for Behavioral Genet. and Dept. of Psychology and Neurosci., Univ. of Colorado Boulder, Boulder, CO

Abstract: Despite the deleterious effects resulting from cigarette use almost 20% of the U.S. adult population currently smokes tobacco, and 80% of adult smokers begin smoking during adolescence (CDC, 2011; USDHHS, 1994). Adolescents consume ethanol more than any other drug of abuse, and binge drinking among teens has become increasingly popular over the years (CASA, 2011; SAMHSA, 2002). Nicotine and ethanol activate the mesolimbic dopamine pathway and increase synaptic dopamine levels in the nucleus accumbens, in part through stimulation of nicotinic acetylcholine receptors on dopamine neurons in the ventral tegmental area (Blomqvist et al., 1997; Corrigall et al., 1994). Because of the way these drugs affect the reward pathway, and the fact that the adolescent brain undergoes extensive psychological development and gross neuroanatomical changes during this developmental period, adolescent use of these two drugs is particularly concerning (Spear, 2000). Thus, it is necessary to investigate reward-related outcomes and potential neurobiological underpinnings that result from exposure to nicotine and alcohol in adolescence, especially in females where rates of use for both these drugs are increasing (CASA, 2011; Pogun & Yararbas, 2009). Twenty- six adolescent female mice were exposed to either water (n=12) or nicotine (n=14) via an oral administration paradigm (200ug/ml) for 7 days. All mice were exposed then to a 4-day drinking in the dark protocol. Adolescent mice exposed to nicotine consumed a significantly more ethanol (g/kg) and displayed higher blood ethanol concentrations than control mice [$Fs(1,25) \geq 4.21$]. Autoradiographic analysis of nAChR density in regions of the brain reward pathway revealed higher levels of epibatidine binding in frontal cortical regions in mice exposed to nicotine compared to control [$Fs(1,25) \geq 2.05$]. Additionally, BEC was a significant positive predictor of α–bungarotoxin binding in the cingulate cortex, striatum, and dorsal tegmental area [$\beta s \geq 0.47$]. Overall, findings from this study suggest that nicotine exposure during adolescence may increase subsequent binge ethanol consumption through increased activation of the brain reward pathway as indexed by increases in nAChR density. Additionally, ethanol may play a significant role in modulating activating of reward pathway through stimulation of these nAChRs in several reward pathway brain regions.

Disclosures: **A.R. Revitsky:** None. **M.J. Marks:** None. **L.C. Klein:** None.

Poster

033. Adolescent Drug Exposure: Alcohol and Nicotine

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 33.07/B23

Topic: A.10. Adolescent Development

Support: DA 017949

Title: The interaction of nicotine and stress during adolescence on long-term impairments on a hippocampus-dependent task

Authors: *E. HOLLIDAY[1], S. LOGUE[2], T. GOULD[2];
[1]Temple Univ., Philadelphia, ; [2]Temple Univ., Philadelphia, PA

Abstract: Despite the known risks associated with tobacco use and decline of tobacco consumption in the last 50 years, nearly 1 in 5 people in the United States are everyday smokers. One reason for the steadfast smoking prevalence rates is adolescent smoking. In rodent models, nicotine treatment during adolescence leads to impairments in hippocampus-dependent tasks later in life. Adult mice under the same treatment conditions do not display long-term cognitive impairments. The results from this study suggest that these long-term impairments result from an interaction between stress and nicotine during adolescence. This study examined the interaction of stress and nicotine in adolescent (p38) and adult (p54) C57BL/6 mice that were either shipped from an animal facility in Maine to our animal facility in Philadelphia (STRESS) or bred in our animal facility (NO STRESS). All animals were implanted with osmotic mini-pumps to deliver 12.6mg/kg of nicotine per day for a period of 12 days, at which time the pumps were removed. Thirty days later animals were trained and tested in our contextual fear conditioning paradigm. Only adolescent animals shipped in and administered nicotine showed long-term impairments. Additionally, blood was collected from animals not designated for behavioral tasks and serum was used for corticosterone analysis. Only adolescent animals in the shipped condition showed elevated levels of corticosterone. Non-shipped adolescents, non-shipped adults, and shipped adults all displayed similar concentrations of corticosterone. This study suggests stress and nicotine interact during adolescence, leading to long-term cognitive changes. These changes may broadly impact

Disclosures: **E. Holliday:** None. **S. Logue:** None. **T. Gould:** None.

Poster

033. Adolescent Drug Exposure: Alcohol and Nicotine

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 33.08/B24

Topic: A.10. Adolescent Development

Support: DA033358

Title: Effects of adolescent caffeine consumption on anxiety behaviors, plasma corticosterone, and neural activity

Authors: J. STAFFORD[1], C. E. O'NEILL[1], R. J. NEWSOM[1], S. C. LEVIS[1], T. SCOTT[1], *R. K. BACHTELL[2];
[2]Dept. of Psychology & Neurosci., [1]Univ. of Colorado, BOULDER, CO

Abstract: Caffeine is the most commonly used psychoactive substance worldwide, and consumption by children and adolescents has risen dramatically in recent years. Previous studies have found that energy drink use is associated with anxiety young adult males, and can induce panic attacks in patients diagnosed with panic disorder. These experiments examine the effects of adolescent caffeine consumption on anxiety related behaviors, basal and stress-induced plasma corticosterone (CORT), and stress-induced c-*fos* mRNA expression. Beginning on post-natal day 28 (P28), Sprague-Dawley rats consumed caffeine (0.3 g/L) for 28 days (P28-55). Age-matched control rats consumed water. Caffeine and water consumption were monitored throughout the procedure, with no significant differences between groups on fluid consumption or body weight gain. Following 28 days of caffeine consumption, the caffeine solution was replaced with water for the remainder of the experiment. Behavioral testing occurred at three different time points: during caffeine exposure (P52-55), short-term withdrawal (24 hrs) from caffeine (P56), and long-term withdrawal (1 week) from caffeine (P62-66). Animals exposed to caffeine during adolescence exhibited increased anxiety in the open field at both withdrawal time points, 24 hrs and 1 week post caffeine, but not during the caffeine exposure. Chronic adolescent caffeine consumption also decreased social interaction with age-matched conspecifics at both short- (24 hr) and long-term withdrawal (1 week), but not during caffeine exposure. Finally, caffeine-exposed rats demonstrated increased anxiety on an elevated plus maze at all three time points. Because systemic caffeine administration has been shown to increase plasma CORT levels, we examined the effect of adolescent caffeine consumption on basal and stress-induced CORT levels during caffeine withdrawal. Animals exposed to caffeine had increased plasma CORT at the circadian trough, however no differences were observed at the circadian peak. Stress-induced CORT was measured 30 min after animals were placed on a small pedestal elevated 2 feet off the ground for 5 minutes. Stress-induced CORT was significantly lower in animals exposed to caffeine during adolescence. Together these findings suggest that adolescent caffeine consumption alters emotional reactivity using behavioral and neuroendocrine measures. We are currently processing the tissue to examine stress-induced c-*fos* mRNA expression in several stress-responsive brain areas including the frontal cortex, the extended amygdala, lateral septum, and hypothalamic regions.

Disclosures: **J. Stafford:** None. **R.K. Bachtell:** None. **C.E. O'Neill:** None. **R.J. Newsom:** None. **S.C. Levis:** None. **T. Scott:** None.

Poster

033. Adolescent Drug Exposure: Alcohol and Nicotine

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Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 33.09/B25

Topic: C.17. Drugs of Abuse and Addiction

Support: NIH grant AA017359

Title: Alterations in neuronal nitric oxide synthase in the hippocampus of female adolescent rats exposed to repeated ethanol administrations

Authors: I. KARPICHEV, L. MALAVE, S. CAIN, S. KRONENBERG, *R. SIRCAR;
The City Col. of New York, NEW YORK, NY

Abstract: Earlier, we have reported that ethanol impairs spatial learning and memory in adolescent female rat, and that the N-methyl-D-aspartate (NMDA) receptor is involved in mediating ethanol-induced memory deficits. In the brain activation of the NMDA receptor by stimulating the enzyme neuronal nitric oxide synthase (nNOS), produces nitric oxide (NO). NO has been shown to be important in synaptic plasticity, and learning and memory. Increasing evidence indicates that NO is involved in many of ethanol's effects such as ethanol consumption, ethanol tolerance, and ethanol withdrawal symptoms. The present study was undertaken to investigate the effects of ethanol exposure on hippocampal NOS in adolescent rats. Female adolescent rats were treated with ethanol (2 g/kg, i.p.) for five consecutive days. Animals were sacrificed at specific time points following ethanol administration, their hippocampi removed and frozen. Control rats received equivalent volumes of vehicle for the same number of days and sacrificed along with the ethanol-treated rats. Levels of nNOS in hippocampal lysates were measured using western blot analysis. The results showed that ethanol-treated adolescent female rats exhibited increased levels of hippocampal nNOS. These data indicate that hippocampal nNOS may be important in mediating some of the cognitive impairing effects of ethanol in adolescent rat.

Disclosures: I. Karpichev: None. **L. Malave:** None. **S. Cain:** None. **S. Kronenberg:** None. **R. Sircar:** None.

Poster

033. Adolescent Drug Exposure: Alcohol and Nicotine

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Program#/Poster#: 33.10/B26

Topic: A.10. Adolescent Development

Support: U01 AA019972-NADIA Project

Title: Intermittent ethanol exposure during adolescence: Effects on stress-induced social alterations in adulthood

Authors: *E. I. VARLINSKAYA, E. M. TRUXELL, L. P. SPEAR;
Dept Psychol, Binghamton Univ., BINGHAMTON, NY

Abstract: Binge patterns of alcohol consumption are common in human adolescents and may contribute to alcohol-related and affective problems later in life, with exposure to different stressors further exacerbating these alterations. Using a rat model of adolescence, we have shown long-lasting and sex-dependent detrimental consequences of repeated exposure to ethanol during the juvenile-early adolescent period. Males, but not females, demonstrated social anxiety-like behavioral alterations, indexed via significant decreases in social investigation and social preference when tested as adults. The present study was designed to assess whether the social anxiety-like behavior associated with adolescent intermittent ethanol (AIE) exposure can be further exacerbated by exposure to a stressor later in life. Adolescent male and female Sprague-Dawley rats were exposed intragastrically to ethanol (3.5 g/kg, 25% solution in tap water) or an isovolumetric amount of water every other day between postnatal days (P) 25-45, for a total of 11 exposures. Three weeks following AIE half of the animals from each adolescent exposure condition were restrained (stress condition) for 90 min/day for 5 days (P66-P70), whereas the other half remained non-stressed. Social behavior and social preference were assessed 30 min following the offset of the stressor on the last exposure day (P70, immediate stress effects) as well as 7 days following exposure to restraint (P77, delayed effects) in a modified social interaction test under familiar circumstances. Non-stressed males (but not females) repeatedly exposed to ethanol showed significant decreases in social behavior and social preference relative to water-exposed non-stressed controls at P70 and P77, an effect not evident in their stressed male counterparts. Socially suppressing effects of stress, both immediate and delayed, were evident only in water-exposed males and females, with adolescent exposure to ethanol curbing the sensitivity of animals to the social consequences of repeated restraint during adulthood. AIE and stress effects on EtOH intake under social circumstances (four same-sex littermates drinking together in a novel cage for 30 min) were assessed in these animals on P80-P91. Neither AIE nor stress had any effects on social drinking, however each independently increased adolescent-typical play fighting in males, but not females, under these social circumstances. Thus, although later stress did not exacerbate the social anxiety seen after AIE in males, both manipulations

reinstated adolescent-typical social facilitation in adult males drinking ethanol in a social context.

Disclosures: **E.I. Varlinskaya:** None. **E.M. Truxell:** None. **L.P. Spear:** None.

Poster

033. Adolescent Drug Exposure: Alcohol and Nicotine

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 33.11/B27

Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grant AA020022

Title: The effect of adolescent intermittent binge ethanol on neurogenesis and oligodendrogenesis in the subventricular zone and anterior corpus callosum of adult rats

Authors: *W. LIU, F. T. CREWS;
Bowles Ctr. for Alcohol Studies, Univ. of North Carolina-Chapel Hill, Chapel Hill, NC

Abstract: Binge drinking is common in adolescent humans. Previous studies have found that adolescent intermittent binge ethanol (AIE) caused a persistent change in hippocampal neurogenesis lasting into young adulthood. The current study examined the effect of AIE compared with water controls on neurogenesis and oligodendrogenesis in the subventricular zone (SVZ) and anterior corpus callosum (acc) of adult rat brain. Male Wistar rats were bred and reared in our vivarium. On the day following birth (P1), litters were culled to 10 pups. At weaning on P21 (Postnatal days 21), male offspring were weight matched and pair-housed. Animals were assigned into four groups. Two groups of them were treated with ethanol (AIE, 5g/kg/day, i.g., P25-P55; 2 days alcohol, 2 days off) and the other two groups were given with water as controls. Animals of the four groups (two controls and two AIE) were injected with a single dose of BrdU (300 mg/kg, i.p.) at P57 and P95, respectively, and were sacrificed 2 hours later. The impacts of AIE on neurogenesis and oilgodendrogenesis were investigated using immunohistochemistry with quantification of immunoreactivity to BrdU, Nestin, sox2, DCX, Dlx2 (neurogenesis markers) and NG2 (oligodendrogenesis progenitor cells, OPCs), olig2 (OPCs and mature oligodendrocytes) and olig1 (oligodendrocytes). In the subventricular zone, AIE reduced neurogenesis marker, sox2+IR (18%, $p<0.05$), DCX+IR (32%, $p<0.001$), and Dlx2+IR (13%, $p<0.05$) at P95. There were no change with BrdU+IR and nestin+IR. The study has showed that there were age-related (from P57 to P95) decline in olig2+IR and NG2+IR cells in

control group, however, there was no age-related decline in AIE group. NG2+IR cells in AIE group showed significantly higher than in control group (56%, $p<0.001$) at P95. In anterior corpus callosum, where OPCs generated in the SVZ migrate into, AIE induced the increase in nestin+IR (33%, $p<0.05$), sox2+IR (33%, $p<0.01$), DCX+IR (78%, $p<0.05$), olig2+IR (59%, $p<0.01$) at P95, and olig1 at P57 (90%, $p<0.01$), compared with control group. These data indicate that adolescent intermittent binge ethanol persistently reduced neurogenesis in the forebrain subventricular zone and increased oligodendrogenesis in anterior corpus callosum of adult rats. (Funded by the NADIA from NIAAA).

Disclosures: **W. Liu:** None. **F.T. Crews:** None.

Poster

033. Adolescent Drug Exposure: Alcohol and Nicotine

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 33.12/B28

Topic: C.17. Drugs of Abuse and Addiction

Support: T32 AA007573

Title: Adolescent binge exposure alters the adult brain response to stress

Authors: *T. J. WALTER[1], R. VETRENO[2];
[1]Univ. of North Carolina - Chapel Hill, Carrboro, NC; [2]Univ. of North Carolina - Chapel Hill, Chapel Hill, NC

Abstract: Binge drinking is common among adolescents and has several long-term effects that can last into adulthood. One of these effects is a change in brain stress response to alcohol during adulthood. This study sought to determine whether adolescent binge exposure changes the adult brain stress response to stressors other than alcohol. This was done by exposing adolescent male Wistar rats to either an intermittent adolescent ethanol (AIE) treatment or water treatment from post-natal day 25 to 55. On post-natal day 95, after a period of abstinence, the AIE-treated and water-treated rats were each subdivided into two groups - stressed or non-stressed - for a total of four groups: Water-No Stress, AIE-No Stress, Water-Stress, and AIE-Stress. The stressed groups received 2 hours of restraint and partial submersion in water. The rats were sacrificed two hours after the end of the stressor and immunohistochemical stains were performed on the brains. The neuronal response to stress was examined using c-Fos and EGR1, and the microglial response to stress was examined using CD11b. Five stress-responsive brain regions, the mPFC, vBNST,

PVN, CeA and PAG were examined. Stress significantly increased c-Fos in all regions. Furthermore, there was a significance treatment-by-stress effect for c-Fos in the PVN. Stress also significantly increased EGR1 in all regions, and AIE significantly decreased EGR1 in the CeA. The AIE-Stress group also showed significantly lower EGR1 in the CeA than the Water-Stress group. Stress significantly increased CD11b in all examined regions and AIE significantly increased CD11b in the mPFC, the CeA and PAG. There was no treatment-by-stress interactions for CD11b in any examined region. Pearson correlations between c-Fos densities in the different brain regions were used to examine connectivity of these regions at baseline and under stress. Similar analyses were performed by correlating EGR1 densities across brain regions. AIE caused subtle differences in correlations both at baseline and under stress. These results suggest that adolescent binge exposure causes some changes in the adult brain response to stress during adulthood.

Disclosures: **T.J. Walter:** None. **R. Vetreno:** None.

Poster

033. Adolescent Drug Exposure: Alcohol and Nicotine

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 33.13/B29

Topic: C.17. Drugs of Abuse and Addiction

Title: Corticosterone response to the route of ethanol administration in adolescent and adult animals

Authors: *J. HOFFMAN[1], R. M. PHILPOT[2], C. L. KIRSTEIN[3];
[1]Psychology, Usf-Psychology, Tampa, FL; [2]Mosani Col. of Med., USF, Tampa, FL;
[3]Psychology, USF-Psychology, Tampa, FL

Abstract: Nearly half (48.6%) of alcohol use involves individuals under the age of 20, indicating the critical importance of research that focuses on the effects of alcohol exposure during the adolescent period. Because of the short period of adolescent development in rodents, studies using these animals to model adolescent alcohol exposure are predominately restricted to alcohol administered by the experimenter, most frequently intraperitoneal (i.p.) injections or oral intragastric (i.g.) administration. Although the i.g. method of administration is physiologically more similar to human alcohol use than i.p. administration, it is often thought of as especially stressful. Since adolescent animals exhibit larger and/or more prolonged corticosterone (CORT) responses to stressors than adult animals, the use of a stressful route of administration could

confound the results of studies comparing adolescent to adult alcohol responses. Therefore, the present study determined whether alterations in circulating CORT levels are larger following acute i.p. or i.g administration of alcohol, whether this response is altered following chronic intermittent ethanol exposure (CIE) and whether these responses are similar in adolescent and adult animals. Blood was collected via tail nick from adolescent and adult male Sprague-Dawley rats prior to and 15, 30 and 60 minutes following i.p or i.g administration of ethanol (2g/kg), saline (i.p.) or water (i.g.). Animals were subsequently exposed to 2g/kg ethanol (i.p or i.g) for 21 days using a CIE schedule (2 days on/1 day off). On the final day of ethanol exposure, blood was again collected prior to and 15, 30 and 60 minutes following ethanol exposure. Results indicate that: 1) adolescents have significantly higher basal circulating CORT than adults; 2) adolescents manifest larger CORT responses (area under the curve) than adults to acute saline (i.p.) or water (i.g.); 3) compared to adults, adolescents exhibit significantly greater increases in CORT following acute i.p., but not i.g., ethanol administration and; 4) during adolescence, but not adulthood, both i.p. and i.g. CIE administration suppress basal and ethanol stimulated circulating CORT. These results indicate that the route of ethanol administration is a relevant consideration when comparing acute and CIE effects between adolescent and adult animals, in particular if experimental measures are affected by CORT.

Disclosures: **J. Hoffman:** None. **R.M. Philpot:** None. **C.L. Kirstein:** None.

Poster

033. Adolescent Drug Exposure: Alcohol and Nicotine

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 33.14/B30

Topic: A.10. Adolescent Development

Support: R01DA019375

R01DA003194

P30DA015663

Title: Interaction of developmental nicotine exposure and the nicotine risk variant in the alpha 5 nicotinic subunit changes nicotine seeking in adolescent mice

Authors: ***H. C. O'NEILL**, C. WAGEMAN, S. R. GRADY, M. J. MARKS, J. A. STITZEL; Inst. For Behavioral Genet. , Univ. of Colorado, Boulder, CO

Abstract: A single nucleotide polymorphism (rs16969968) in the nicotinic acetylcholine receptor (nAChR) alpha5 subunit (a D397N substitution) has been linked to early onset smoking behavior and increased risk for nicotine dependence. The interaction of this polymorphism and developmental exposure to nicotine (DNE) with subsequent adolescent nicotine consumption has not been investigated. Recently, we developed a knockin mouse with this human SNP to facilitate further investigation. Prior to breeding and until weaning of the pups at day PN21, dams drank nicotine (100 µg /ml) in 0.2% saccharin water (developmental nicotine exposed, DNE) or 0.2% saccharin water alone (vehicle) as their sole source of fluid. For adolescent drinking studies, at 30 days of age offspring were given a choice between water or nicotine (100-400 µg/ml nicotine, 4 days at each concentration). Adolescent vehicle offspring homozygous for the high risk allele (N397) drank more nicotine than adolescent vehicle mice homozygous for the wild-type variant (D397). Adolescent DNE N397 mice drank more nicotine at the highest dose than all other groups, while DNE D397 mice appeared to actively avoid nicotine resulting in significantly less nicotine consumption compared to all other groups. No difference between vehicle and DNE D397 mice were noted for nicotine-stimulated synaptosomal striatal dopamine (DA) release, while DA release was decreased significantly in DNE N397 mice compared to all other groups. No differences were noted between D397 and N397 vehicle alpha5*-nAChR binding sites measured by [125I]-epibatidine binding and DNE had no effect on these sites in either variant. These data suggest that decreased DA function in the striatum may lead to increased nicotine seeking in N397 DNE mice. Recent studies have implicated the habenula in nicotine aversion. Functional assays measuring synaptosomal nAChR-mediated 86Rb efflux indicated decreased function in the habenula in N397 DNE mice, indicating a potential mechanism for reduced aversion and thus increased intake of nicotine in these mice. Binding studies in the habenula demonstrated no significant changes in nAChR binding populations; however, an interesting trend towards decreased alpha3 beta4-nAChR binding was evident in N397 DNE mice. Together these data indicate interplay of mechanisms that may contribute to increased nicotine intake and decreased aversion in N397 DNE mice. Further studies are warranted to investigate mechanisms underlying increased aversion in D397 DNE mice.

Disclosures: **H.C. O'Neill:** None. **C. Wageman:** None. **S.R. Grady:** None. **M.J. Marks:** None. **J.A. Stitzel:** None.

Poster

033. Adolescent Drug Exposure: Alcohol and Nicotine

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 33.15/B31

Topic: A.10. Adolescent Development

Support: ADD P50 AA017823

Title: Age differences in conditioned place preferences and taste aversions to nicotine

Authors: *C. DANNENHOFFER, L. P. SPEAR;
Psychology, Binghamton Univ., Binghamton, NY

Abstract: Nicotine is an addictive substance that produces both rewarding and aversive effects within an individual simultaneously. These opposing effects can be examined in animal models using conditioned taste aversion (CTA) and conditioned place preference (CPP) paradigms. Given that adolescents and adults have been reported to respond differently to rewarding and aversive effects of drugs and other stimuli in humans and laboratory animals, the current experiment investigated age differences in nicotine sensitivity using a combined CTA and CPP procedure. Adolescent (P25-35) and adult (P70-80) male Sprague-Dawley rats were pair-housed in their home cage with ad lib food and water throughout the experiment. Every other day for 8 days, animals were first given 30 min access to supersac (3% sucrose, 0.125% saccharin in water) in the home cage, followed by injection with nicotine (0.0, 0.2, 0.4, and 0.8mg/kg; s.c.), and placement on one side of the CPP chamber for 20 min post-injection; on alternative days, water access was followed by saline injection and placement on the alternative side of the CPP chamber. On CPP test day, animals were allowed access to both sides of the apparatus and place preference was determined. CTAs were defined as attenuated supersac intake relative to saline controls over training/test days. Under these training/test conditions, adolescents displayed stronger CPP while tending to show weaker CTA than adults. To determine whether use of the combined CTA/CPP procedure contributed to the relatively modest age-associated attenuation in CTA seen in adolescents in this study relative to prior reports, a CTA alone group was added at each age, with animals remaining in the home cage post-injection. Analysis of data from this CTA-only condition revealed no age effects. The lack of notable age differences in nicotine CTA seen in these studies contrasts with prior studies showing attenuated nicotine CTA in adolescents relative to adults. Differences may be related to the low stressfulness of the CTA conditioning procedures used here: i.e., conditioning of pair-housed, ad libitum food/water animals in the home cage. While confirming the enhanced sensitivity of adolescents to the rewarding properties of nicotine, these data suggest that observed age differences in the aversive effects of nicotine may be dependent on testing conditions, perhaps including the relative stressfulness (i.e., deprived vs. non-deprived; home cage vs. novel context) of the training/test protocol. [ADD
Support: Supported by P50 AA017823.

Disclosures: **C. Dannenhoffer:** None. **L.P. Spear:** None.

Poster

033. Adolescent Drug Exposure: Alcohol and Nicotine

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 33.16/B32

Topic: A.10. Adolescent Development

Title: Neuronal injury associated with prenatal exposure of wistar rats to crude extract of vernonia amygdalina (bitter leaf)

Authors: *W. G. BALOGUN[1], A. AMIN[2], A. E. COBHAM[1], A. O. ISHOLA[1], W. I. ABDULMAJEED[2], O. B. AKINOLA[1];
[1]anatomy, Univ. of Ilorin, Ilorin Kwara State, Nigeria; [2]Physiol., Univ. of Ilorin, Ilorin, Nigeria

Abstract: Background: Bitter leaf is widely consumed by pregnant women in Africa for the treatment of many diseases during the various phases of pregnancy. But whether this treatment is deleterious to developing prefrontal cortex requires clarification. AIM: This study investigated some histological and histochemical effects of prenatal exposure of aqueous bitter leaf extract on the developing prefrontal cortex. METHODS: Twenty-five pregnant Wistar rats with an average weight of 200g were randomly divided into five groups (n=5). The experimental groups were administered bitter leaf (400mg/kg) on the gestational days 1-7 (group B), 8-14 (group C), 15-21 (group D) and 1-21 (group E) while the control (group A) was given normal saline from gestational days 1-21. After parturition, the litters in each group were weighed and sacrificed by euthanized on postnatal day 35. The brain was weighed and the prefrontal cortices were excised, fixed in formol calcium and processed. Tissue sections were stained with: Feulgen reaction for DNA substances and Cresyl Fast Violet for Nissl substance. while the homogenate was used to assayed the level of GDP, LDH and ALP. RESULTS: Using CFV, there was partial loss of Nissl substances in the litters exposed to bitter leaf on E8-E14 and E1-21 while there was more DNA loss in the litters exposed to bitter leaf on E8-E14 and E1-21. The GDP, ALP and LDH levels in the litters exposed to bitter leaf on E8-E14 and E1-21 was statistically significant from the control group. CONCLUSION: The above findings suggest that prenatal exposure of young Wistar rats to oral bitter leaf at 400 mg/kg is associated with neuronal loss in the prefrontal cortex. Keyword: bitter leaf, histological studies, biochemical studies

Disclosures: **W.G. Balogun:** None. **A. Amin:** None. **A.E. Cobham:** None. **A.O. Ishola:** None. **W.I. Abdulmajeed:** None. **O.B. Akinola:** None.

Poster

033. Adolescent Drug Exposure: Alcohol and Nicotine

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 33.17/B33

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: NIH Grant DA034912

Title: Timing deficits are improved by nicotine pre-treatment in the neonatal quinpirole model of schizophrenia

Authors: E. M. ODINEAL[1], M. I. PALMATIER[1], E. D. CUMMINS[1], A. B. SHEPPARD[1], *R. W. BROWN[2];
[1]Psychology, [2]East TN State Univ., JOHNSON CITY, TN

Abstract: Schizophrenia is a developmental disorder characterized by alterations in dopamine (DA) and glutamate neurotransmitter systems in the forebrain. Among the myriad symptoms of schizophrenia, deficits in time perception may be especially dependent on hyper-sensitivity of DA D2-like receptors. Our laboratory has developed a model of schizophrenia in which neonatal quinpirole (QUIN) treatment causes long-term increases in the sensitivity of dopamine D2-like receptors without a change in receptor number. The present study investigated the effects of neonatal QUIN treatment on timing using a peak-interval procedure in rats. From postnatal days (P)1-21 male rats received daily intraperitoneal injections of QUIN (1mg/kg) or saline (SAL). After weaning the rats were socially housed and raised to adulthood (P60). All rats were then shifted to single-housing, feed restricted, and shaped to press a nose-key for a sucrose reward. After initial shaping sessions, a peak-interval procedure was used in which most rewards were delivered approximately 60 s apart (fixed-interval or FI 60s, 28 trials each session). To measure the distribution of responses around the expected time of reward delivery, two peak trials were also included - these trials lasted 180s and no reinforcer was delivered. We hypothesized that anticipation of the reward would result in maximal response-rates approximately 60s into peak-interval trials. However, potent disruptions in timing were observed for rats that were exposed to neonatal QUIN - response rates increased and decreased sporadically with no clear peak during test-trials. After 6 initial acquisition tests, subcutaneous nicotine injections (0.4mg/kg) were administered 15 minutes prior to testing. Nicotine pretreatment resulted in a gradual increase in response rates and the development of a peak interval that was centered between 55 and 70 s. However, when nicotine was subsequently replaced with placebo, response-rates gradually

declined and returned to a temporally disorganized pattern. These findings indicate that hypersensitivity in DA D2-like receptors interferes with performance on the peak-interval task and that pre-treatment with nicotine improves performance and timing. These findings also suggest that nicotine alleviates cognitive or affective deficits that interfere with performance of reward-based cognitive tasks in schizophrenia.

Disclosures: **E.M. Odineal:** None. **R.W. Brown:** None. **M.I. Palmatier:** None. **E.D. Cummins:** None. **A.B. Sheppard:** None.

Poster

033. Adolescent Drug Exposure: Alcohol and Nicotine

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 33.18/B34

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: NIH Grant 1 R15 DA034912-01A1

Title: Neonatal quinpirole treatment enhances nicotine self-administration in adult rats

Authors: *E. CUMMINS, E. M. ODINEAL, A. B. SHEPPARD, M. I. PALMATIER, R. W. BROWN;
Psychology, East Tennessee State Univ., Johnson City, TN

Abstract: Individuals with schizophrenia are 3 to 4-fold more likely to smoke cigarettes than the general population, but no mechanism for this comorbidity has been delineated. One potential mechanism for increased smoking may be alterations in dopamine (DA) function - especially increased sensitivity of DA D2-like receptors. In our pre-clinical model of schizophrenia, neonatal quinpirole (QUIN) treatment results in long-term increases in the sensitivity of dopamine D2-like receptors and also increases behavioral and neurochemical responses to nicotine (NIC) in adulthood. The present study investigated whether neonatal QUIN treatment increased NIC self-administration in adult rats. Male rats were given single intraperitoneal injections of QUIN (1mg/kg) or saline (SAL) from postnatal days (P)1-21. Rats were then socially housed and raised to adulthood (P60). All rats were then shifted to single-housing, feed restricted and shaped to lever-press for a sucrose reward before being instrumented for intravenous (IV) NIC self-administration. Meeting the schedule of reinforcement on the active lever resulted in delivery of NIC (60 ug/kg/infusion, base) and a ‘time-out’ cue (30 s extinction of the houselight) - no additional reinforcers were available during the time-out. Responses on a

second inactive lever were recorded but had no consequence. The schedule of reinforcement during 60-min sessions was increased from an FR1 to FR5. During acquisition, no differences between neonatal QUIN and SAL rats were observed. Once stable responding on the FR5 schedule was observed, all rats were shifted to a progressive ratio (PR) reinforcement schedule in which each reinforcer earned increased the number of responses required to earn subsequent reinforcers. Breaking point (BP) was defined as the final ratio completed before 60 min elapsed with no infusions earned. This breaking point indicates the point at which the motivation to obtain NIC is outweighed by the amount of effort required to earn the next infusion. Under the PR schedule QUIN rats showed increased motivation to self-administer NIC compared to SAL controls across all NIC doses tested (7.5-60 ug/kg/infusion), no differences in responding for placebo (0 ug/kg/infusion) were observed. The findings confirm that developmentally-induced increases in DA D2-like receptor sensitivity enhances the motivation to self-administer NIC in adulthood and provides further validation of the neonatal QUIN model of schizophrenia. These findings also suggest that enhanced motivational effects of NIC may explain the robust comorbidity between schizophrenia and smoking.

Disclosures: **E. Cummins:** None. **E.M. Odineal:** None. **A.B. Sheppard:** None. **M.I. Palmatier:** None. **R.W. Brown:** None.

Poster

033. Adolescent Drug Exposure: Alcohol and Nicotine

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 33.19/B35

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: NIH 1 R15 DA034912-01A1

Title: The role of the α7 and α4β2 nicotinic receptors in nicotine sensitization and neural plasticity of adolescent rats neonatally treated with quinpirole

Authors: *D. PETERSON, E. D. CUMMINS, C. M. BARDO, R. W. BROWN;
East Tennessee State Univ., Johnson City, TN

Abstract: Substance abuse comorbidity is a common problem in schizophrenia and other psychotic disorders. In addition, smoking typically begins in adolescence, a period of development in which substance abuse commonly initiates. Nicotine has been shown to increase dopaminergic activity, which mediates positive reinforcement and may reduce some of the

negative symptoms of schizophrenia as well as cognitive impairments associated with schizophrenia. Dopaminergic activity also mediates behavioral sensitization to psychostimulants, including nicotine. Previous research from our lab has shown that rats neonatally treated with quinpirole, a dopamine D_2/D_3 agonist, from postnatal days (P) 1-11, 1-21, or 21-35 produces an increase in the sensitivity of the D_2 receptor throughout the animal's lifetime. This increase in D_2 sensitivity is a hallmark characteristic of schizophrenia. The purpose of the current study was to examine the roles of the α7 and α4β2 nicotinic acetylcholine receptors (nAChRs) in nicotine sensitization in adolescent rats neonatally treated with quinpirole. Adolescent rats (tested from postnatal days 33-49 every second day) were neonatally treated with either quinpirole (1 mg/kg) or saline from postnatal day (P)1-21. Results revealed that neonatal quinpirole enhanced sensitization to nicotine as compared to animals neonatally treated with saline and administered nicotine, and that the α4β2 antagonist dihyro-beta erythrodine (DhβEl 1 and 2.5 mg/kg) was more effective at blocking nicotine sensitization than methyllycaconitine (MLA; 2 and 4 mg/kg), regardless of neonatal drug treatment. In fact, rats that received DhβE demonstrated activity levels below that of controls treated with saline. Interestingly, DhβE was more effective in blockade of increases in activity produced by nicotine in males as compared to females on the final day of sensitization. In contrast, MLA failed to block sensitization to nicotine in both males and females, regardless of neonatal treatment condition. Interestingly, MLA (2 and 4 mg/kg) effectively blocked the acute hypoactive response to nicotine in males, and the higher dose of MLA (4 mg/kg) reduced sensitization more effectively in males, although this was not blocked to control levels. Brain tissue from the nucleus accumbens (NAc), the dorsal striatum, and hippocampus will be analyzed for both brain-derived neurotrophic factor (BDNF) and the mammalian target of rapamycin (mTOR), a protein signal downstream of BDNF. These results indicate that the α4β2 receptor, a common target for smoking cessation treatments plays a critical role in nicotine sensitization in the neonatal quinpirole model of schizophrenia.

Disclosures: **D. Peterson:** None. **E.D. Cummins:** None. **C.M. Bardo:** None. **R.W. Brown:** None.

Poster

034. Dopamine and Dopamine Receptor Function

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 34.01/B36

Topic: B.03. G-Protein Linked Receptors

Support: NIDA IRP funds

Title: Low constraint-associated D4 receptor variant does not elicit differential G-protein activation

Authors: *M. SANCHEZ[1,2], N.-S. CAI[1], S. FERRE[1], H. YANO[1];
[1]NIDA/NIH, BALTIMORE, MD; [2]Univ. de Barcelona, Barcelona, Spain

Abstract: Dopamine D4 receptors (D4R) are expressed in the prefrontal cortex and in the basal ganglia. They play a fundamental role in the modulation of cortico-basal ganglia circuits involved in intentional volitional control, which determines the personality trait constraint. The gene encoding the human D4R shows an interesting polymorphism that consists of a variable number of tandem repeat of 2 to 11 repeats of a 48-base pair unit in exon 3, which codes for the third intracellular loop of the receptor. The D4.7R variant (with 7 repeats) is associated with low constraint, which constitutes an endophenotype of ADHD, OCD and substance use disorders. D4R is coupled to Gi proteins and its activation leads to inhibition of adenylyl cyclase. It has been suggested that activation of the D4.7R produces a less efficient adenylyl cyclase signaling compared to the other D4R. However, since the second messenger readouts may vary depending on the cellular environments, these reports may have overlooked the expression differences of receptors and effector species such as G protein subtypes. Thus, we turned to a biophysical approach in order to study D4R-mediated specific and quantifiable effector activation for all G protein species expressed in the cortex and striatum. We set up a bioluminescence resonance energy transfer (BRET) assay that detects a conformational change between the α and the γ subunit of the G protein. Upon receptor activation, the G protein binds to the receptor and changes its conformation in such a way that a light donor fused to the α subunit and an acceptor fused to the γ subunit move away resulting in a decreased BRET signal. Using this technique, we have studied the activation of five Giα isoforms by the three main receptor variants found in humans (D4.4R, D4.7R, D4.2R, in order of global allelic frequency) in response to its endogenous ligands and selective agonists and antagonists. On the contrary to reported studies, our results indicate that three D4R variants couple to all tested Gi protein subtypes to virtually the same extent. Therefore, using this controlled expression method, we have demonstrated for the first time that D4.7R variant does not present differences in G-protein coupling or activation. We are also implementing a BRET-based arrestin recruitment assay to look for differential effects for the different variants. The lack of differences in the signaling properties of the D4R variants when expressed alone will support a main role of the reported differential biochemical properties of heteromers of D4R variants with D2R (or other receptors) in the differential modulation of cortico-basal ganglia circuits and the personality trait constraint.

Disclosures: **M. Sanchez:** None. **N. Cai:** None. **S. Ferre:** None. **H. Yano:** None.

Poster

034. Dopamine and Dopamine Receptor Function

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 34.02/B37

Topic: B.03. G-Protein Linked Receptors

Support: NIH Grant RR024158

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NIH Grant DA026434

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Lieber Center for Schizophrenia Research

NIH NIDA IRP program

Title: Development of novel Gs / Golf biosensors: Gs-Golf functional selectivity in dopamine D1 receptors

Authors: *H. YANO[1], D. PROVASI[2], M. FILIZOLA[2], A. BONCI[1], S. FERRE[1], J. A. JAVITCH[3,4];
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Abstract: Numerous neurotransmitters and neuromodulators mediate their effects directly through G protein-coupled receptors (GPCRs). Therefore, understanding the structure-function relationship of GPCRs is key to better development of neuropharmacological agents. Conformational changes associated with GPCR activation have been revealed in remarkable details by the crystal structure of agonist-bound beta2 adrenergic receptor coupled to Gs. However, the extent to which conformational changes are conserved in other G protein species and how different agonists may lead to different conformations remain to be determined. We are working to develop assays that can answer these questions. The dopamine D1 receptor can couple to both Gs and Golf (89% identical to Gs). As the expression patterns of Gs and Golf greatly differ in the brain and Golf is enriched in the striatum, the discovery of Golf-selective ligands would provide a novel tool for exploring the relevance of the various signaling pathways and thereby help to advance therapeutics for neuropsychiatric disorders. We first investigated conformational changes in the Gs heterotrimer after activation of D1R. Using a library of novel Gs biosensors with either luciferase or GFP inserted at various positions throughout the structure, we studied conformational changes by bioluminescence resonance energy transfer (BRET) and

compared the results to the crystal structures of the inactive and active conformations. We also studied conformational changes in the Gs protein induced by other receptors. Finally, taking advantage of the significant homology, we constructed Golf biosensor constructs and subsequently validated them in BRET-based assays of receptor activation. Our studies using these biosensors show for the first time that conformational changes within the Gs heterotrimer are very similar for different Gs-coupling receptors. Also comparison between the Gs and Golf sensor readouts suggests that activation of Gs and Golf lead to similar conformational changes. Using this set of biosensors, efficacy of ligands and activation preference between Gs and Golf can be addressed. This enables the screening of functionally selective D1 ligands that differentially target these related G proteins.

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Poster

034. Dopamine and Dopamine Receptor Function

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 34.03/B38

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: CIHR

Parkinson Society Canada

Brain Canada

Krembil Foundation

Title: Dopamine- and glutamate-containing axon terminals are segregated in distinct domains in dopamine neurons and regulated by striatal neurons

Authors: G. M. FORTIN[1], M.-J. BOURQUE[1], Z. SANEEI[1], C. PACELLI[1], R. K. VARASCHIN[1], N. GIGUERE[1], M. BRILL[1], S. SINGH[3], P. W. WISEMAN[3], *L.-E. TRUDEAU[2];
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Abstract: A subset of midbrain DA neurons has been shown to express the type 2 vesicular glutamate transporter (VGLUT2), supporting their capacity for glutamate release from some of their axon terminals. Glutamate release from DA neurons is found mainly by neurons of the VTA and can be detected at terminals contacting ventral but not dorsal striatal neurons, suggesting the possibility that target-derived signals regulate the neurotransmitter phenotype of DA neurons. Whether glutamate can be released from the same terminals that release DA or from a segregated subset of axon terminals is also undetermined. Here we examined the localization of VGLUT2 both *in vitro*, in a primary co-culture system with ventral or dorsal striatal neurons, and *in vivo* in mouse striatal brain sections obtained from either conditional VGLUT2 knockout (cKO) mice or DAT-Cre mice expressing a floxed fluorescent reporter protein selectively in DA neurons. We find that while VGLUT2 is colocalized with tyrosine hydroxylase (TH) in a subset of terminals when mesencephalic DA neurons are cultured alone, VGLUT2 is mostly segregated from TH when DA neurons are cultured with ventral, but not striatal neurons. In striatal brain sections, we also confirm that VGLUT2 is found in axonal varicosities emanating from DA neurons but expressing very little if any TH. More advanced image analysis using the 2-color SpIDA technique also confirms low or random colocalization of TH and VGLUT2 signal *in vivo*. Our work unveils a fundamental feature of dopamine-glutamate cotransmission and suggests that striatal neurons can regulate the neurotransmitter phenotype of DA neurons.

Disclosures: **G.M. Fortin:** None. **M. Bourque:** None. **Z. Saneei:** None. **R.K. Varaschin:** None. **N. Giguere:** None. **M. Brill:** None. **S. Singh:** None. **P.W. Wiseman:** None. **L. Trudeau:** None. **C. Pacelli:** None.

Poster

034. Dopamine and Dopamine Receptor Function

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Topic: B.01. Neurotransmitters and Signaling Molecules

Support: NIH-1P20GM103653-01A1-NIGMS

Title: Neuro-modulatory role of the D2-like dopamine auto-receptor in *C. elegans*

Authors: ***S. MANDALAPU**, R. FORMISANO, M. MERSHA, P. HAN, H. S. DHILLON; Dept of Biol. Sci., Delaware State Univ., Dover, DE

Abstract: Dopamine influences a wide range of neural processes and is implicated in several neurological disorders. Degeneration of dopaminergic neurons in the midbrain of Parkinson's disease patients leads to decreased dopamine levels that may involve D2-like auto-receptors. In *C. elegans,* the D2-like dopamine receptor DOP-2 has been proposed to be the dopamine auto-receptor that modulates dopamine release during the process of associative learning. Our goal is to understand the auto-receptor functional of DOP-2 in modulating dopamine levels in the synaptic cleft. Work in our lab has shown that DOP-2 physically interacts with GPA-14 an inhibitory G-alpha subunit (Gαi), and that both *dop-2* and *gpa-14* deletion mutants habituate at a significantly faster rate as compared to wild-type. Furthermore, *gpa-14* deletions also show associative learning deficits similar to those reported for *dop-2* previously. In addition to dopamine, our results indicate that a TRP cation channel may be modulating DOP-2 auto-receptor function. Preliminary results for the *dop-2* and *trp-4* mutants show faster habituation and in the *trp-4;dop-2* double mutants the habituation is the same as the single mutants. We thus hypothesize that *dop-2* and *trp-4* act in the same pathway to modulate neurotransmitter release. Besides the genetic and behavioral approach outlined above, we are also using fluorescence recovery after photobleaching (FRAP) to test our hypothesis. Results from our experiments will provide insight into the molecular mechanisms involved in the modulation of the dopamine neurotransmitter release.

Disclosures: S. Mandalapu: None. **R. Formisano:** None. **M. Mersha:** None. **P. Han:** None. **H.S. Dhillon:** None.

Poster

034. Dopamine and Dopamine Receptor Function

Location: Halls A-C

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Program#/Poster#: 34.05/B40

Topic: B.01. Neurotransmitters and Signaling Molecules

Title: Coordination and remodeling of cortico-limbic network dynamics by dopaminergic neuronal activity

Authors: *H. K. DECOT[1], W. GAO[2], P. A. KANTAK[3], Y.-C. J. KAO[4], M. DAS[4], J. H. JENNINGS[1], I. B. WITTEN[5], K. DEISSEROTH[6], Y.-Y. I. SHIH[4], G. D. STUBER[3]; [1]Neurobio., [2]Radiology, [3]Psychiatry, [4]Neurol., Univ. of North Carolina At Chapel Hill, Chapel Hill, NC; [5]Psychology, Princeton Univ., Princeton, NJ; [6]Bioengineering, Stanford Univ., Stanford, CA

Abstract: Ventral Tegmental Area (VTA) dopaminergic neurons encode reward prediction errors and signal the incentive salience of sensory cues. Burst firing of these neurons result in phasic dopamine release in cortical and limbic terminal fields such as the medial prefrontal cortex (mPFC) and nucleus accumbens (NAc), which acts to modulate postsynaptic neuronal firing to promote changes in motivated behavioral output. However, it remains unclear how VTA dopaminergic activity affects large-scale brain network functional connectivity. This project aims to investigate whether the selective activation of DA neurons within the midbrain alters the functional connectivity between these neurons and their postsynaptic target regions, as well as global patterns of brain connectivity to provide clinically relevant insight into the effects of dopaminergic dysregulation on neural circuit mechanisms in the intact brain. To target DA neurons within this region, tyrosine hydroxylase (TH)-Cre adult Long Evans rats were microinjected into the ventral midbrain with a Cre-inducible adeno-associated virus carrying the gene encoding channelrhodopsin-2 (ChR2), a light-gated cation channel fused to an enhanced yellow fluorescent protein (EYFP) (THVTA::ChR2 rats) or only EYFP (THVTA::control rats). Chronic optical fibers were stereotactically implanted bilaterally above the VTA to permit light delivery to selectively activate DA neurons within this region. Single shot, single sampled GE-EPI sequences were acquired using a Bruker 9.4T MR scanner and custom surface coil. Data were averaged across subjects in order to provide group-averaged fMRI activation maps. Selective optogenetic activation of DA neurons within the midbrain not only caused significant regional CBV increases in downstream targets of the VTA including the dorsal striatum and nucleus accumbens in THVTA::ChR2 rats ($p<0.05$ corrected for multiple comparisons) but also coordinated dynamic shifts in global patterns of functional connectivity. Intravenous application of the D1 receptor antagonist, SCH23390 significantly attenuated the optical stimulation mediated CBV responses within these forebrain targets. In addition, pairing VTA dopamine neuronal activity with somatosensory stimuli dramatically enhanced the neuronal representation of the sensory stimulus and also remodeled functional connectivity. These data suggest that aberrant DA neuromodulation may alter the neuronal activation patterns seen within cortico-limbic networks, providing mechanistic insight into how DA signaling alters global patterns of brain connectivity.

Disclosures: **H.K. Decot:** None. **W. Gao:** None. **P.A. Kantak:** None. **Y.J. Kao:** None. **M. Das:** None. **J.H. Jennings:** None. **I.B. Witten:** None. **K. Deisseroth:** None. **Y.I. Shih:** None. **G.D. Stuber:** None.

Poster

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Topic: B.01. Neurotransmitters and Signaling Molecules

Support: NS083410

Title: Dopamine, MMP activity, and NMDA stimulated calcium signal in striatal neurons

Authors: Y. LI[1], J. G. PARTRIDGE[2], S. VICINI[2], *K. CONANT[3];
[1]Neurosci. (Georgetown University), Georgetown Univ. and State Key Lab. of Biotherapy, West China Hospital, Sichuan Univ., Washington (U.S.A.) and Chengdu (China), DC; [2]Pharmacol., [3]Neurosci., Georgetown Univ., Washington, DC

Abstract: Dopamine is a neuromodulator known to influence glutamatergic transmission in striatal spiny neurons. While dopamine signaling may activate varied effectors of excitatory transmission, interactions with dopamine D1 receptor (D1R) bearing neurons in particular may stimulate PKA dependent phosphorylation of glutamate receptor subunits. Herein we focus on the question of whether D1 receptor activation stimulates superimposed changes in matrix metalloproteinase (MMP) activity to further influence glutamatergic transmission in the striatum. MMP levels are increased in the background of excess dopaminergic activity and these proteases are increasingly recognized as important effectors of glutamatergic transmission. We observe that dopamine and a D1R agonist, SKF81297, increase MMP activity in extracts from striatal slices as determined by cleavage of the substrate β-dystroglycan. Using transgenic mice engineered to express the calcium indicator protein GCaMP3 in D1R or D2R cell populations, we also observe that SKF81297 pretreatment of slices (10 μM, 40 min) can potentiate NMDA stimulated intracellular calcium increases in both cell types. The change in D2R cells is of particular interest in that it is consistent with the possibility that one or more soluble factors may be released from D1R bearing cells to in turn influence NMDA receptor dependent changes in neighboring cells. Present studies are underway to determine the extent to which specific MMPs contribute to D1R enhancement of NMDA function in subpopulations of striatal neurons.

Disclosures: **Y. Li:** None. **K. Conant:** None. **J.G. Partridge:** None. **S. Vicini:** None.

Poster

034. Dopamine and Dopamine Receptor Function

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Topic: B.01. Neurotransmitters and Signaling Molecules

Support: NIH Grant NS086107

Title: Heterogeneous evoked dopamine plasticity in the rat striatum

Authors: S. H. WALTERS, *A. C. MICHAEL;
Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Fast scan cyclic voltammetry (FSCV) enables real-time quantitative measurements of subsecond concentration changes of several neurotransmitters in living brains. Our laboratory has previously published (Moquin 2009) unique, domain-correlated, differential evoked dopamine response plasticity at the timescale of seconds. This observation was obtained at microcylinder electrodes, which spatially average the response from a large number of dopamine terminals. We hypothesized that additional forms of plasticity might be observed at microdisk electrodes, which sample the response from a smaller number of terminals, and are less prone to "average out" physiological variability. Measurements within and across animals in the *in vivo* anesthesized rat striatum revealed the existence of multiple discrete response patterns to consistent stimulus conditions, which we confirmed to be a feature of the recording site rather than an instrumental artifact. This confirms our hypothesis that the differential response stability is a form of localized differences in dopamine response plasticity. Combined mathematical modeling and experimental efforts to elucidate the mechanism or mechanisms of the observed dopamine response plasticity are ongoing.

Disclosures: **S.H. Walters:** None. **A.C. Michael:** None.

Poster

034. Dopamine and Dopamine Receptor Function

Location: Halls A-C

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Program#/Poster#: 34.08/B43

Topic: B.03. G-Protein Linked Receptors

Support: NIDA IRP Funds

Title: Applying label-free dynamic mass redistribution technology to analyze ligand efficacy on dopamine D1 receptor signaling in living cells

Authors: *N. CAI, H. YANO, M. SANCHEZ-SOTO, X. GUITART, A. H. NEWMAN, S. FERRE;
NIDA/NIH, BALTIMORE, MD

Abstract: Both the canonical dopamine D_1 receptor (D_1R) agonist SKF-38393 and the canonical D_1^R antagonist SCH 23390 are benzazepine derivatives that have been widely used as pharmacological tools and radioligands. Intriguingly, both have been reported to be partial agonists on adenylyl cyclase signaling. We first addressed the validity and degree of the partial agonism of SCH-23390 by using label-free dynamic mass redistribution technology in transfected HEK-293 cells, which measures G protein-mediated signaling. As expected, agonist-like responses were obtained with dopamine and, with lower efficacy, with SKF-38393 in cells stably transfected with D_1R, but not in non-transfected cells. Similarly, SCH-23390 showed an agonist-like concentration-response in D_1R-transfected but not in non-transfected cells, albeit with lower efficacy than SKF-38393 in D_1R-transfected cells. In agreement with previous studies, and only in D_1R-transfected cells, the non-selective dopamine receptor antagonists flupenthixol and (+)-butaclamol showed an inverse-agonist-like response and they antagonized the effects of dopamine, SKF-38393 and SCH-23390. As SKF 38393 and SCH 23390 have been widely reported to have opposing effects on locomotor activity in the experimental animal, these data suggest a role of G protein-independent signaling in the locomotor effects of D_1R ligands. To expand on these findings, we analyzed the efficacies of a series of previously synthesized benzazepine derivatives and discovered varying partial agonist efficacies in D1R-transfected cells that were also counteracted by flupenthixol and (+)-butaclamol. Behavioral evaluation of these compounds is in progress to determine if G protein-independent signaling plays a significant role on D_1R-mediated locomotor activation.

Disclosures: **N. Cai:** None. **H. Yano:** None. **M. Sanchez-Soto:** None. **X. Guitart:** None. **A.H. Newman:** None. **S. Ferre:** None.

Poster

034. Dopamine and Dopamine Receptor Function

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Topic: B.01. Neurotransmitters and Signaling Molecules

Support: Parkinson's UK Grant H-1003

Medical research council

Title: The roles of voltage-gated calcium channels (VGCCs) in the control of striatal dopamine release are variable and dynamically regulated

Authors: *K. BRIMBLECOMBE[1], C. GRACIE[2], S. J. CRAGG[2];
[1]DPAG, [2]Dept. of Physiol. Anat. and Genet., Univ. of Oxford, Oxford, United Kingdom

Abstract: Voltage-gated calcium channels (VGCCs) operate differently in dopamine (DA) neurons of Substantia nigra pars compacta (SNc) compared to ventral tegmental area (VTA), neurons which are respectively sensitive and resistant to degeneration in Parkinson's disease. There is still much to understand about the VGCCs on the vast striatal axonal arbors of DA neurons that control striatal DA release. Previous studies will have been confounded by the VCGGs that operate on cholinergic interneurons and regulate ACh release, which in turn modulates and drive DA release. We have investigated which VGCC subtypes on DA axons operate in dorsal and ventral striatum to control DA release. We used fast-scan cyclic voltammetry (FCV) at carbon-fibre microelectrodes in mouse acute striatal slices to determine the roles of VGCCs subtypes in striatal DA release using specific blockers of N, P/Q, L, and T-type channels (ω-Conotoxin GVIA, 100 nM; ω-Agatoxin IVA, 200 nM; Isradipine, 5 μM; and NNC 55-0396, 1 μM respectively), in the presence of the nicotinic receptor antagonist DHßE (1 μM) to remove the confounding effects of ACh on DA terminals. We determined that at 2.4 mM extracellular Ca2+, N>P/Q>T and L-type channels control DA release in dorsal striatum whereas only N>P/Q-type channels contribute in ventral striatum. However, "silent" L- and T-type channels in ventral striatum had a role unmasked by increasing extracellular Ca2+. We also reveal that DA release in response to tonic versus phasic patterns of activity is a general function of calcium availability and not in fact dependent on specific VGCC subtypes operating at specific frequencies. Furthermore, data suggest that auxiliary α2δ subunits also modulate the VGCCs that control DA release . In summary the VGCCs that control striatal release are of many types, and are dynamically involved according to striatal region, Ca2+ concentration, auxiliary subunits and stimulation frequency.

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Poster

034. Dopamine and Dopamine Receptor Function

Location: Halls A-C

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Topic: B.03. G-Protein Linked Receptors

Support: R37MH073853

U19MH082441

Title: Physiological relevance of dopamine 2 receptor functional selectivity assessed in mice

Authors: S. M. PETERSON[1], D. J. URBAN[1], N. M. URS[1], O. LICHTARGE[2], *M. G. CARON[3];
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Abstract: Schizophrenia symptoms are alleviated with antipsychotics, a class of drugs that preferentially target the dopamine 2 receptors (D2Rs). However atypical antipsychotics such as aripiprazole and clozapine have partial agonist activity and weak antagonistic activity when compared to typical antipsychotics such as haloperidol and display fewer extrapyramidal side effects, potentially due to their distinct D2R pharmacology. D2R belongs to the superfamily of G protein-coupled receptors, are highly expressed in the striatum in indirect pathway medium spiny neurons (MSNs), and mediate the neuronal function of dopamine. D2R signals through many diverse intracellular signal transduction molecules including the inhibitory class of G proteins and the multifunctional adaptor β-arrestin. D2R's inhibition of cAMP and neuronal excitability through G proteins have long been established, while there is evidence that β-arrestin can function as a scaffold of signaling kinases and phosphatases. In order to gain a better understanding of the function of D2R and facilitate manipulation of each signaling pathway, we used the Evolutionary Trace approach to generate functionally selective receptors. Two mutants, termed [Gprot]D2R and [βarr]D2R, capable of functioning through either the G protein or β-arrestin interaction pathways and were used to catalog cellular signaling *in vitro*. In addition, using adeno-associated viral constructs, these D2R mutants were overexpressed in the indirect MSNs of the striatum as well as re-expressed in these MSNs with endogenous D2R removed by Cre recombinase. Amphetamine-induced locomotor activity in mice, a model to assess the activity of antipsychotics, was used to assess the behavioral outcome of these manipulations. [βarr]D2R expressing mice had enhanced amphetamine induced hyperlocomotion when compared to [Gprot]D2R, wild-type D2R or a control inactive D2R protein. Thus functional selectivity of D2Rs may provide an approach to identify selective behavioral consequences and previously unappreciated molecular targets for the development of more efficacious therapies for dysfunction of the brain dopamine system.

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Poster

034. Dopamine and Dopamine Receptor Function

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Topic: B.01. Neurotransmitters and Signaling Molecules

Support: CNPq

Faperj

Proppi/UFF

Capes

Title: Dopamine regulates CREB nuclear activity via EPAC2 in retinal cells

Authors: *R. PAES-DE-CARVALHO, C. E. NOGUEIRA, R. SOCODATO;
Neurobio., Fluminense Federal Univ., Niteroi, Brazil

Abstract: Cell communication in the CNS occurs mainly at synapses where electrical signals in the pre-synaptic terminal are translated in the release of neurotransmitters and activation of post-synaptic receptors. Synapses undergo dramatic morphological and biochemical modifications and such synaptic plasticity occurs in many neurological functions like learning, memory and cognition. Dopamine is an important neurotransmitter in several CNS areas and previous works showed its ability in regulating neurite outgrowth and growth cone motility in retinal neurons. Dopamine regulates two classes of receptors classified as D1-like and D2-like receptors, which are respectively coupled to activation or inhibition of adenylyl cyclase and cAMP accumulation. A classical cAMP/PKA pathway promotes the phosphorylation of the transcription factor CREB (cyclic AMP responsive element binding protein), which is also modulated by calcium/calmodulin kinase and the ERKs pathway. The accumulation of cAMP also stimulates the guanine nucleotide exchange factor EPAC (exchange protein activated by cAMP), which regulates ERK activity through the small GTPase Rap1. Therefore, this pathway could potentially modulate CREB activation. The aim of the present work was to study the signaling pathways involved in CREB phosphorylation induced by dopamine in retinal cells. Retinal cells were obtained from 8-day-old chick embryos and cultured for 3 days. Cultures were washed with Hanks saline, incubated for different times with dopamine and then processed for western blot or fixed for immunocytochemistry and confocal image analysis. Dopamine stimulated CREB

phosphorylation at Ser133 in a concentration and time-dependent manner. Maximal stimulation was attained with 1 μM dopamine after 15 minutes of incubation. The same phosphorylation level was observed after 20 minutes but completely disappeared after 45 or 60 minutes of incubation. Confocal microscopy showed that dopamine increased pCREB in cell nuclei and that this effect was reduced by infection of cultures with lentiviruses carrying shRNAs specific for EPAC2. Interestingly, ERK2 phosphorylation also increased in cell nuclei after stimulation with dopamine and could be inhibited by knocking down EPAC. Moreover, CREB phosphorylation was completely inhibited when ERK2 was knocked down with specific shRNAs. In conclusion, our present data show that dopamine increases CREB phosphorylation in cultured retinal cells and that EPAC2 is part of a signaling pathway promoting activation and migration of ERK2 into the cell nucleus where it activates the transcription factor CREB.

Disclosures: **R. Paes-de-Carvalho:** None. **C.E. Nogueira:** None. **R. Socodato:** None.

Poster

034. Dopamine and Dopamine Receptor Function

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Topic: B.01. Neurotransmitters and Signaling Molecules

Support: NIH Grant GM071558-06A1

Title: Allosteric regulation of phosphodiesterase-2 controls dopamine-induced GluA1 membrane insertion

Authors: R. S. SONG, B. MASSENBURG, *S. R. NEVES;
Pharmacol., Mount Sinai Sch. of Med., New York, NY

Abstract: The strength and duration of synaptic transmission is largely determined by the number of post-synaptic AMPA receptors (AMPARs). Modulation of synaptic strength is achieved by changing AMPAR numbers in a process that requires receptor trafficking from intracellular vesicles to the plasma membrane. Trafficking of GluA1-containing AMPARs is regulated by PKA, a cAMP-dependent kinase. Phosphodiesterase-4 (PDE4), an enzyme that degrades cAMP, regulates PKA activity and controls dopamine-induced GluA1 membrane insertion. Here we asked whether additional PDEs expressed in medium spiny neurons also regulate GluA1 membrane insertion. Our approach combines live-cell imaging of cAMP and cGMP dynamics, along with GluA1 trafficking measurements and computational modeling of

signaling to explore the contribution of each PDE to GluA1 trafficking in striatal neurons. We found that inhibiting PDE1, a cAMP/cGMP PDE, resulted in a counterintuitive decrease in dopamine-induced cAMP levels and GluA1 membrane insertion. This decrease was due to the allosteric activation of PDE2 by cGMP, as simultaneous inhibition of both PDE1 and PDE2 abolished the decrease in GluA1 membrane insertion. Moreover, PDE1 regulation of GluA1 trafficking was limited to distal dendritic segments, suggesting selective spatial control. These results show that the interplay of multiple PDE activities can lead to unanticipated regulation of dopamine-induced GluA1 trafficking.

Disclosures: **R.S. Song:** None. **S.R. Neves:** None. **B. Massenburg:** None.

Poster

034. Dopamine and Dopamine Receptor Function

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Topic: B.01. Neurotransmitters and Signaling Molecules

Support: CIHR - Canadian Institutes of Health Research

CNPq - National Council of Scientific and Technological Development - Brazil

Title: Histamine H3 receptor modulation of dopamine release in mouse striatum

Authors: ***R. K. VARASCHIN**[1], L.-É. TRUDEAU[2];
[1]Pharmacol., [2]Pharmacol. and Neurosciences, GRSNC, Univ. de Montréal, Montréal, QC, Canada

Abstract: Histamine H3 receptors are widely distributed in the mammalian brain. Activation of these Gi-coupled receptors reduces neuronal activity and inhibits release not only of histamine itself, but also of acetylcholine, glutamate and serotonin. Although these receptors are abundantly expressed in the dorsal and ventral striatum, their modulatory role on activity-dependent dopamine (DA) release has not been directly evaluated. Here, we employed pharmacological tools and the fast-scan cyclic voltammetry technique to address the hypothesis that histamine H3 receptor activation diminishes DA release in the dorsal and ventral striatum. Acute brain slices from C57/BL6 mice were obtained using a protective slicing method. A stimulating bipolar electrode was placed either in the dorsal or ventral striatum and slices were stimulated every 2 min. Slices were then perfused with histamine (10 μM), the selective H3

agonist alpha-methyl-histamine (1 μM) or vehicle. In the dorsal striatum, neither histamine nor alpha-methyl-histamine had any significant effect on evoked DA overflow. In the ventral striatum however, alpha-methyl-histamine reduced the amplitude of DA overflow by about 50 %, whereas histamine only induced a modest, non-significant reduction. None of the drugs affected vesicle mobilization, as estimated by comparing responses to pairs of stimuli at 100 Hz. These results lead us to speculate that histamine is an important modulator of DA release in the ventral striatum. Interestingly, in this region, histamine H3 receptors may also be expressed post-synaptically by medium spiny neurons and cholinergic interneurons. Therefore, at least two converging mechanisms may account for histamine H3 modulation of DA release: one direct, implicating H3 receptor-mediated inhibition of DA release from dopaminergic axons; and another indirect, involving H3 receptor-mediated inhibition of cholinergic interneurons, otherwise known to positively modulate DA release. Experiments are currently being undertaken to evaluate these two possibilities. These findings may serve as proof-of-principle for the development of novel pharmacotherapeutic strategies to treat diseases where DA release in this region is abnormal, such as in drug addiction.

Disclosures: **R.K. Varaschin:** None. **L. Trudeau:** None.

Poster

034. Dopamine and Dopamine Receptor Function

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Topic: B.01. Neurotransmitters and Signaling Molecules

Support: AMRF grant 3703809

Title: The effect of flow rate on D2 receptor modulation of dopamine release measured in brain slices with fast-scan cyclic voltammetry

Authors: M. BURRELL, *J. LIPSKI;
Dept Physiol, Univ. Auckland, Auckland, New Zealand

Abstract: Fast-scan cyclic voltammetry (FSCV) has been widely used to measure stimulated release and uptake of dopamine (DA) and some other neurotransmitters in brain slices and *in vivo.* In contrast to constant potential amperometry (CPA), a technique in which calibration highly depends on flow rate, calibration performed in solutions with FSCV is relatively flow-independent. However, the flow dependence of the effects of stimulated DA release in brain

slices has not been systematically studied, in spite of the fact that many studies used ACSF flow rates as low as 1 ml/min, which may compromise oxygen delivery to the slice and affect DA overflow and activation of D2 autoreceptors. Our aim was to assess the effect of different ACSF flow rates through the recording chamber (volume, 1.2 ml) on the inhibitory action of D2 receptor activation during DA release evoked by bursts of electrical stimuli (2 s,10 Hz) delivered in dorsal striatum in submerged brain slices (300-350 μm; 32 ± 0.5°C) obtained from P21-28 Wistar rats. DA release was detected with FSCV using cylindrical carbon fibre microelectrodes (7 μm diameter) placed in the immediate vicinity of bipolar stimulating electrodes. At relatively high flow rate (3.5 ml/min), there was no significant effect of sulpiride (10 μM, n=4) or raclopride (1 μM, n=4) on the peak amplitude or the area under the curve (AUC) of stimulated DA release. However, the amplitude and the AUC were increased when flow rate was reduced to 1.0-1.75 ml/min (both $p<0.005$; combined sulpiride and raclopride effects). Reduction of flow rate from 3.5 to 1.0-1.75 ml/min did not increase the amplitude and AUC of burst stimulation-induced DA release in the absence of D2 receptor block. Our data show that flow rate should be taken into consideration when studying the effects of released DA using FSCV. Although high flow rates (>3 ml/min) are recommended for electrophysiological studies performed in brain slices to maintain network activity (eg. Hajos et al. 2009 Eur J Neurosci 29:319), they may reduce the inhibitory effect of D2 autoreceptor activation on DA release. This could be due to the reduced activation of these receptors by accelerated DA diffusion, and/or by hyperoxygenation and increased level of reactive oxygen species (eg. Matott et al. 2014 Neuroscience 270:98).

Disclosures: **M. Burrell:** None. **J. Lipski:** None.

Poster

034. Dopamine and Dopamine Receptor Function

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 34.15/B50

Topic: B.01. Neurotransmitters and Signaling Molecules

Title: Computational modeling suggests low striatal dopamine release probability

Authors: *K. ROONEY, L. J. WALLACE;
Pharmacol., The Ohio State Univ., Columbus, OH

Abstract: The hypothesis being evaluated is that dopaminergic receptors at varying locations near a dopamine release site might be exposed to different concentrations of dopamine. Such a study is important as dopamine in the striatum signals saliency of the current environmental

input and is involved in the learned formation of appropriate responses. One key component of background information to support the hypothesis is that the majority of dopamine signaling appears to involve a volume or neighborhood phenomenon rather than a synaptic paradigm. Given published densities of dopaminergic varicosities in the dorsal striatum, the volume associated with a release site is about 10 μm3. Potential dopamine targets in such a volume include D1 receptors on medium spiny neurons and D2 receptors on medium spiny neurons, cholinergic interneurons, and dopamine axon terminals. A computer model using the program MCell was developed to estimate extracellular dopamine and potential gradients in such a volume. The model contained a dopamine release site and a ring of 1800 dopamine transporters located at a distance of about 4 μm from the release site. Kinetic values for dopamine transporter were determined from studies simulating uptake experiments. The number of dopamine molecules in a single vesicle was calculated to be about 1500 based on the concentration of dopamine in rodent brain and an estimate of 250 vesicles per varicosity. Simulations of a firing rate of five per second with each event releasing the contents of a single vesicle resulted in an average extracellular concentration of 10 μM, much greater than the approximately 5 nM reported by no-net flux micro-dialysis. Less than 5 dopamine molecules would need to be released per firing event to create a 5 nM extracellular concentration, which is less than 0.3% of the number of dopamine molecules in a single vesicle. These data suggest that if no-net flux micro-dialysis is an accurate measure of extracellular dopamine, then dopamine signaling is characterized by probability of a vesicle release associated with each firing event less than 1% and/or a mechanism such as kiss and run releasing a very small fraction of vesicular contents. Additional modeling experiments simulated published data of dopamine transients elicited by electrical stimulation of striatal slices. The results suggest that a single supra-maximal electrical pulse can release 88% of the contents of one vesicle from each varicosity. This result also suggests that dopamine signaling involves a very small fraction of available dopamine.

Disclosures: **K. Rooney:** None. **L.J. Wallace:** None.

Poster

034. Dopamine and Dopamine Receptor Function

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

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Topic: B.01. Neurotransmitters and Signaling Molecules

Support: NIH Grant R21DA027358

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the Pediatric Psychopharmacology Philanthropic fund

Title: Hyperactivity and working memory deficits induced by prenatal nicotine exposure are associated with dopamine D1 and D4 receptor dysfunction

Authors: K. P. LEE[1], N. PINEDA[1], T. BRUNE[1], K. PATEL[1], A. GANNON[1], T. J. SPENCER[2], J. BIEDEMAN[2], P. G. BHIDE[1], *J. ZHU[1];
[1]Ctr. for Brain Repair, Biomed. Sci., Florida State Univ. Col. of Med., Tallahassee, FL; [2]Clin. and Res. Programs in Pediatric Psychopharmacology and Adult ADHD, Massachusetts Gen. Hospital, Harvard Med. Sch., Boston, MA

Abstract: Attention deficit/hyperactivity disorder (ADHD) affects about 5-10% of children worldwide. ADHD symptoms include inattention, hyperactivity, impulsivity, and deficits in executive function/working memory, with often a predominance of only a subset of the symptoms. Stimulant medications such as methylphenidate and amphetamine remain the most commonly prescribed drugs for ADHD treatment. Stimulants block dopamine reuptake, increase extracellular dopamine levels and thereby mitigate ADHD symptoms. However, stimulant medications are not equally effective against the full set of ADHD symptoms, nor is the dopamine receptor signaling mechanism(s) underlying the therapeutic effects of stimulant treatment fully understood. Here we report that hyperactivity and working memory deficits in a prenatal nicotine exposure (PNE) mouse model of ADHD are associated with impairment of specific dopamine receptor signaling mechanisms and that these symptoms can be treated by rectifying the specific impairments. We have reported previously that PNE is associated with locomotor hyperactivity and deficits in working memory/attention. Here we used a Y-maze test to assay separately working memory/attention performance (spontaneous alternation) and locomotor activity (number of arm entries). Selective dopamine D1 or D4 receptor agonists were administered 30 minutes prior to the Y-maze assay in PNE and control mice. The selective D1 receptor agonist, A-77636 (0.1 or 1μg/kg, i.p.) reduced the total number of arm entries dose-dependently (Mean±SEM, 0, 0.1 vs. 1μg/kg: 56±4, 45±3 vs. 40±2, one-way ANOVA, $F_{(2,20)}$=19.68, P<0.001), but had no significant effect on spontaneous alternation (55±2, 55±3 and 56±3, $F_{(2,20)}$=-24.54, P>0.05). Administration of the selective D4 agonist, PD168,077 (1, 10, 20mg/kg, i.p.), however, improved spontaneous alternation dose-dependently, (0, 10, 20 mg/kg: 67±3, 62±4, and 48±3, $F_{(2,31)}$=9.13, P<0.001), but did not have significant effects on the total number of arm entries (51±5, 54±4, and 56±5, $F_{(2,31)}$=1.23, P>0.05). Thus, hyperactivity and working memory deficits appear to be dissociable at the level of individual dopamine receptor function in the PNE mouse model of ADHD. Our data provide novel insights into the molecular mechanisms associated with individual components of the ADHD symptomatology, and underscore the potential for developing personalized treatments that target specific symptom domains.

Disclosures: **K.P. Lee:** None. **J. Zhu:** None. **N. Pineda:** None. **T. Brune:** None. **K. Patel:** None. **A. Gannon:** None. **T.J. Spencer:** None. **J. Biedeman:** None. **P.G. Bhide:** None.

Poster

034. Dopamine and Dopamine Receptor Function

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 34.17/B52

Topic: B.03. G-Protein Linked Receptors

Support: FONDECYT grant N° 1110392

Millennium Science Initiative grant N° P10-063/F

Title: Influence of dopamine d1 receptor recycling in its heteromerization with type-2α corticotropin hormone receptors

Authors: *H. E. YARUR, K. GYSLING;
PUC, Santiago, Chile

Abstract: G-protein coupled receptors (GPCR) are involved in many physiological responses and are the target of more than 50% of the drugs present in the market. Heteromerization of GPCRs is a phenomenon known as the interaction of at least two GPCRs. In our laboratory, we have shown that type-2 corticotropin hormone receptors (CRHR2α) and dopamine D1 receptors (D1R) are capable of heteromerize after heterologous co-expression. D1R expressed alone is readily trafficked to the plasma membrane while CRHR2α is observed mainly in the endoplasmic reticulum. Interestingly, we have observed a change in the subcellular localization of both receptors when they are co-expressed. We hypothesize that the known recycling capacity of D1R influences its heteromerization with CRHR2α. For this purpose, we are evaluating the interaction of CRHR2α with a mutated form of D1R lacking its recycling domain, using protein immunoprecipitation and inmunofluorescence microscopy. We expect that the lack of D1R recycling domain will diminish the level of D1R/CRHR2 heteromerization and the consequent observed change in their sub-cellular localization induced by the heteromerization.

Disclosures: **H.E. Yarur:** None. **K. Gysling:** None.

Poster

034. Dopamine and Dopamine Receptor Function

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 34.18/B53

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: NIH Grant AA010761

Title: Dopamine and serotonin alter neuronal excitability of lateral orbitofrontal cortex neurons

Authors: *S. NIMITVILAI, J. J. WOODWARD;
Neurosciences, Med. Univ. of South Carolina, Charleston, SC

Abstract: The orbitofrontal cortex (OFC), a brain region within the prefrontal cortex (PFC), plays an important role in integrating sensory information, and participates in learning, prediction and decision making for emotional and reward-related behaviors. The OFC reciprocally interacts with several brain areas, including the dorsal and ventral striatum, medial PFC (mPFC), amygdala, dorsal raphe nucleus and midbrain. Many studies have revealed that the OFC is extensively innervated by monoamines, including serotonin, dopamine and noradrenaline. Dysfunction of the OFC is associated with numerous neuropsychiatric disorders such as schizophrenia, obsessive-compulsive disorder and alcohol and substance abuse disorders, and drugs that target monoamine receptors have been used in the treatment of these psychiatric diseases. However, little is known about how monoamine neurotransmitters modulate excitability of OFC neurons. In this study, we addressed this question and used whole-cell patch-clamp electrophysiology to examine the effects of serotonin and dopamine on current evoked firing of neurons in deep layers of the lateral OFC. Dopamine decreased the evoked excitability of OFC neurons in a concentration-dependent manner and this effect was largely reversed by a D2 receptor antagonist. Similarly, serotonin produced a reduction in number of spikes of OFC neurons and the role of 5-HT1 and other 5-HT receptor subtypes were examined. These data suggest that the intrinsic activity of lateral OFC neurons is reduced by either dopamine or serotonin and suggest that dysfunction of either of these neuromodulators may underlie some of the changes in OFC observed in various neuropsychiatric diseases including alcohol dependence. Supported by AA010761.

Disclosures: **S. Nimitvilai:** None. **J.J. Woodward:** None.

Poster

034. Dopamine and Dopamine Receptor Function

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 34.19/B54

Topic: B.03. G-Protein Linked Receptors

Support: NIDA IRP funds

Ministerio de Ciencia y Technologia

Government Catalonia

CIBERNED

Title: Calcium levels determine differential signaling of adenosine A2A-dopamine D2 receptor heteromers in striatal neurons

Authors: *S. FERRE[1], D. AGUINAGA[2], E. MORENO[2], A. CORTES[2], J. MALLOL[2], V. CASADO[2], C. LLUIS[2], E. CANELA[2], P. MCCORMICK[2,3], G. NAVARRO[2];
[1]NIDA, IRP, NIH, DHHS, BALTIMORE, MD; [2]UNIVERSITY OF BARCELONA, BARCELONA, Spain; [3]UNIVERSITY OF EAST ANGLIA, Norwich, United Kingdom

Abstract: The pharmacological significance of the adenosine A2A receptor (A2AR)-dopamine D2 receptor (D2R) heteromer is well established, but the physiological factors that control its biochemical properties are still unknown. Using resonance energy transfer techniques and proximity ligation assay and determining adenylyl cyclase and MAPK signaling in HEK-293T transfected cells and striatal neurons in culture, we demonstrate a significant role of Ca2+ in the regulation of A2AR-D2R heteromer function. This is mediated by allosteric modulations mediated by the neuronal Ca2+-binding proteins NCS-1 and calneuron-1, which exert differential effects on the antagonistic modulation that A2AR exerts on D2R-mediated MAPK activation and adenylyl-cyclase inhibition. In transfected cells, NCS-1 and calneuron-1 compete for the same receptor epitopes to interact with A2AR-D2R heteromers. In striatal neurons, different Ca2+ levels promote the binding of either NCS-1 or calneuron-1 to the A2AR-D2R heteromer and its functional modulation. Preferential activation of A2AR in the A2AR-D2R heteromer leads to both cAMP accumulation and MAPK signaling. Upon co-activation of A2AR and D2R, the presence of low Ca+2 levels, with the corresponding binding of NCS-1, leads to only MAPK signaling; with high Ca+2 levels, co-activation leads to a very diminished cAMP and MAPK signaling response. In this way, A2AR-D2R heteromer constitutes a cellular device that provides a fine-tune modulation of adenosine and dopamine signals that, depending on the concentration of each neurotransmitter and the Ca+2 levels, determines the degree of adenylyl-cyclase or MAPK signaling. The results also shed light on the physiological role of reciprocal

antagonistic interactions within the A2AR-D2R heteromer, considered as a relevant target for the treatment of neuropsychiatric disorders.

Disclosures: **S. Ferre:** None. **D. Aguinaga:** None. **E. Moreno:** None. **A. Cortes:** None. **J. Mallol:** None. **V. Casado:** None. **C. Lluis:** None. **E. Canela:** None. **P. McCormick:** None. **G. Navarro:** None.

Poster

034. Dopamine and Dopamine Receptor Function

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 34.20/B55

Topic: B.01. Neurotransmitters and Signaling Molecules

Title: Computational model evidence for a complex containing DAT, VMAT, and D2 receptors and for pH regulation of dopamine leak from storage vesicles

Authors: ***L. J. WALLACE**, K. H. CIERNIAK, A. T. ZURLINDEN, A. D. KLAUSING; Ohio State Univ., COLUMBUS, OH

Abstract: The combination of an antipsychotic drug and an inhibitor of dopamine transporter (DAT) exerts several effects on dopamine nerve terminals (varicosities) that have not yet been explained. The goal of this project is to explain these effects using computer simulation models of dopaminergic varicosities. The simulation model utilizes rate equations for dopamine synthesis and metabolism and for transport of dopamine and its metabolite, DOPAC, between compartments. Drug effects are simulated by changing values of parameters thought to be impacted by the drugs. When such changes failed to provide a model output that matches published experimental data, additional changes were made using a trial and error process until model output matched experimental data. A set of parameters was found that provided model output closely matching published data for extracellular dopamine, extracellular DOPAC, tissue dopamine, tissue DOPAC, and rate of dopamine synthesis. The results document that : • D2 receptor antagonism increases extracellular dopamine by 63% with a compensatory increase in rate of dopamine synthesis • Some but not all antipsychotic drugs increase the rate of passive diffusion of dopamine out of storage vesicles. Rate of dopamine synthesis increases proportionally to the rate of increase of diffusion • D2 receptor antagonism decreases rate of DOPAC disappearance by 50% • DAT inhibition decreases rate of dopamine removal from signaling space • DAT inhibition decreases rate of secretion of dopamine by 70% • Combination of drugs reverses decreased rate of secretion elicited by inhibitors of DAT alone • Combination

of drugs decreases rate of dopamine storage by vesicular monoamine transporter (VMAT) by 60% We postulate the following biological explanation for these observations. (1) Some (but not all) antipsychotic drugs are lipophilic weak bases. These accumulate in storage vesicles, partially alkalinize the vesicle, and increase amount of diffusible neutral dopamine. Some tyrosine hydroxylase is located on storage vesicles, and activity of this enzyme increases in proportion to increases in rate of passive diffusion of dopamine out of vesicles. (2) DAT, VMAT, and D2 receptors exist in a complex such that pharmacological inhibition of both D2 receptors and DAT results in decreased activity of VMAT.

Disclosures: **L.J. Wallace:** None. **K.H. Cierniak:** None. **A.T. Zurlinden:** None. **A.D. Klausing:** None.

Poster

034. Dopamine and Dopamine Receptor Function

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 34.21/B56

Topic: B.03. G-Protein Linked Receptors

Support: NIDA IRP funds

Ministerio de Ciencia y Tecnología

Government of Catalonia

CIBERNED

Title: The adenosine A2A receptor-dopamine D2 receptor heterotetramer: Understanding the molecular mechanisms of caffeine

Authors: *J. BONAVENTURA[1,3], G. NAVARRO[3], K. AZDAD[4], W. REA[1], V. CASADÓ[3], M. BRUGAROLAS[3], E. ANGELATS[3], J. MALLOL[3], E. I. CANELA[3], A. CORTÉS[3], C. LLUÍS[3], N. D. VOLKOW[2], S. N. SCHIFFMANN[4], V. CASADÓ[3], S. FERRÉ[1];
[1]NIDA/NIH, Baltimore, MD; [2]NIDA/NIH, Rockville, MD; [3]Univ. de Barcelona, Barcelona, Spain; [4]Universtité Libre de Bruxelles, Bruxelles, Belgium

Abstract: G-protein coupled receptors (GPCRs) form homomers and heteromers. The pentameric structure constituted by one GPCR homodimer and one heterotrimeric G protein may provide a main functional unit, and it is being hypothesized that GPCR heteromers are

constituted by heteromers of homodimers. Allosteric mechanisms determine a multiplicity of possible unique pharmacological properties of GPCR heteromers. Binding of a ligand to one of the receptors in the heteromer can modify the affinity of ligands for the other receptor. The most reproduced allosteric modulation of ligand-binding properties in a GPCR heteromer is the ability of adenosine A2A receptor (A2AR) agonists to decrease the affinity of dopamine D2 receptor (D2R) agonists in the A2AR-D2R heteromer. Since this mechanism could not explain recent results of PET experiments showing that caffeine significantly enhances binding of the D2R antagonist [11C]raclopride in human striatum, we evaluated other possible allosteric interactions in the A2AR-D2R heteromer involving antagonists. Experiments were performed in membrane preparations from sheep and human striatum and from HEK-293T cells transfected with D2R and wild type or mutant A2AR. The same as for A2AR agonists, A2AR antagonists (and caffeine) reduced D2R agonist (and antagonist) affinity, and this effect was significantly attenuated upon concomitant exposure to an A2AR agonist and an A2AR antagonist. Similar interactions of A2AR and D2R ligands were observed at the level of cell signaling (MAPK activation), striatal neuronal function (patch-clamp experiments) and locomotor activity (in rats) and indicated an additional modulation of the intrinsic efficacy of D2R agonists by A2AR agonists and antagonists. Our results indicate the presence of two binding sites for A2AR ligands, corresponding to two orthosteric sites, in the A2AR-D2R heteromer. Only their occupancy with either an A2AR agonist or antagonist, but not their simultaneous occupancy with an A2AR agonist and an A2AR antagonist, leads to allosteric modulations of D2R ligands. These results also suggest that A2AR-D2R heterotetramers constitute main functional units, which was supported with BRET experiments with double bi-molecular complementation of bioluminescent and fluorescent proteins.

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Poster

034. Dopamine and Dopamine Receptor Function

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 34.22/B57

Topic: B.03. G-Protein Linked Receptors

Support: NIH RO1 DA32701

T32NS082145

Title: Decreasing the availability of intracellular calcium enhances potency and efficacy of dopamine on D2 autoreceptor-mediated currents in DBA/2J mouse brain slices

Authors: *A. J. AVELAR[1], A. SHARPE[2], M. J. BECKSTEAD[1];
[1]Dept. of Physiol., UT Hlth. Sci. Ctr. San Antonio, San Antonio, TX; [2]Univ. of the Incarnate Word, San Antonio, TX

Abstract: Dopamine neurons in the substantia nigra are involved in the initiation of voluntary movement and reward-related processes. D2-type autoreceptors on these neurons powerfully inhibit cell firing and have been negatively associated with psychostimulant use. Previous work from our lab suggests that intracellular calcium inhibits maximal autoreceptor-mediated outward currents in dopamine neurons from mouse brain slices. Additionally, we have observed that methamphetamine self-administration decreases maximal D2 autoreceptor-mediated outward currents in a calcium-dependent manner. However, since under physiological conditions only some of the autoreceptors are activated at any given time it is important to determine the effects of calcium on outward currents induced by lower concentrations of agonist. The objective of this study was to investigate the calcium dependence of the concentration-response curve for dopamine activation of D2 dopamine autoreceptor-mediated outward currents in naive and methamphetamine self-administration mice. To address this, we performed whole-cell patch-clamp electrophysiology of dopamine neurons in horizontal brain slices from DBA/2J mice to record outward currents from D2 autoreceptor opening of G protein coupled inwardly rectifying potassium (GIRK) channels. Naïve and methamphetamine self-administering mice were used to compare concentration-response curves between groups. The internal solution of the recording pipette contained either 10 mM BAPTA (high calcium chelation) or 0.025 mM EGTA (control conditions, low calcium chelation). Dopamine iontophoresis (1M) indicated the maximal D2 autoreceptor-mediated outward current that could be obtained from each cell, and bath perfusion of dopamine (3-300 μM) was used to construct agonist concentration-response curves. We tested the hypothesis that calcium chelation produces a leftward shift in the dopamine concentration-response curve at D2 autoreceptors. Our results show that decreasing the availability of intracellular calcium enhances both the potency and efficacy of dopamine on D2 autoreceptor-mediated currents. This calcium-mediated regulation of dopamine neuron physiology could have implications for the effects of psychostimulants on dopaminergic neurotransmission.

Disclosures: **A.J. Avelar:** None. **M.J. Beckstead:** None. **A. Sharpe:** None.

Poster

034. Dopamine and Dopamine Receptor Function

Location: Halls A-C

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Program#/Poster#: 34.23/B58

Topic: B.03. G-Protein Linked Receptors

Support: NIH R01AG019230

TSRI grant

Title: Dopamine-induced synaptic plasticity is mediated by molecular interaction of ghrelin and dopamine-1 receptor in hippocampal neurons

Authors: *A. KERN, R. G. SMITH;
Metabolism & Aging, The Scripps Res. Inst., Jupiter, FL

Abstract: Employing *ghsr-IRES-tau-GFP* mice we found that ghrelin receptor (GHSR1a) and dopamine-1 receptor (DRD1) are co-expressed in dentate gyrus, CA1, CA2 and CA3 regions of hippocampus. We detected dependence on GHSR1a for DRD1 agonist-mediated expression of early response genes associated with synaptic plasticity, as well as phosphorylation of CaMKII, translocation of pCaMKII, exocytosis of glutamate receptors and induction of synaptic plasticity. In neurons co-expressing GHSR1a and DRD1 the DRD1 agonist, SKF81297, activates non-canonical DRD1 signal transduction via $G\alpha_q$-PLC-IP_3-Ca^{2+}; and independent of ghrelin, $G\alpha_s$ coupling and signaling through cAMP. This non-canonical pathway is inactive in hippocampal neurons from *Ghsr-/-* mice and is blocked by a neutral GHSR1a antagonist in neurons of *Ghsr+/+* mice. Confocal FRET microscopy is consistent with formation of trimeric complex in hippocampal cell bodies and neurite processes composed of GHSR1a:DRD1 heteromers in close proximity to $G\alpha_q$ (5.45 nm). The dynamic molecular interaction between GHSR1a and DRD1 was confirmed by using single molecule TIRF microscopy in real-time at the plasma membrane of hippocampal neurons. The molecular interaction between GHSR1a and DRD1 increased in the presence of SKF81297. Our demonstration that SKF81297-induced synaptic plasticity in hippocampal neurons is dependent on forming GHSR1a:DRD1 heteromers to result in DRD1 coupling to $G\alpha_q$ at the expense of $G\alpha_s$, is a new finding with profound implications for mechanisms involved in learning and memory. These results also provide an allosteric basis for indirectly fine-tuning dopamine action in GHSR1a:DRD1 expressing neurons using GHSR1a antagonists.

Disclosures: **A. Kern:** None. **R.G. Smith:** None.

Poster

034. Dopamine and Dopamine Receptor Function

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 34.24/B59

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: NIMH Intramural Research Program

Title: Genetic predictors of *in vivo* human DOPA decarboxylase activity

Authors: *D. P. EISENBERG[1], J. C. MASDEU[1], P. D. KOHN[1], C. HEGARTY[1], B. KOLACHANA[2], D. R. WEINBERGER[3], K. F. BERMAN[1];
[1]Section on Integrative Neuroimaging, CBDB, [2]CBDB, NIMH, NIH, DHHS, Bethesda, MD; [3]Lieber Inst. for Brain Develop., Baltimore, MD

Abstract: BACKGROUND: DOPA decarboxylase (DDC) is an important enzyme in the synthesis of neuroactive molecules, including dopamine, serotonin, and trace amines. Rare genetic mutations abolishing the activity of this enzyme result in a syndrome that includes marked mental and motor developmental delays. Common genetic variation in DDC has been suggested to contribute to risk for several neuropsychiatric conditions and to illness onset in schizophrenia, in which DDC activity is exaggerated. Yet, it is unknown whether common variation in the DDC gene impacts its product's function *in vivo*. METHODS: To address this knowledge gap, we performed both DDC genotyping and DDC (18F-DOPA) PET studies in 119 healthy adults (59 women, 60 men) under 55 years of age. Twenty three markers across DDC with minor allele frequencies greater than 5% and providing coverage of HapMap annotated common variants in the CEU sample (at r-squared greater than 0.8 by 2- and 3-marker tagging as implemented by Haploview) were genotyped from peripheral blood samples with Taq-Man 5'-exonuclease assay. Haplotypes were created using PHASE software. For PET studies, after carbidopa pretreatment and at least 6 hours of fasting, 8-16 mCi of 18F-DOPA were injected intravenously and 90 minutes of dynamically binned images were acquired. These scans were attenuation-corrected, realigned, and coregistered to a structural MRI obtained in a separate session. Four regions of interest (ROIs) - dorsal caudate, dorsal putamen, ventral striatum (including nucleus accumbens) and midbrain - and one cerebellar reference region were delineated on each individual's structural MRI and applied to their PET data. The Patlak-Gjedde graphical method was employed using PMOD software to calculate the specific uptake constant Ki. General linear model analyses were performed in SPSS. RESULTS: Five haplotypes with frequencies of greater than 5% were generated and were independent of age and sex. Haplotype 2 and haplotype 4 provided nominal predictive value for specific tracer uptake, which, in post-hoc univariate tests, were shown to be driven largely by associations with midbrain ($p=0.004$)

and ventral striatum (p=0.01) Ki. DISCUSSION: Common variation in DDC predicts measureable differences in central 18F-DOPA uptake, suggesting a possible impact on DDC cis-regulation and warrenting genetic investigation of its potential molecular underpinnings. By offering evidence for functional effects of DDC polymorphisms in the living human brain, this study lays groundwork upon which to pursue hypotheses linking this candidate gene and aspects of neuropsychiatric conditions with mesolimbic involvement.

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Poster

034. Dopamine and Dopamine Receptor Function

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 34.25/B60

Topic: C.05. Aging

Support: CIHR

NSERC

OMHF

Title: Age-related decrease in gene expression for conserved dopamine neurotrophic factor (CDNF) in humans

Authors: *A. SIDDIQI[1], K. TERPSTRA[1], M. SEHMBI[2], L. E. CUDNEY[3], R. B. SASSI[4], B. N. FREY[1], R. K. MISHRA[1];
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Abstract: Introduction: Aging is a complex, biological progression that increases the likelihood of developing neurodegenerative diseases such as Alzheimer’s and Parkinson’s disease. Neurotrophic factors provide an avenue of study into the aging process, as these secreted peptides promote survival, differentiation and maintenance of neurons, as well as the establishment of synaptic connections during development. The loss of neurotrophic support for specific neurotransmitter populations could confer susceptibility to various neurodegenerative disorders. Conserved dopamine neurotrophic factor (CDNF) is a novel neurotrophic factor that

has not yet been investigated in the aging process. Several scientists have proposed that the circulating blood may act as a "sentinel tissue", reflecting states of health or disease within the body for which human brain tissue biopsy samples are unavailable. Therefore, the objective of the present study was to measure CDNF levels in peripheral whole blood of healthy individuals, to examine CDNF mRNA expression in the human population over the course of development. Methods: Venous whole blood was collected from 26 healthy individuals at St. Josephs Healthcare in Hamilton, Ontario (all protocols approved by Hamilton Integrated Research Ethics Board and the Research Ethics Board at McMaster University). Whole blood mRNA was purified and extracted from PAXgene blood RNA tubes. CDNF mRNA gene expression was analysed using real-time reverse-transcriptase polymerase chain reaction (RT-PCR). PCR forward (F) and reverse (R) CDNF primers were as follows (5' 3'): F AAAGACGCAGCCACAAAGAT, R AGGATCTGCTTCAGCTCTGC. Results: Patients were divided into 3 age groups; A) Children [0-18 (n=6)], B) Young adults [18-50 (n=16)], and C) Older adults [50+ (n=5)]. One-way ANOVA was used to determine differences in CDNF mRNA expression levels between groups. Results indicated a trend towards decreasing CDNF levels with aging (p=0.07). Conclusions: This preliminary result suggests that CDNF mRNA levels may decrease with aging. Reductions in CDNF over the course of development may be particularly important in Parkinson's disease, a disorder stemming from dopamine degeneration. While larger studies are awaited to confirm this hypothesis, the examination of CDNF levels in peripheral blood may serve as a viable biomarker in the understanding of the selective dopaminergic degeneration within this disorder. This study was supported by: Canadian Institutes of Health Research (CIHR), Natural Sciences and Engineering Research Council (NSERC), and the Ontario Mental Health Foundation (OMHF).

Disclosures: **A. Siddiqi:** None. **K. Terpstra:** None. **M. Sehmbi:** None. **L.E. Cudney:** None. **R.B. Sassi:** None. **B.N. Frey:** None. **R.K. Mishra:** None.

Poster

035. Opiate, Cytokines, and Other Neuropeptides

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 35.01/C1

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: Stony Brook Medical Center Dept. of Pediatrics

Title: Hypoxia induces rapid production and release of enkephalins and cytokines in mouse pheochromocytoma cells

Authors: *M. J. EVINGER[1], A. S. CALERO[2], H. H. VILLANUEVA[2], P. GIRI[2], J. F. POWERS[3], A. S. TISCHLER[3];
[2]Pediatrics, [1]Stony Brook Univ., Stony Brook, NY; [3]Pathology, Tufts Univ. Sch. of Med., Boston, MA

Abstract: Adrenal chromaffin cells function as O_2 sensors during the third trimester of pregnancy and early post-natal life prior to maturation of the sympathetic nervous system. The ability to detect and mount appropriate physiological responses is important for neonate survival of apnea and hypoxic events frequently encountered during labor, delivery and early postnatal life. Mouse pheochromocytoma cells (MPC 10/9/96CR) are one of several adrenergic chromaffin-like cell lines characterized by epinephrine production and release (Powers et al., *Cell Tissue Res*., 2000). Complementing our previous demonstration that hypoxia is a potent stimulator of PNMT gene expression in these cells (Evinger et al., *Ann N Y Acad Sci.* 2002), this study hypothesizes that reduced oxygen concentrations[O_2] are likewise a significant stimulus for release of other chromaffin cell products important for survival of hypoxic episodes. Using MPC 10/9 cells subjected to 10% [O_2](hypoxia) for specific intervals, we demonstrate that hypoxia induces rapid and robust release of the catecholamine epinephrine with concentrations in media increasing from 0 to 25 ng/ml following 15 min incubation at 10% [O_2]. Hypoxia-stimulated production and release of enkephalin (ENK) parallels that of catecholamines: a ~2-fold increase of Met-ENK is detected by ELISA in the medium within 15 min. Following an initial decrease in cellular ENK content, the amount of ENK in MPC 10/9 cell extracts then rises by an average of 50% by 60 min. Synthesis of preproenkephalin mRNA likewise increases up to 3-fold within 30 min exposure to 10% O_2. Additionally, hypoxia stimulates production and release of chromaffin cell cytokines IL-1α and IL-1β from MPC 10/9 cells, with significant changes in IL-1β detectable within 15 min, as revealed using antibody-coupled Luminex detection . A compensatory increase of the cytokine mRNAs occurs following incubation in reduced [O_2]. Therefore, hypoxia-stimulated release of ENK and of cytokines IL-1α and IL-1β parallels the effects of reduced [O_2] on MPC 10/9 cell catecholamine content. Thus, in addition to metabolic enhancement provided by chromaffin cell catecholamine release, hypoxia evokes significant release of opioid peptides capable of modulating reflex-regulated cardiac contractility (especially during asphyxia-induced hypotension) and pro-inflammatory cytokines with associated influences on peptide hormone, catecholamine and steroid production. As seen in effects on CNS neurons, hypoxia evokes the rapid release and compensatory re-synthesis of adrenal medullary factors that enhance metabolic and physiological survival during neonatal O_2 deprivation.

Disclosures: M.J. Evinger: None. **A.S. Calero:** None. **H.H. Villanueva:** None. **P. Giri:** None. **A.S. Tischler:** None. **J.F. Powers:** None.

Poster

035. Opiate, Cytokines, and Other Neuropeptides

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 35.02/C2

Topic: B.03. G-Protein Linked Receptors

Support: NIH Grant DA023281

NIH Grant DA031777

Title: NOP-eGFP mice: Immunohitochemical characterization of the NOP-eGFP distribution in the spinal cord and DRG

Authors: *A. OZAWA[1], J. WU[1], D. MERCATELLI[1], G. BRUNORI[1], S. LOW[2], G. SCHERRER[2], B. L. KIEFFER[3], L. TOLL[1];
[1]Torrey Pines Inst. For Mol. Studies, Port St Lucie, FL; [2]Stanford Univ. Sch. of Med., Palo Alto, CA; [3]Douglas Institute, McGill Universit, Montreal, QC, Canada

Abstract: NOP receptors and N/OFQ are found throughout the brain, in the spinal cord, dorsal root ganglia, and a variety of peripheral tissues. The NOP-N/OFQ system regulates a wide range of biological actions including nociception. To better characterize the anatomical distribution patterns of the receptor, immunohistochemical experiments are conducted on mice in which the NOP receptor is replaced by an active NOP-eGFP receptor C-terminal fusion protein. Tissue sections from the dorsal root ganglia and spinal cord are stained with anti-GFP antibodies, as well as with antibodies to NF200, CGRP, PKCγ, and with biotinylated IB4. In the DRG, NOP-eGFP receptors are expressed on large, medium, and relatively small NF200+ (myelinated) cells that are mostly CGRP-, suggesting that they are not the peptidergic Aδ nociceptors. This also implies that the NOP-eGFP receptors might be on myelinated low-threshold mechanosensory neurons, including down-hair afferents. Down-hair afferents are the most sensitive mechano-sensitive somatosensory neurons and contribute to touch perception. They have myelinated axons and relatively small diameter cell bodies, and are NF200+. These cells contain neither mu nor delta receptors. They project to the ventral border of inner lamina II of the spinal cord where PKCγ+ spinal neurons are located. There are also a number of small cells that are NOP-eGFP+, NF200-, CGRP+ (or mu receptor positive): these are likely the typical peptidergic nociceptive C-fibers. Small cells are also observed that stain only for NOP-eGFP, implying the possibility that these cells might be involved in nonpeptidergenic nociceptive responses. In the spinal cord, NOP-eGFP receptors are highly expressed in laminae I-III, where CGRP (lamina I and lamina II

outer) immunoreactive terminals, PKCγ (ventral border of lamina II inner) interneurons and the terminals of IB4 positive neurons (dorsal border of lamina II inner) are located; immunoreactivity extends into lamina X. A moderate GFP immunoreactivity also appears ventrally throughout the spinal cord. This expression pattern of NOP-eGFP in spinal cord is consistent with the previously reported *in vitro* autoradiography in rat spinal cord. In most of the cells visualized, whether they are from the spinal cord, DRG, or brain, NOP receptors appear to be located throughout the cell, rather than being localized to the plasma membrane. In summary, immunohistochemical characterization provides us with information regarding the cell types containing NOP receptors in DRG and the lamina location in the spinal cord. It will be useful information for understanding the mechanism by which NOP receptors alter a nociceptive response.

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Poster

035. Opiate, Cytokines, and Other Neuropeptides

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 35.03/C3

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: NASU Biotechnology and Functional Genomics and Metabolomics Grants

DFFD F46.2/001 and F47/066 Grants

Title: Hippocalcin signaling underlying slow afterhyperpolarization in hippocampal neurons

Authors: *N. I. KONONENKO[1,2], A. DOVGAN[1,2], V. CHERKAS[1,2], T. TSUGORKA[1,2], P. BELAN[1,2];
[1]Inst. Physiol, Kiev, Ukraine; [2]Key State Lab. of Mol and Cell Biol, Kiev, Ukraine

Abstract: Hippocalcin (HPCA) is a neuronal Ca2+ sensor protein that mediates many cellular functions. In particular, it is thought that HPCA mediates expression of a slow afterhyperpolarization (sAHP) in response to an increase in a free intracellular Ca2+ concentration ([Ca2+]i) in a neuronal dendritic tree. In the current work, we studied molecular mechanisms of sAHP induction by HPCA in cultured hippocampal neurons. First, we pharmacologically and electrophysiologically characterized channels underlying sAHP in these

neurons. We demonstrated that sAHP was mediated by potassium Ca2+-dependent conductance, which was not significantly reduced by a specific KCNQ channel blocker, XE991, indicating that this class of potassium channels does not contribute to sAHP current (IsAHP). Interestingly, that well-known sAHP inhibitors, UCL2077 or UCL1848 also did not affect IsAHP. At the same time, IsAHP was almost completely inhibited by activation of M1 cholino- or beta-adrenergic receptors. By means of loss of function and overexpression strategies we demonstrated that amplitude of IsAHP is strongly correlated with a level of HPCA expression implying that HPCA does function as a Ca2+ sensor for the sAHP in the hippocampal neurons. Simultaneously recording IsAHP and HPCA-YFP translocation induced in the same neuron by activation of voltage-gated Ca2+ channels we demonstrated overlapping of their time courses in certain sites of dendritic tree. It suggested that HPCA insertion into the plasma membrane in these sites may induce IsAHP. Using modification of FRET approach, which allowed us to measure HPCA concentration in the plasma membrane, a direct correlation between this concentration in the apical part of dendritic tree and IsAHP value was observed during the current decay. These results strongly suggest that the HPCA bound to certain sites of apical dendrite induces potassium Ca2+-dependent conductance responsible for sAHP and that sAHP conductance is proportional to HPCA concentration in these sites. It should be noted that a proportion of neurons revealing HPCA insertion into the plasma membrane upon an increase of [Ca2+]i did not produce a measurable IsAHP. It suggests that HPCA insertion into the plasma membrane in these neurons occurs in sites lacking the respective potassium conductance. All these findings demonstrate that sAHP is a complex phenomenon, in which HPCA integrates spatio-temporal changes of [Ca2+]i into its widespread insertion into the dendritic plasma membrane. This, in turn, results in activation of K+ conductance only in the sites where the respective still unidentified potassium channels are located.

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Poster

035. Opiate, Cytokines, and Other Neuropeptides

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 35.04/C4

Topic: B.03. G-Protein Linked Receptors

Support: NSC Neuroscience Promotion Grant NSC102-2321-B-002-066;

NSC NRPB Grant NSC102-2325-B-002-047

NHRI IRG NHRI-EX103-10251NI

Title: A novel mechanism for stress-induced analgesia: Involvement of orexin, substance P, and mGluR5 in the periaqueductal gray

Authors: *L.-C. CHIOU[1,2,3], Y.-C. CHIU[2];
[1]Dept. of Pharmacol., Natl. Taiwan University, Med. Col., Taipei, Taiwan; [2]Grad. institute of Pharmacol., Taipei, Taiwan; [3]Grad. institute of Brain and Mind Sci., Taipei, Taiwan

Abstract: Substance P has been known to be antinociceptive at the supraspinal level, primarily mediated by the neurokinin-1 receptor (NK1R). However, its action mechanism(s) remain unclear. An electrophysiological study in the ventrolateral periaqueductal gray (vlPAG) showed that substance P can activate glutamatergic neurons to release massive glutamate that activate perisynaptic $mGluR_5$, yielding endocannabinoids (eCBs) that inhibit GABA release via presynaptic cannabinoid 1 receptors (CB1Rs), producing retrograde disinhibition. Previously, we found orexin A induced antinociception also through eCB-mediated retrograde disinhibition in the vlPAG.[1] Both orexins and substance P are involved in stress-induced analgesia (SIA). Therefore, we investigated whether orexin A induces analgesia through releasing substance P in the vlPAG via this glutamate-$mGluR_5$-eCB-CB1R signaling cascade, and if this mechanism contributes to SIA. Intra-vlPAG microinjection (*i.pag.*) of substance P (5 nmol) significantly increased the paw withdrawal latency in the hot plat test in C57BL/6 mice (male, 8-12 weeks). This antinociceptive effect was blocked by *i.pag.* MPEP (50 nmol, an $mGluR_5$ antagonist) and AM251 (30 nmol, a CB1R antagonist), respectively. As reported previously, *i.pag.* orexin A (1 nmol.) also produced significant antinociceptive effect. Interestingly, this antinociceptive effect was blocked by *i.pag.* L-703,606 (10 nmol, an NK1 receptor antagonist) or MPEP. Interestingly, a 30-min restraint stress in mice significantly increased the withdrawal latency, and this SIA was blocked by *i.pag.* L-703,606 and MPEP. Both antagonists did not affect spontaneous locomotor activity in mice. Besides, substance P levels, measured by enzyme immunoassay, in vlPAG homogenates were significantly elevated in restrained mice, as compared to un-restrained ones (95.4±13.1 *vs.* 55.7±2.0 pg/μg protein, $p<0.05$). It is suggested that during stress, hypothalamic orexin neurons are activated, and released orexin A induces analgesia through releasing substance P that produces disinhibition via NK1Rs in the vlPAG by releasing glutamate to activate the $mGluR_5$-eCB-CB1R signaling cascade. [1]Ho, YC, Lee, HJ, Tung, LW, Liao, YY, Fu, SY, Teng, SF, Liao, HT, Mackie, K & Chiou, LC. (2011) Activation of orexin 1 receptors in the periaqueductal gray of male rats leads to antinociception via retrograde endocannabinoid (2-arachidonoylglycerol)-induced disinhibition. J Neurosci 31:14600.

Disclosures: **L. Chiou:** None. **Y. Chiu:** None.

Poster

035. Opiate, Cytokines, and Other Neuropeptides

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 35.05/C5

Topic: B.03. G-Protein Linked Receptors

Title: Nuclear export of OGFr is dependent on a nuclear export signal

Authors: N. P. KREN, I. S. ZAGON, *P. J. MCLAUGHLIN;
Dept Neural & Behav Sci., Penn State Univ. Coll Med., HERSHEY, PA

Abstract: The opioid growth factor (OGF) - OGF receptor (OGFr) axis regulates cell replication during normal development, tissue homeostasis, and neoplasia. Unlike classical opioid receptors, OGFr is not a transmembrane receptor, but does share some pharmacological properties with mu, delta, and kappa opioid receptors. OGFr binds the pentapeptide OGF, chemically termed [Met5]-enkephalin, to activate cyclin-dependent inhibitory kinas pathways and repress cell replication. This function of Gofer is dependent on nuclear localization. OGFr has three nuclear localization signals, and transport into the nucleus is mediated by karyopherinβ and Ran. Mechanisms of OGFr export from the nucleus are unknown. A variety of techniques were utilized to determine whether OGFr is actively exported from the nucleus. An evolutionarily conserved region of OGFr has 1 predicted nuclear export signal (NES) comprised of four leucines at residues 217, 220, 223 and 225. OGFr-eGFP was overexpressed in COS-7 cells, lacking other classical receptors, in order to visualize nuclear trafficking. Treatment with leptomycin B, an inhibitor of CRM-1 (chromosome region maintenance 1), demonstrated accumulation of OGFr-eGFP as well as endogenous OGFr in the nucleus, indicating that OGFr is exported in a CRM-1 dependent manner. Site-directed mutagenesis of specific leucines 217, 220, 223, and 225 individually or in combination resulted in decreased nuclear to cytoplasmic ratios, rather than accumulation within the nucleus. There are two plausible explanations for this unexpected result 1) OGFr could homodimerize with endogenous OGFr and be exported by the functional NES found in endogenous OGFr or 2) OGFr could be degraded within the nucleus. When cells expressing deltaNES were treated with leptomycin B nuclear accumulation increased by 22%, indicating that receptor dimerization may be only partially responsible. In similar experiments utilizing COS-7 cells expressing deltaNES treated with MG132, a proteasome inhibitor, there was no effect on nuclear localization, indicating that degradation does not account for exclusion of deltaNES from the nucleus. In conclusion, OGFr is actively exported from the nucleus by the nuclear export signal identified as residues 217-225, however regulation of this event warrants further studies.

Disclosures: **N.P. Kren:** None. **I.S. Zagon:** None. **P.J. McLaughlin:** None.

Poster

035. Opiate, Cytokines, and Other Neuropeptides

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 35.06/C6

Topic: B.03. G-Protein Linked Receptors

Support: Helsinn Healthcare

Title: Netupitant and palonosetron trigger NK_1 Receptor internalization in NG108-15 cells

Authors: ***C. ROJAS**, A. G. THOMAS, M. STATHIS, B. S. SLUSHER;
Johns Hopkins Sch. of Med., Baltimore, MD

Abstract: Current therapy for chemotherapy induced nausea and vomiting includes the use of both 5-HT_3 and NK_1 receptor antagonists. Acute emesis has largely been alleviated with the use of 5-HT_3 receptor antagonists while an improvement in preventing delayed emesis has been achieved with NK_1 receptor antagonists. Delayed emesis however, remains a problem with a significant portion of cancer patients receiving highly emetogenic chemotherapy. Like other drugs in its class, palonosetron, a 5-HT_3 receptor antagonist, has shown efficacy against acute emesis. However, palonosetron has also shown consistent improvement in the suppression of delayed emesis. Since both 5-HT_3 and NK_1 receptor antagonists are often simultaneously administered to patients, the question remains if palonosetron's effect on delayed emesis would remain distinct when co-administered with an NK_1 receptor antagonist. Recent mechanistic studies using NG108-15 cells have shown that palonosetron and netupitant, an NK_1 receptor antagonist currently in phase 3 clinical trials, exhibited synergistic effects when inhibiting the Substance P response. The present studies showed that both netupitant and palonosetron induced NK_1 receptor internalization in NG108-15 cells and that when used together receptor internalization was additive. Palonosetron-induced NK_1 receptor internalization was dependent on the presence of the 5-HT_3 receptor. Results provide a possible explanation for palonosetron's enhancement of the inhibition of the SP response and suggest that the effect of palonosetron and NK_1 receptor antagonists on prevention of delayed emesis could be additive.

Disclosures: **C. Rojas:** None. **A.G. Thomas:** None. **M. Stathis:** None. **B.S. Slusher:** Other; Helsinn Healthcare.

Poster

035. Opiate, Cytokines, and Other Neuropeptides

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 35.07/C7

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: AFOSR FA9550-10-1-0385

NINDS (NIH) R01NS39600

Keck Nakfi

ONR MURI N00014-10-1-0198

Title: Hippocampome.org: A neuroinformatics tool for biomarker profiling

Authors: *C. WHITE, C. L. REES, D. W. WHEELER, D. J. HAMILTON, A. O. KOMENDANTOV, S. VENKADESH, G. A. ASCOLI;
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Abstract: Comprehending the dynamics of complex biological systems like the mammalian brain requires understanding the cells involved. Fundamental to this endeavor is knowledge of the molecular biomarkers they express. These biomarkers may enable the chemical identification of neuron types while providing informative signatures of functional mechanisms. A wealth of relevant literature on this topic exists; however, its optimal utilization requires that it be collected, organized, and integrated in easily accessible, centralized locations. Hippocampome.org provides such an opportunity by characterizing the morphology, electrophysiology, and biomarker expression of neuron types in the rodent hippocampal formation (dentate gyrus, CA1, CA2, CA3, subiculum, and entorhinal cortex). This knowledge base defines neuronal types on the basis of their primary neurotransmitter and the anatomical distributions of their axons and dendrites. The scientific literature then is mined for experimental biomarker data from individual morphologically-defined neurons corresponding to these types. Hippocampome.org also includes data on spatial expression patterns within the hippocampal subregions and layers that can be linked to specific neuron types by their somatic location. Hippocampome.org contains information on over 60 biomarkers. Those with expression information known for the most neuron types include the calcium binding proteins, calbindin, calretinin, and parvalbumin and the neuropeptides, cholecystokinin and somatostatin.

Approximately two-thirds of the more than 100 neuron types in the current instantiation of Hippocampome.org have expression information for at least one biomarker. From these data, potentially unique biomarker profiles can be identified for some hippocampal neuron types.

Disclosures: **C. White:** None. **C.L. Rees:** None. **D.W. Wheeler:** None. **D.J. Hamilton:** None. **A.O. Komendantov:** None. **S. Venkadesh:** None. **G.A. Ascoli:** None.

Poster

035. Opiate, Cytokines, and Other Neuropeptides

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 35.08/C8

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: NIDA DA018310

NINDS NS031609

NSF 0965918 IGERT

Title: Neuropeptide release in response to mechanical stimulation of cultured dorsal root ganglion cells

Authors: *E. G. TILLMAAND[1,2], C. A. CROUSHORE[3,1], S. S. RUBAKHIN[1], T. A. SAIF[4], J. V. SWEEDLER[3,2,1];
[1]Beckman Inst. For Advanced Sci. and Technol., Urbana, IL; [2]Neurosci., [3]Chem., [4]Mechanical Sci. and Engin., Univ. of Illinois at Urbana-Champaign, Urbana, IL

Abstract: The pervasiveness of peptides as intercellular signaling molecules in the nervous system, along with their involvement in multiple disease states, underscores the importance of understanding key processes responsible for their function, including constitutive and/or stimulated release from healthy and pathologically modified cells. While the responses of neurons to chemical stimulation have been studied in detail, responses to mechanical stimuli are not as well understood. Results of recent investigations show that cells can specifically respond to mechanical stimuli and environments (Engler, AJ et al. Cell. 2006) and that tension plays a role in neuronal function (Ahmed, WW et al. Cell Mol Bioeng. 2012). However, the role of mechanical stimuli in the modulation of intercellular communication mechanisms remains elusive as studies linking neuropeptide release to changes in the mechanical environment are lacking. Here, we study dorsal root ganglion (DRG) cell neurochemistry as a function of changes

in mechanical environment. Toward this end, DRG cells are isolated from male Sprague-Dawley rats and cultured on a polydimethylsiloxane (PDMS) mechanical platform. After approximately 14 days in culture, tensile strain is applied to the PDMS substrate and, therefore, to the cultured cells. Samples of the extracellular media containing released peptides are collected before, during, and after the mechanical stimulation. Off-line matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) is used to study the peptide profile of the releasates and results are compared with that of DRG cells stimulated via an increase in extracellular K+. A number of signals within the peptide molecular mass region are observed, with current efforts aimed at identifying the peptides using liquid chromatography coupled to tandem mass spectrometry. Several peptides have been tentatively identified including those derived from proteins involved in neuronal functions such as S100-beta and various splice forms of Myelin Basic Protein S, and those involved in receptor signaling. Interestingly, application of the two different stimuli to the DRG cells (mechanical versus chemical) resulted in the release of distinct peptide profiles with some overlap, suggesting that there are may be unique signaling pathways for the two methods of stimulation. By coupling the mechanical strain system with mass spectrometric microanalysis, we are able to focus on mechanically induced peptide release in order to further our understanding of the roles mechanical forces play in cell-to-cell communication.

Disclosures: **E.G. Tillmaand:** None. **C.A. Croushore:** None. **S.S. Rubakhin:** None. **T.A. Saif:** None. **J.V. Sweedler:** None.

Poster

035. Opiate, Cytokines, and Other Neuropeptides

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 35.09/C9

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: CONACyT project no. 60789

CONACyT grant no. 209646

Title: Dopamine D3, but not D2 or D4 receptor subtypes, mediate the inhibition of the vasodepressor sensory CGRPergic outflow in pithed rats

Authors: *G. MANRIQUE-MALDONADO, A. H. ALTAMIRANO-ESPINOZA, E. RIVERA-MANCILLA, B. VILLANUEVA-CASTILLO, C. M. VILLALÓN; Farmacobiología, Cinvestav-IPN, México, D.F., Mexico

Abstract: Primary sensory nerves play an important role in the modulation of several physiological functions including vascular tone, contributing to blood pressure control by the release of vasodilator neuropeptides, primarily calcitonin gene related peptide (CGRP). Indeed, electrical stimulation of the perivascular sensory outflow in pithed rats results in vasodepressor responses mainly mediated by CGRP receptor stimulation. Moreover, our group has recently reported that these vasodepressor responses are inhibited by prejunctional (quinpirole-sensitive) D_2-like receptors. Since D_2-like receptors consist of the D_2, D_3 and D_4 subtypes, this study was designed to identify, by using selective antagonists, the specific subtypes (D_2, D_3 and/or D_4) involved in the above quinpirole-induced inhibition. For this purpose, after anaesthesia, 40 male Wistar rats were pithed, artificially ventilated and pretreated intravenously (i.v.) with 25 mg/kg gallamine, i.v. continuous infusions of 2 mg/kg.min hexamethonium (to block autonomic ganglia) and 20 µg/kg.min methoxamine (for maintaining an increased arterial blood pressure at around 130 mm Hg). Then, the animals were divided into 8 groups (n=5 each) that subsequently received: (i) nothing (control group); (ii) saline (0.02 ml/min); (iii) quinpirole (D_2-like receptor agonist; 0.1 µg/kg.min); (iv) saline (0.02 ml/min) + ascorbic acid 5% (vehicle to dissolve the antagonists; 1 ml/kg); (v) quinpirole + ascorbic acid 5%; (vi) quinpirole + L-741,626 (D_2 antagonist; 300 µg/kg); (vii) quinpirole + SB-277011-A (D_3 antagonist; 300 µg/kg); and (viii) quinpirole + L-745,870 (D_4 antagonist; 100 µg/kg). Based on functional studies in pithed rats, the doses of these antagonists are high enough to completely block their respective receptors without modifying *per se* the electrically-induced vasodepressor responses. Under these conditions, electrical stimulation (0.56, 1, 1.8, 3.1 and 5.6 Hz; 50 V and 2 ms) of the thoracic spinal cord (T_9-T_{12}) resulted in frequency-dependent vasodepressor responses which: (i) remained unchanged during saline infusion or after i.v. bolus injection of ascorbic acid 5%; and (ii) were inhibited by quinpirole. Moreover, quinpirole-induced inhibition was: (i) unaltered after i.v. treatment with ascorbic acid 5% or the antagonists L-741,626 (D_2) and L-745,870 (D_4); and (ii) abolished after SB-277011-A (D_3). In conclusion, our results suggest that quinpirole-induced inhibition of the vasodepressor sensory CGRPergic outflow is mainly mediated by activation of prejunctional dopamine D_3 receptors, with no clear-cut evidence for the role of prejunctional D_2 or D_4 receptor subtypes.

Disclosures: **G. Manrique-Maldonado:** None. **A.H. Altamirano-Espinoza:** None. **E. Rivera-Mancilla:** None. **B. Villanueva-Castillo:** None. **C.M. Villalón:** None.

Poster

035. Opiate, Cytokines, and Other Neuropeptides

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 35.10/C10

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: NIH HL98589

NIH HL71830

Title: Expression and actions of AT1, AT2 and Mas receptors in the guinea pig intrinsic cardiac plexus

Authors: E. N. POWERS[1], K. A. LUCKETT[1], S. A. ROSEN[1], E. M. SOUTHERLAND[2], J. L. ARDELL[2], *J. C. HARDWICK[1];
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Abstract: The intrinsic cardiac (IC) nervous system integrates inputs from multiple sources to coordinate cardiac function. These inputs include parasympathetic efferent, sympathetic efferent and sensory afferent fibers in addition to local hormonal factors. A major local factor is angiotensin II (Ang II) which is found in the blood and is also produced by proteases within the cardiac interstitium. Ang II acts via AT1 or AT2 receptors. In addition, Ang II can be cleaved by ACE2 to formed Ang(1-7), a peptide that has been found to have significant effects on neurons via activation of Mas receptors. In this study, we looked at the expression of the different angiotensin peptide receptors and their actions on neurons of the guinea pig intrinsic cardiac plexus. All three receptors, AT1R, AT2R, and MasR, were detected in homogenates of the isolated cardiac ganglion by Western blot analysis. Previous studies showed that AT2R can increase the neuronal responses to adrenergic and muscarinic signals. Using a whole mount *in vitro* preparation of the cardiac plexus, intracellular voltage recordings were made from individual IC neurons. We found that Ang(1-7) synergistically increases neuronal excitability responses to NE and muscarinic agonists. We also examined the effects of Ang II on synaptic function in the ganglion by stimulating intraganglionic fibers leading to IC neurons of interest. Application of Ang II reduced the synaptic efficacy, as indicated by a reduced number of postsynaptic action potentials generated with increasing frequency suprathreshold stimulation. Addition of the AT1R antagonist losartan inhibited this effect. To examine potential changes in AngII receptor function with chronic heart disease, myocardial infarction (MI) was surgically induced in animals, and the cardiac ganglion examined 8 weeks later. Western blot analysis showed a decrease in AT1R expression in ganglia from MI animals, with no significant change in either AT2R or MasR expression levels. However, prior functional studies of the effects of Ang II on IC neurons demonstrated a MI-induced loss in sensitivity to exogenous Ang II application. Combined, these results indicate that MI induces a down regulation in presynaptic AT1 receptors, as well as a decrease in signaling via AT2R. Thus, increases in Ang II production, such as occurs with chronic heart disease, can result in significant alterations in function within this ganglion.

Disclosures: **E.N. Powers:** None. **K.A. Luckett:** None. **S.A. Rosen:** None. **E.M. Southerland:** None. **J.L. Ardell:** None. **J.C. Hardwick:** None.

Poster

035. Opiate, Cytokines, and Other Neuropeptides

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 35.11/C11

Topic: B.02. Ligand-Gated Ion Channels

Support: Dean of College at Princeton University

Lambert Neuroscience Award

Princeton University Department of Ecology and Evolutionary Biology

Title: Adaptive changes in lampyridae illuminate evolutionary trends: A study of toxin resistance in sodium-potassium pumps

Authors: *L. COBBS[1], P. ANDOLFATTO[2];
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Abstract: Two Lampyridae species, Photinus and Photuris, have evolved the ability to safely produce and consume, respectively, lucibufagin, a cardiac glycoside toxin which targets the alpha subunit (ATPα) of sodium-potassium protein pumps. Photuris, the consumers of exogenous lucibufagin, have accumulated adaptive mutations in their ATPα to resist cardiac glycoside binding. The path of Photuris ATPα's evolution has been shaped by negative pleiotropic constraints and gene duplication events. Previous studies have examined how these constraints and forces direct the speed and orientation of evolution, but debate continues over early models of gene duplicate maintenance and the significance of negative pleiotropy. I examined the amino acid sequences, cardiac glycoside binding affinities, and tissue-specific expression patterns of Photuris' ATPα gene copies to better parse the constraints and forces guiding adaptive evolution. My findings show that Photuris have employed many of the same molecular strategies to evolve lucibufagin resistance as other insects which also safely consume cardiac glycosides. This parallelism across ecological systems suggests that there are general rules governing adaptive evolution. Independent of ecological context, it seems that gene duplication eases constraints imposed by negative pleiotropy to allow for fixation of nonsynonymous mutations. In addition to providing insight into evolutionary dynamics, my

findings have implications for treatment of human diseases involving the sodium-potassium pump, including Digitalis therapy for Congestive Heart Failure and several neural disorders.

Disclosures: **L. Cobbs:** None. **P. Andolfatto:** None.

Poster

036. Muscarinic Acetylcholine and Metabotropic Glutamate Receptors

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 36.01/C12

Topic: B.03. G-Protein Linked Receptors

Support: Regione Autonoma della Sardegna, L.R. 7/2007-CRP10810/2012

Title: Activation of M_3 muscarinic acetylcholine receptors protects against interferon-β-induced neuronal cell death

Authors: *M. C. OLIANAS, S. DEDONI, P. ONALI;
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Abstract: The clinical use of type I interferons (IFNs), such as IFN-α and IFN-β, is known to be frequently associated with the occurrence of central nervous system (CNS) side effects, including anxiety, confusion, cognitive deficits, mania, psychosis, depression and suicidal behaviour. In addition, chronically elevated IFN-α production in the CNS is considered a pathogenic factor in Aicardi-Goutieres syndrome, a genetically determined encephalopathy. At the cellular level, long-term exposure to type I IFNs induces neuronal cell death through activation of apoptosis. In the present study we used the SH-SY5Y cell line as a human neuronal cell model to identify neurochemical mechanisms capable of preventing type I IFN neurotoxicity. We found that cell treatment with either carbachol (CCh), a cholinergic receptor agonist, or oxotremorine M, a selective muscarinic acetylcholine receptor (mAChR) agonist, markedly suppressed IFN-β-induced neuronal cell death. The neuroprotective effect of CCh was antagonized by 4-DAMP, a M_3 mAChR preferring antagonist, but not methoctramine, a M_2 mAChR preferring antagonist, or MT-7, a selective M_1 mAChR antagonist. Concurrent cell treatment with CCh inhibited IFN-β-induced cytochrome c release from mitochondria, caspase activation and cleavage of poly-(ADP ribose) polymerase. CCh induced a long-lasting activation of either p38 or ERK 1 and 2 (ERK1/2) MAP kinases. Pharmacological inhibition of ERK1/2 but not p38 MAP kinase prevented the antiapoptotic effect of CCh. These data indicate that in SH-

SY5Y neuronal cells activation of M_3 mAChR counteracts IFN-β-induced neuronal apoptosis through activation of ERK1/2 pathway.

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

Poster

036. Muscarinic Acetylcholine and Metabotropic Glutamate Receptors

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 36.02/C13

Topic: B.03. G-Protein Linked Receptors

Support: F32 MH095285

RO1 MH073676

P50 NS071669

Title: M4-muscarinic receptors attenuate dopamine release via production of H2O2 in direct pathway medium spiny neurons

Authors: ***D. J. FOSTER**, Z. XIANG, P. J. CONN;
Vanderbilt Ctr. For Neurosci. Drug Discovery, Nashville, TN

Abstract: Two seminal clinical studies have demonstrated that the M1/M4 preferring agonist xanomeline can provide significant therapeutic efficacy in treating psychosis in patients with Alzheimer's disease or scizhophrenia. Unfortunately gastrointestinal side-effects, likely mediated by M2/M3 receptors, have removed xanomeline from consideration for clinical use. Here we utilize a subtype-selective M4 positive allosteric modulator (VU0467154) to elucidate the role of M4 in regulating striatal dopamine release. Application of the non-selective muscarinic receptor agonist Oxo-M decreased electrically-evoked striatal dopamine release as monitored via fast scan cyclic voltammetry in acute brain slices. The inhibition of dopamine release induced by sub-maximal Oxo-M concentrations was potentiated by inclusion of VU0467154, demonstrating that M4 activation reduces dopaminergic signaling. In the striatum M4 is found primarily on direct pathway medium spiny neurons and is also reported to be expressed on cholinergic interneurons and cortical afferents. However, M4 is not thought to be located on dopamine terminals, raising the question of how M4 activation alters dopamine overflow in acute striatal brain slices. Hydrogen peroxide (H2O2) produced in medium spiny neurons has been shown to inhibit dopamine release via activation of KATP channels on dopamine terminals. By monitoring

H_2O_2 directly in striatal slices with the fluorescent indicator H2DCFDA, we found that activation of M4 led to an increase in striatal H_2O_2 production. Furthermore, M4-mediated inhibition of dopamine release was potentiated by mercaptosuccinate (an inhibitor of H_2O_2 degredation) and attenuated by glybenclamide (a KATP blocker). These results demonstrate that M4 activation attenuates dopamine release via H_2O_2 production in direct pathway medium spiny neurons, a signaling pathway that may be of therapeutic interest to developing novel treatments for schizophrenia.

Disclosures: **D.J. Foster:** None. **Z. Xiang:** None. **P.J. Conn:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Astra Zeneca, Bristol Meyer Squibb.

Poster

036. Muscarinic Acetylcholine and Metabotropic Glutamate Receptors

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 36.03/C14

Topic: B.03. G-Protein Linked Receptors

Support: NIH U01 MH087965

NIH R01 MH073676

NIH U54 MH084659

NIH R01 NS065867

Astrazeneca

Title: Evaluation of the therapeutic efficacy of M1 muscarinic acetylcholine receptor potentiation in chronic phencyclidine-treated mouse model of schizophrenia

Authors: *A. GHOSHAL, J. M. ROOK, J. W. DICKERSON, R. D. MORRISON, S. R. STAUFFER, C. K. JONES, J. S. DANIELS, C. M. NISWENDER, C. W. LINDSLEY, Z. XIANG, P. J. CONN;
Pharmacology, Vanderbilt Ctr. for Neurosci. Drug Discovery, Vanderbilt Univ. Med. Ctr., Nashville, TN

Abstract: Current available treatments for schizophrenia reduce the positive symptoms of the disease, but have no clear efficacy in reducing the negative and cognitive symptoms. Interestingly, a recent clinical study suggested that the M1/M4-preferring muscarinic acetylcholine receptor (mAChR) agonist xanomeline may have efficacy in reducing all symptom clusters in schizophrenia patients. However, xanomeline and other mAChR agonists induce profound adverse effects due to the absence of subtype selectivity and have failed to advance in further clinical development. In recent years, our lab and others have developed highly selective positive allosteric modulators (PAMs) of M1 that provide unprecedented subtype selectivity and are now advancing in preclinical and clinical development. In this study, we have characterized a highly selective and systemically active M1 PAM, VU0453595. It induces a robust potentiation of ACh-induced activation of M1 but has no activity at M2-M5. It also strongly potentiates an M1-dependent long term depression (LTD) mediated by 50 μM carbachol (CCh) observed at the layer II/III to layer V synapse of the rodent medial prefrontal cortex (mPFC). VU0453595 has an excellent pharmacokinetic profile with significant brain penetration and has robust efficacy in improving cognitive function in multiple rodent models. Moreover, this compound does not cause any adverse effects associated with previous M1 agonists. In this study, we also used VU0453595 to investigate its efficacy in a preclinical rodent model of schizophrenia. Chronic NMDA receptor antagonism has been shown to be a reliable model for the negative and cognitive symptoms of schizophrenia. We found that daily treatment of rats with phencyclidine (PCP; 10 mg/kg) for 7 days induced profound electrophysiological and behavioral deficits. This treatment caused deficits in M1 receptor-mediated LTD in the mPFC induced with 50 μM CCh application. However, other responses to M1 activation in mPFC pyramidal neurons remained unaltered after the PCP administration as no deficits were observed in CCh-induced increases in spontaneous EPSCs and a CCh-induced inward current. We also observed profound cognitive deficits, including recognition memory, after chronic PCP treatment. Interestingly, pretreatment of 10 μM VU0453595 reversed the LTD deficits in the PCP-treated mice. Ongoing studies are looking at the behavioral efficacy of VU0453595 in the chronic PCP mouse model of schizophrenia. These results provide critical data in validating the potential utility of M1 PAMs as a novel therapeutic approach for treatment of negative and cognitive symptoms in schizophrenia patients.

Disclosures: **A. Ghoshal:** None. **J.M. Rook:** None. **J.W. Dickerson:** None. **R.D. Morrison:** None. **S.R. Stauffer:** Other; Astrazeneca and BMS. **C.K. Jones:** Other; Astrazeneca and BMS. **J.S. Daniels:** Other; Astrazeneca and BMS. **C.M. Niswender:** Other; Astrazeneca and BMS. **C.W. Lindsley:** Other; Astrazeneca and BMS. **P.J. Conn:** Other; Astrazeneca and BMS. **Z. Xiang:** None.

Poster

036. Muscarinic Acetylcholine and Metabotropic Glutamate Receptors

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 36.04/C15

Topic: B.03. G-Protein Linked Receptors

Support: MH87965

MH73676

MH84659

NS065867

Title: M1 muscarinic receptor expression levels influence the effect of positive allosteric modulators

Authors: *M. NOETZEL[1], J. M. ROOK[1], A. LAMSAL[1], R. D. MORRISON[1], C. HAN[1,2], H. P. CHO[1], C. M. NISWENDER[1], J. S. DANIELS[1], S. R. STAUFFER[1,2], C. W. LINDSLEY[1,2], P. J. CONN[1];
[1]Dept. of Pharmacol. and Vanderbilt Ctr. for Neurosci. Drug Discovery, [2]Dept. of Chem., Vanderbilt Univ., Nashville, TN

Abstract: There is evidence to suggest that selective enhancement of cholinergic transmission through the M1 muscarinic acetylcholine receptor (mAChR) may reduce or ameliorate the loss of cognitive function that is observed in patients with Alzheimer's disease. Previous efforts to develop compounds that selectively activate M1 receptors have failed in clinical trials due to a true lack of specificity and adverse effects associated with the activation of other mAChR subtypes. We have focused our efforts on the development of novel compounds that do not activate G-protein coupled receptors (GPCRs) directly, but rather act at allosteric sites on the receptor to potentiate the activation of the receptor by its endogenous ligand; in the case of the M1 receptor, acetylcholine (ACh). Even activation through the allosteric site does not guarantee that these compounds will not display adverse effects as demonstrated with other classes of GPCRs, such as metabotropic glutamate receptor subtype 5 where allosteric agonist activity or positive allosteric modulation (PAM) coupled with agonist activity (ago-PAM) was shown to be correlated with the induction of seizures; however, PAM activity alone did not result in the induction of seizures. In addition, previous research has demonstrated that allosteric modulators may display signaling bias which could have fundamentally different effects on CNS function and affect their utility as therapeutics in a compound dependent manner. We were interested in determining if M1 allosteric agonist activity can be correlated to adverse effects observed with administration of a set of M1 modulators. We utilized cells lines in which the M1 receptor was expressed using an inducible promoter. With increasing levels of receptor expression, there was

a shift from pure PAM activity to ago-PAM activity, but the level of receptor expression that was needed to induce the shift varied depending on the compound tested. Furthermore, the potency of the PAM response did not change significantly with the change in receptor expression. *In vivo* studies in rodents assessed the adverse effect liability of various M1 PAMs from multiple distinct chemical scaffolds relative to the degree of agonist activity observed in *in vitro* assays. Further studies are underway to gain insight into the role of receptor expression in allosteric modulator activity to avoid the potential for adverse effects.

Disclosures: **M. Noetzel:** None. **J.M. Rook:** None. **A. Lamsal:** None. **R.D. Morrison:** None. **H.P. Cho:** None. **C.M. Niswender:** None. **J.S. Daniels:** None. **S.R. Stauffer:** None. **C.W. Lindsley:** None. **P.J. Conn:** None. **C. Han:** None.

Poster

036. Muscarinic Acetylcholine and Metabotropic Glutamate Receptors

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 36.05/C16

Topic: B.03. G-Protein Linked Receptors

Title: The discovery of highly selective muscarinic M1 agonists: Application of novel structure-based drug design with G protein-coupled receptors

Authors: *A. BROWN[1], G. BROWN[2], M. CONGREVE[2], J. DIAS[3], E. HURRELL[1], M. PICKWORTH[2], F. MARSHALL[4];
[1]Pharmacol., [2]Chem., [3]Structural Biol., [4]Heptares Therapeut. Ltd, Welwyn Garden City, United Kingdom

Abstract: Despite significant research there remains a major unmet medical need in the treatment of Alzheimer's disease. Current marketed drugs (e.g. Donepezil, Aricept®) function through inhibition of acetylcholinesterase activity resulting in widespread enhanced cholinergic activity. However the cognitive benefits from targeting this mechanism are modest and are accompanied by a range of dose-limiting cholinergic side effects due to the non-specific activation of all muscarinic receptor sub-types. Pre-clinical and clinical evidence suggests that specific activation of the muscarinic M_1 receptor activation plays a critical role in the beneficial elemnts of acetylcholinesterase inhibitors in Alzheimer's disease whereas activation of muscarinic M_2 and M_3 receptors are responsible for many of the side effects. Our objective has been to utilise a novel structure based drug discovery approach to design highly selective agonists for the M_1 receptor devoid of dose limiting activity at other muscarinic receptors.

Heptares' have solved the structure of the muscarinic M_1 receptor in an agonist conformation with multiple agonist ligands and the structural insights gained from this work have been instrumental in the design of highly selective M_1 agonists with good ligand efficiency and drug-like properties. The lead compound emonstrates ~1000fold selectivity against the muscarinic M_2, M_3 and M_5 receptors and is active in a primary rat hippocampal CA1 cell firing model. Oral administration at 3, 10 and 30mg/kg reversed a scopolamine deficit in a rat passive avoidance model and increased discrimination following a delay induced deficit in the rat novel object recognition model with an ED_{50} of approximately 4mg/kg. In addition these effects were additive with donepezil. This work has led to the first highly selective M_1 agonist candidate that entered Ph I clinical trials in late 2013.

Disclosures: **A. Brown:** A. Employment/Salary (full or part-time):; Heptares Therapeutics Limited. **G. Brown:** A. Employment/Salary (full or part-time):; Heptares Therapeutics Limited. **M. Congreve:** A. Employment/Salary (full or part-time):; Heptares Therapeutics Limited. **J. Dias:** A. Employment/Salary (full or part-time):; Heptares Therapeutics Limited. **E. Hurrell:** A. Employment/Salary (full or part-time):; Heptares Therapeutics Limited. **M. Pickworth:** A. Employment/Salary (full or part-time):; Heptares Therapeutics Limited. **F. Marshall:** A. Employment/Salary (full or part-time):; Heptares Therapeutics Limited.

Poster

036. Muscarinic Acetylcholine and Metabotropic Glutamate Receptors

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 36.06/C17

Topic: B.03. G-Protein Linked Receptors

Support: ARC Grant DP110100687

NHMRC Grant 519461

Australian Postgraduate Award

Title: Novel bitopic ligands for the M_1 muscarinic acetylcholine receptor

Authors: *B. J. DAVIE, S. N. MISTRY, C. VALANT, B. CAPUANO, P. J. SCAMMELLS, A. CHRISTOPOULOS;
Monash Inst. of Pharmaceut. Sci., Parkville, Australia

Abstract: Molecules capable of simultaneously interacting with the orthosteric pocket and an allosteric site of a G protein-coupled receptor are termed 'bitopic ligands'. Rationally designing such hybrid molecules by linking a high potency orthosteric pharmacophore to a subtype-selective allosteric pharmacophore may result in a high affinity/potency and more selective drug. The objective of this project is to synthesize and pharmacologically evaluate putative bitopic ligands for the M_1 muscarinic acetylcholine receptor (M_1 mAChR); a prospective therapeutic target for the treatment of the cognitive deficits experienced in Alzheimer's disease and schizophrenia. Iperoxo, a highly potent non-selective orthosteric mAChR agonist, and BQCA, a highly selective M_1 mAChR positive allosteric modulator, were selected as our building-block molecules. In order to rationally design bitopic ligands, we first investigated the effect of alkyl linkers of various lengths on each pharmacophore. Six compounds were synthesized and pharmacologically evaluated for the preservation of their respective orthosteric and allosteric binding and functional profiles, in M_1 mAChR-expressing Chinese Hamster Ovary cells, using both [^{3}H]NMS radioligand binding and ERK1/2 phosphorylation assays. Interestingly, despite most of our novel analogues showing diminished agonist and allosteric properties, they all appeared to retain their overall respective pharmacological profiles, indicating that the introduction of linkers is tolerated. Encouraged by these results, the synthesis of complete hybrid orthosteric/allosteric molecules for the M_1 mAChR was subsequently undertaken. We can conclude from our preliminary results that our novel putative bitopic ligand design exhibiting a linker between orthosteric and allosteric pharmacophores for the M_1 mAChR is synthetically accessible, and may lead to the generation of a ligand able to bridge both the orthosteric pocket and an allosteric site of the receptor via a bitopic mode of binding. Future work will require [^{3}H]NMS radioligand dissociation kinetics assays and mutagenesis studies to conclusively confirm a bitopic mode of action for these hybrid molecules. Bitopic ligands may be useful pharmacological tools to further study the M_1 mAChR, and potentially pave the way towards novel, improved therapeutics for cognitive deficits.

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Poster

036. Muscarinic Acetylcholine and Metabotropic Glutamate Receptors

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 36.07/C18

Topic: B.03. G-Protein Linked Receptors

Support: NIH Grant AG005214

Title: Mutations enable positive allosteric modulation by benzyl quinolone carboxylic acid (BQCA) at non-M1 muscarinic receptors

Authors: *J. ELLIS, G. J. ELMSLIE;
Psychiatry and Pharmacol., Penn State Univ., Hershey, PA

Abstract: The muscarinic acetylcholine receptors (mAChRs) comprise a family of G-protein-coupled receptors, all of which are expressed in the CNS. Physiological, behavioral, and knock-out mouse studies all suggest that these receptors play crucial roles in many CNS functions and disorders. Unfortunately, the structures of the five subtypes of mAChRs are very highly conserved in the transmembrane regions where ACh binds; this has hampered the development of subtype-selective agonists and antagonists. Additionally, the mAChRs also play essential roles in the autonomic system; this means that non-selective muscarinic agents have serious dose-limiting side effects. One approach to developing ligands with sufficient selectivity has been to target allosteric sites on the receptors, which are typically much less conserved than the orthosteric site. BQCA is a premier example of a positive allosteric modulator that is highly selective for the M_1 mAChR, which is a subtype whose enhancement is likely to benefit cognitive deficits, including those of Alzheimer's Disease. BQCA enhances the potency of ACh by 100-fold or more at M_1, while having no effect at the other subtypes. Such selectivity can be due either to highly selective affinity for M_1 or to neutral cooperativities at the other subtypes, and it is of significant interest to determine which mechanism is at work for this prototypical agent. We have used mutagenesis to identify residues that are key to the selective PAM activity. In agreement with others, we found that the aromatic nature of $M_1Y^{5.29}$ is essential (superscript numerals refer to the system of Ballesteros and Weinstein); Y=>A mutation abolishes activity, while Y=>F preserves it. Of the five subtypes, only M_5 lacks Y or F at this site. We found that the M_5Q=>$Y^{5.29}$ mutant displays modest PAM activity. Additionally, the PAM activity of the double mutant M_1E=>$A^{7.32}$,E=>$A^{7.36}$ is markedly attenuated, whereas the M_5YEE mutant has PAM activity equal to M_1. (The M_2EE mutant also exhibits moderate PAM activity). Finally, the interactions between BQCA and ACh in inhibiting the binding of the labeled orthosteric antagonist [^{3}H]N-methylscopolamine to M_1 vs M_1AA and M_5Y vs M_5YEE were evaluated using a mathematical allosteric model. The mutations caused marked changes in the cooperativity between BQCA and ACh, but did not alter the affinity of BQCA for the receptor. This indicates that the region near the top of TM7 that contains $E^{7.32}$ and $E^{7.36}$ is not part of the binding site. In agreement with this, BQCA was not found to be competitive with the allosteric modulator W84, which has previously been shown to bind within this region.

Disclosures: **J. Ellis:** None. **G.J. Elmslie:** None.

Poster

036. Muscarinic Acetylcholine and Metabotropic Glutamate Receptors

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 36.08/C19

Topic: B.03. G-Protein Linked Receptors

Support: Department of Pharmacology, University of Toledo

Title: Development of M5 muscarinic antagonists: Interaction of GZ-002-05 with muscarinic receptor subtypes

Authors: *W. S. MESSER, JR[1], T. YANG[2], C. MITCHELL[3], G. ZHENG[4];
[2]Pharmacol., [3]Neurosci., [1]Univ. of Toledo, TOLEDO, OH; [4]Pharmaceut. Sci., Univ. of Arkansas for Med. Sci., Little Rock, AR

Abstract: Selective antagonists for M_5 muscarinic receptors might be useful in the treatment of drug abuse. GZ-002-05 was identified previously as a novel, M_5-selective muscarinic antagonist. Muscarinic receptor selectivity was characterized by studying the effects of acetylcholine in the presence or absence of the compound using CHO cells expressing human M_1, M_3, or M_5 receptors. A [^{3}H] arachidonic acid release assay measured receptor activity and helped delineate the nature of the interaction(s) between the compound and muscarinic receptor subtypes. At M_1 receptors, 10 μM GZ-002-05 did not change the maximal response elicited by acetylcholine, however, it lowered significantly the EC_{50} value for acetylcholine from 1.8 μM to 36 μM. The data suggest a competitive interaction at M_1 receptors. In contrast, at 10 μM GZ-002-05 changed neither the maximal response nor the EC_{50} value of acetylcholine at M_3 receptors, suggesting that the compound did not influence agonist activation of M_3 receptors at that concentration. At M_5 receptors, 10 μM GZ-002-05 reduced the maximal response produced by acetylcholine (72 ± 8.8% of control stimulation) with no significant effect on the EC_{50} value, suggesting that the compound does not interact in a competitive manner at M_5 receptors. Overall, the results indicate unique mechanisms of interactions with muscarinic subtypes. The data suggest that GZ-002-05 may be useful as a lead compound in the development of potential therapeutic agents, however, further work is needed to understand how it acts at each muscarinic receptor subtype.

Disclosures: **W.S. Messer:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Dr. Messer holds patents for muscarinic receptor ligands.. **T. Yang:** None. **C. Mitchell:** None. **G. Zheng:** None.

Poster

036. Muscarinic Acetylcholine and Metabotropic Glutamate Receptors

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 36.09/C20

Topic: B.03. G-Protein Linked Receptors

Support: Nancy Lurie Marks Family Foundation

Lefler Center for the Study of Neurodegenerative Disorders Postdoctoral Fellowship

NIH Grant T32 NS07484-15

NIH Grant R01 NS046579

Title: Cholinergic modulation of PKA activity

Authors: *Y. CHEN[1,2], B. SABATINI[1,2];
[1]Neurobio., Harvard Med. Sch., BOSTON, MA; [2]Howard Hughes Med. Inst., Boston, MA

Abstract: Neuromodulators have profound effects on circuits and behavior, but the dynamics of their intracellular effectors has remained unclear. The adenylate cyclase/cyclic AMP/protein kinase A (PKA) module is a critical intracellular effector. This module is regulated by multiple behaviorally important neuromodulator receptors. Furthermore, PKA activity is necessary for the induction of many forms of synaptic plasticity and for the formation of long-term memory. In order to monitor PKA activity in brain tissue, we have developed a PKA activity sensor for quantitative analysis of endogenous GPCR signaling via 2-photon fluorescence lifetime imaging microscopy. We have used the PKA reporter to examine cholinergic modulation of PKA activity in the mouse hippocampus. Our results reveal new mechanisms for cholinergic action, with implications on synaptic transmission and plasticity.

Disclosures: **Y. Chen:** None. **B. Sabatini:** None.

Poster

036. Muscarinic Acetylcholine and Metabotropic Glutamate Receptors

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 36.10/C21

Topic: B.03. G-Protein Linked Receptors

Support: NIH/NIDCD Grant DC008794

NIH/NEI Grant EY022338

Title: Modulatory effects of activation of presynaptic metabotropic glutamate receptors on the inhibitory responses in thalamic relay cells in the mouse

Authors: *T. LIU, I. PETROF, S. M. SHERMAN;
Neurobio., Univ. of Chicago, Chicago, IL

Abstract: We have previously shown that activation of presynaptic group II metabotropic glutamate receptors (mGluRs) reduces the gain of GABAergic inputs in both primary visual and auditory cortices (V1 and A1). In the present study, we sought to determine if activation of mGluRs can also affect the inhibitory input in thalamus. We used whole cell recording in a slice preparation in the mouse and studied two GABAergic inputs to relay cells: that of the thalamic reticular nucleus (TRN) to cells of the ventral posteromedial nucleus (VPM), activated by glutamate uncaging and electrical stimulation in TRN, and that of interneurons to cells of the lateral geniculate nucleus (LGN), activated disynaptically by electrical stimulation of the optic tract. We tested effects of mGluRs on these inputs by applying various agonists to the slice. In the pathway involving stimulation in TRN and recording in VPM, we found that activation of mGluRs significantly reduced the amplitude of IPSCs evoked from TRN inputs to VPM cells. Because the effects were not blocked by the addition of GDPβS to the recording electrode, and because mGluR agonists did not affect responses to photostimulation of GABA in a low Ca^{2+} and high Mg^{2+} bathing solution, we conclude that the reduction of the amplitude of GABAergic IPSCs was due to activation of presynaptic mGluRs. Furthermore, using specific mGluR agonists, we found that both group I and group II mGluRs were involved in these effects. Similar results were found in the interneuron inputs to LGN cells. Here, activation of presynaptic group I (especially type 1) and group II mGluRs significantly reduced the amplitude of evoked IPSCs. However, activation of type 5 mGluR increased the frequency of spontaneous IPSCs but not their amplitude in LGN cells, which we interpret to be via dendrodendritic synapses (F2 terminals). Nonetheless, it appears that groups I and II mGluRs may generally reduce the amplitude of evoked GABAergic IPSCs of axonal inputs to thalamic relay cells, operating through presynaptic mechanisms, and this extends our previous findings in cortex.

Disclosures: **T. Liu:** None. **I. Petrof:** None. **S.M. Sherman:** None.

Poster

036. Muscarinic Acetylcholine and Metabotropic Glutamate Receptors

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 36.11/C22

Topic: B.03. G-Protein Linked Receptors

Support: R01 AG 043430-01A1 to A.F.T.A.

Donation of Percy Sanguinetti Arnsten to L.E.J.

Donation of Elsie Louise Torrance Higgs to C.D.P.

Title: mGluR2/3 influences in prefrontal cortex - potential for therapeutics

Authors: *L. E. JIN[1], M. WANG[1], Y. YANG[1], D. OTTENHEIMER[2], J. STEIN[2], C. D. PASPALAS[1], A. F. ARNSTEN[1];
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Abstract: Schizophrenia is a complex mental disorder associated with impaired dorsolateral prefrontal cortical (dlPFC) function and abnormal glutamatergic transmission. Drugs acting on the group II metabotropic glutamate receptors (mGluR2/3) are of particular interest to the treatment of schizophrenia, especially as mGluR3 have been genetically linked to the illness. mGluR2/3 couple to Gi/Go and inhibit cAMP signaling. In rodent hippocampus, presynaptic mGluR2/3 reduce excitability by inhibiting neurotransmitter release, while postsynaptic mGluR2/3 have an opposite effect, increasing neuronal excitability by inhibiting K+ conductance. However, there is little knowledge of how mGluR2/3 influence higher cortical circuits in primates. Layer III dlPFC circuits in primates mediate the persistent activity needed for working memory via NMDA synaptic connections on spines. These circuits are of key interest, as they are greatly afflicted in patients with schizophrenia, causing dlPFC hypo-activity and cognitive impairments. We here present immuno electron microscopy data from monkey dlPFC layer III circuits showing mGluR2/3 on both pre- and postsynaptic sites, as well as on astrocytes that envelope the axospinous glutamate synapses. To test the roles of mGluR2/3 in regulating working memory, we recorded single-units in monkey dlPFC while the animals performed a spatial working memory task. Compounds targeting mGluR2/3 were applied by iontophoresis during recording. Low doses of the mGluR2/3 agonist, (2R,4R)-APDC, significantly improved the spatially-tuned persistent firing that is needed for working memory. At higher doses, the persistent firing was eroded, producing an inverted-U dose-response. These physiological data suggest that postsynaptic mGluR2/3 may be positioned on spines to inhibit cAMP-K+ channel signaling and strengthen dlPFC network firing at low doses, while firing may be decreased by presynaptic inhibition of glutamate release at higher doses. An inverted-U dose-response was also seen at the behavioral level in monkeys following systemic administration of APDC, with enhancing effects on working memory performance at low doses, and mixed effects

or impairment at higher doses. No evidence of side effects was observed. Local infusion of low doses of (2R,4R)-APDC into rat medial prefrontal cortex also improved working memory performance. Overall, the data indicate that mGluR2/3 modulate working memory circuits in PFC, and if their beneficial actions can be isolated, may have therapeutic potential in treating cognitive deficits in schizophrenia.

Disclosures: L.E. Jin: None. **M. Wang:** None. **Y. Yang:** None. **D. Ottenheimer:** None. **J. Stein:** None. **C.D. Paspalas:** None. **A.F. Arnsten:** None.

Poster

036. Muscarinic Acetylcholine and Metabotropic Glutamate Receptors

Location: Halls A-C

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Topic: B.03. G-Protein Linked Receptors

Support: NINDS Grant NS031372

PhRMA Foundation Postdoctoral Fellowship

Title: Activation of metabotropic glutamate receptor 3 (mGlu3) is required for the induction of long-term depression in medial prefrontal cortex and fear extinction learning

Authors: *A. G. WALKER, C. J. WENTHUR, Z. XIANG, C. W. LINDSLEY, P. J. CONN; Pharmacol. & VCNDD, Vanderbilt Univ. Med. Ctr., Nashville, TN

Abstract: There has been growing interest in the role of group II mGlus (mGlu2 and mGlu3) in the regulation of cognition. In rodents, pharmacological activation of mGlu2/3 in medial prefrontal cortex (mPFC) results in long-term depression (LTD) that is expressed postsynaptically. Interestingly, anatomical studies have indicated that mGlu2 and mGlu3 have distinct pre- and postsynaptic expression, suggesting they may differentially influence neurophysiology and behavior. Unfortunately, the individual contributions of mGlu2 and mGlu3 to cognition and LTD have yet to be investigated, largely due to a lack of compounds that can differentiate between the subtypes. We recently reported a series of selective mGlu3 negative allosteric modulators (NAMs) and have performed a series of studies to determine the role of mGlu3 in LTD and cognition. We recorded extracellular field excitatory postsynaptic potentials (fEPSPs) from layer V of mouse prelimbic mPFC in response to stimulation of layer II/III. Application of the mGlu2/3 agonist LY379268 (LY268; 30-100nM for 10 min) produced a

concentration dependent inhibition of the fEPSP slope with LTD observed at 100nM. Analysis of paired-pulse ratios (PPR) demonstrated that the initial inhibition was accompanied by a robust increase in PPR that decreased to baseline levels during the LTD phase, indicating it is expressed postsynaptically. LTD was blocked by the mGlu2/3 antagonist LY341495 (500nM), but not AP5, indicating NMDA receptor activation is not required. When LY268 was applied in the presence of a selective mGlu3 NAM, VU0469942 or VU0477950 (10uM), only transient inhibition, but no LTD was induced. Moreover, LY268 induced LTD in slices from mGlu2, but not mGlu3 knockout mice. To test the hypothesis that mGlu3 mediated effect is postsynaptic in nature, we monitored intracellular calcium (Ca2+) in layer V pyramidal neurons. Experiments were performed in the presence of tetrodotoxin to isolate postsynaptic receptor actions. Application of LY268 induced an increase in Ca2+ that was attenuated by VU0469942. Taken together, these data indicate that postsynaptic mGlu3 activation is required for induction of LTD in mPFC by an mGlu2/3 agonist. We also tested the effects of the mGlu3 NAM VU0477950 on fear extinction, a cognitive behavior dependent upon mPFC. Relative to vehicle, animals treated with VU0477950 (3-100mg/kg i.p.) displayed a dose dependent delay in fear extinction learning. Thus, our physiology and behavioral experiments provide evidence that mGlu3 strongly influences the function of circuits governing cognition and indicate this receptor as a putative target for treatment of disorders of impaired cognition.

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Poster

036. Muscarinic Acetylcholine and Metabotropic Glutamate Receptors

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

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Topic: B.03. G-Protein Linked Receptors

Support: CIHR grant TGS-109219 to BRS

Title: Sleep-deprivation induces changes in GABA-B and mGlu receptors and reduces the depressant action of agonists at the above receptors on excitatory postsynaptic potentials in rat hippocampal CA1 neurons

Authors: A. KWOK, J. HWANG, H. AZIZI, R. TADAVARTY, *B. SASTRY;
Univ. British Columbia Fac Med., Vancouver, BC, Canada

Abstract: Sleep is a necessity for a majority of animal species and it facilitates memory consolidation. Sleep-deprivation (SD) adversely affects synaptic plasticity and memory consolidation. Previous studies in our laboratory indicate that SD induces an increase in the expression and heterodimerization of $GABA_B$-R1 and mGlu1αR in the CA1 region of rat hippocampus. In the current study, we examined if the SD induced changes in $GABA_B$ and mGlu receptors translate into alterations in the actions of applied agonists. We also tested if the changes recover with time. Experiments were conducted on 3-4 week old male Wistar rats that were (a) allowed to normally sleep, (b) SD for 12 hrs, and (c) SD but allowed to recover for 48 hrs. Field excitatory postsynaptic potentials (EPSPs) were recorded from the CA1 pyramidal layer in response to the stimulation of stratum radiatum, in the hippocampal slice preparation. Agonists were applied to the slice-superfusing medium. The presence of $GABA_B$-R1, $GABA_B$-R2 and mGlu1αR was assessed using immunohistochemistry. $GABA_B$-R1 immunoreactivity in the CA1 pyramidal neurons was enhanced following SD but returned to control levels with a 48 hr recovery from SD. $GABA_B$-R2 expression was not changed following SD, but with the 48 hr recovery, CA1 interneurons showed more staining and pyramidal neurons less staining than in control or SD slices. Immunoreactivity for mGlu1αR, which was increased in SD rats, returned to controls with a 48 hr recovery from SD. We examined the actions of baclofen (GABA-B agonist; 0.25, 0.5, 1.0, 5.0 and 10 μM) and 1-Amino-1,3-dicarboxycyclopentane **(**ACPD, broad spectrum mGluR agonist; 1, 5, 10, 20 and 40 μM). In hippocampal slices from normally sleeping rats, ACPD depressed the EPSP in a dose dependent manner (EPSP as a % of pre-drug control in 10 μM ACPD: 33.95 $\pm$ 8.93, n=8). In SD animals, the depressant action was reduced (EPSP in 10 μM ACPD as a % of control: 57.28 $\pm$ 10.76, n=9). After a 48 recovery from SD, the EPSP was depressed more than in SD (EPSP in μM ACPD as a % of control: 51.82 $\pm$ 11.92, n=6). In slices from normally sleeping animals, baclofen depressed the EPSP in a dose dependent manner (EPSP in 0.5 μM baclofen as a % of pre-drug control: 49.40 $\pm$ 8.62, n=6). In SD animals, the depression was reduced (EPSP in baclofen as a % of control: 90.99 $\pm$ 3.16, n=8). After a 48 recovery from SD, the EPSP was depressed more than in SD animals (EPSP in baclofen as a % of control: 66.06 $\pm$ 5.04, n=6). In conclusion, $GABA_B$-R1 and mGlu1αR and the actions of applied mGlu and $GABA_B$ agonists change following a 12 hr SD and the changes subside with a 48 hr recovery from SD.

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Poster

036. Muscarinic Acetylcholine and Metabotropic Glutamate Receptors

Location: Halls A-C

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Program#/Poster#: 36.14/C25

Topic: B.03. G-Protein Linked Receptors

Support: Jean Templeton Hugill Anesthesiology Research Fund

Title: Metabotropic glutamate group II receptors modulate presynaptic release of GABA in nociceptive thalamus

Authors: *K. A. ASSERI;
Dept. of Anesthesiology, Pharmacol. and Therapeut., Univ. of British Columbia, Vancouver, BC, Canada

Abstract: Metabotropic glutamate receptors (mGluRs) belong to the family C of G-protein coupled receptors. Activation of group II mGluRs inhibits neurons by altering Ca2+ and K+ conductances. Group II mGluRs may regulate release of neurotransmitters at central synapses. Ventrobasal thalamic nuclei process somatosensory information including pain impulses. A mixed group I/II mGluR agonist was used in previous thalamic studies to assess mGluRs involvement in extracellular responses. We examined the analgesic effects of the selective group II agonist (LY354740) on ventrobasal neurons. We hypothesize that this inhibitory action would decrease the release of GABA by activating group II mGluRs on nerve terminals on thalamocortical neurons of ventrobasal nuclei. Whole-cell patch clamp recordings were performed at 23-25°C from Sprague-Dawley rat (P11-12) brain slices. The main sensory input (medial lemniscus) and ventrobasal neurons were visually identified with a differential interference contrast microscope. Inhibitory postsynaptic currents (IPSCs) were evoked by electrical stimulation of medial lemniscus. Kynurenic acid was applied to block ionotropic glutamatergic transmission, isolating the IPSCs. Internal cesium was used to block postsynaptic actions of agonist. Drugs were applied in the bathing solution. When applied alone, group II mGluR agonist (LY354740) had no effects on active or passive membrane properties in 32 neurons. In a concentration-dependent manner, LY354740 reduced the peak amplitude of evoked IPSCs without affecting the decay time constants. With or without action potential-evoked release, LY354740 respectively reduced the frequency of spontaneous and miniature IPSCs. These reductions were blocked by co-application of LY341495, a selective group II mGluR antagonist. When applied alone, LY341495 increased the amplitude of lemniscal IPSCs and increased the frequency of miniature IPSCs, suggesting tonic activation of group II mGluRs. All IPSCs were antagonized by the GABAA antagonist bicuculline. The studies demonstrate that activation of group II mGluRs decreases the release of GABA in ventrobasal nuclei. As heteroreceptors, presynaptic group II mGlu receptors may regulate the release of GABA in thalamic neurons, providing potential target for antinociceptive drugs.

Disclosures: **K.A. Johnson:** None. **D.M. Lovinger:** None.

Poster

036. Muscarinic Acetylcholine and Metabotropic Glutamate Receptors

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 36.15/C26

Topic: B.03. G-Protein Linked Receptors

Support: NIAAA DICBR

Title: Modulation of striatal synaptic transmission by group I metabotropic glutamate receptors

Authors: *K. A. JOHNSON, D. M. LOVINGER;
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Abstract: Group I metabotropic glutamate receptors, which include subtypes mGlu1 and mGlu5, modulate glutamatergic and GABAergic transmission in the CNS via diverse, synapse-specific mechanisms. Activation of postsynaptic group I mGlu receptors has been shown to reduce both glutamatergic and GABAergic transmission in medium spiny neurons (MSNs) in the dorsal striatum via retrograde endocannabinoid signaling and activation of presynaptic CB1 receptors. Interestingly, mGlu receptor-mediated suppression of GABA transmission occurs at more hyperpolarized membrane potentials ("down" state), whereas group I mGlu receptors only substantially inhibit glutamate transmission at slightly depolarized ("up" state) membrane potentials. While group I mGlu receptor-mediated suppression of GABA transmission is readily reversible, we and others have observed long-term depression (LTD) of glutamatergic transmission following treatment of mouse striatal slices with the group I mGlu receptor-selective agonist DHPG. Intriguingly, the differential state-dependence and time course of synaptic modulation suggest that divergent mechanisms contribute to mGlu receptor-induced retrograde endocannabinoid signaling at different inputs to MSNs. Previous studies indicate that activation of striatal group I mGlu receptors with the selective agonist DHPG increases production of 2-arachidonoylglycerol (2-AG) but not anandamide, suggesting that 2-AG is the primary mediator of group I mGlu receptor-induced suppression of synaptic transmission. To test this hypothesis, we evaluated the ability of DHPG to inhibit glutamatergic transmission in mice lacking diacylglycerol lipase α (DGLα), the enzyme primarily responsible for 2-AG production in the striatum. Surprisingly, DHPG produced a significant inhibition of glutamatergic transmission in slices obtained from DGLα knockout mice, suggesting that alternative mechanisms contribute to the ability of group I mGlu receptors to modulate synaptic

transmission in MSNs. Ongoing studies will further elucidate the mechanisms underlying the state-dependent and input-specific modulation of neurotransmission in MSNs by group I mGlu receptors.

Disclosures: **K.A. Johnson:** None. **D.M. Lovinger:** None.

Poster

036. Muscarinic Acetylcholine and Metabotropic Glutamate Receptors

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 36.16/C27

Topic: B.03. G-Protein Linked Receptors

Title: Adaptive changes in the expression of grm4 and grm5 genes in the striatum following induction of parkinsonism in mice

Authors: M. CANNELLA[1], M. MOTOLESE[1], D. BUCCI[1], G. MOLINARO[1], A. TRAFICANTE[1], J. MARROCCO[2], V. BRUNO[1,3,2], F. NICOLETTI[1,3,2], *G. BATTAGLIA[1]; [1]Neurosci., I.R.C.C.S. Neuromed, Pozzilli, Italy; [2]I.R.C.C.S. Neuromed, Sapienza University, Italy, and Univ. of Lille, France, LIA (International Associalted Laboratories), Roma, Italy; [3]Physiol. and Pharmacol., Univ. Sapienza, Roma, Italy

Abstract: It is generally believed that neuroadaptive changes occurring in the indirect pathway of the basal ganglia motor circuit delay the onset of motor symptoms associated with parkinsonism. The identification of these changes may add further insights into the pathophysiology of parkinsonism and better define the time window of intervention with different classes of antiparkinsonian agents. Parkinson's disease (PD) is characterized by the progressive loss of nigro-striatal neurons that lead to a decreased activity of the direct pathway and an increased activity of the indirect pathway, with ensuing inhibition of thalamocortical neurons and motor dysfunction. Activation of mGlu4 metabotropic glutamate receptors inhibits GABA release at the synapse between striatal projection neurons and neurons of the external globus pallidus. This is the first synapse of the indirect pathway of the basal ganglia motor circuit, which is overactive in PD. Drugs that activate mGlu4 receptors cause a robust depression of inhibitory synaptic transmission in the external globus pallidus, relieve motor symptoms and induce neuroprotective effects in animal models of parkinsonism. It is generally believed that during the early "compensated" phase of PD a number of mechanisms reduce the activity of the indirect pathway. We examined the transcripts of mGlu4 receptors and other proteins involved in the regulation of basal ganglia motor circuit in mice challenged with the parkinsonian toxin, 1-

methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP, 30 mg/kg, i.p.) and with the dopamine receptor blocker, haloperidol (1 mg/kg, s.c.). We observed an up-regulation of mGlu4 receptors, CB1 receptors and preproenkephalin-A few days after MPTP and haloperidol injection and a decrease of A2A and mGlu5 receptors. To examine whether the increase in mGlu4 receptor transcript could represent an early compensatory mechanism, we measured the catalepsy in wild-type and mGlu4 receptor knockout mice at different time points (30 min, 1 h, 2 h, and 4 h) after haloperidol injection (0.5 and 1 mg/kg, s.c.). Wild-type and mGlu4 receptor knockout mice showed the same response to haloperidol at 30 min and 1 h (1 mg/kg); in contrast, catalepsy remained high at 2 and 4 h in mGlu4 receptor knockout mice as opposed to wild-type mice. The lower dose of haloperidol (0.5 mg/kg) induced a much higher response and catalepsy in mGlu4 knockout mice at all time points as compared to wild-type mice. These data suggest that an up-regulation of mGlu4 receptors in striatal projection neurons contributes to restrain the activity of the indirect pathway in the early phases of parkinsonism.

Disclosures: **M. Cannella:** None. **M. Motolese:** None. **D. Bucci:** None. **G. Molinaro:** None. **A. Traficante:** None. **V. Bruno:** None. **F. Nicoletti:** None. **G. Battaglia:** None. **J. Marrocco:** None.

Poster

036. Muscarinic Acetylcholine and Metabotropic Glutamate Receptors

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

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Topic: B.03. G-Protein Linked Receptors

Support: Fondazione Istituto Italiano di Tecnologia

Title: Corticostriatal metaplasticity of group I metabotropic glutamate receptors contributes to habit learning

Authors: B. GRECO[1], A. CAVACCINI[1], A. ROCCHI[1], M. TRUSEL[1], V. PAGET-BLANC[1], M. PENNUTO[2], *R. TONINI[1];
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Abstract: Goal-directed control of actions is the ability to adapt the behavior to obtain specific outcomes and to efficiently respond in changing situations. With repetition, actions become more automatic and habitual and are mainly driven by contextual stimuli. Neuronal projections from the limbic and associative cortical areas to the medial part of the dorsal striatum (*cognitive*

control system) are involved in the goal-directed actions and behavioral flexibility, while projections from sensory motor cortices to the dorsolateral striatum (*habit system*) in motor planning and habit formation. Recent theories propose that the cognitive control system and the habit system interact to determine action control. Neuromodulation of these systems could be important in the formation of goal-directed and habitual behaviors, and in the emergence of pathological behaviors such as drug addiction or compulsive disorders. Our study aimed at elucidating the role of synaptic neuromodulators in the neuronal processes underlying the shift from flexible goal-directed actions to habitual strategies that occurs naturally following repeated practice. To this end, we assessed the synaptic and behavioral impact of different training regimes of instrumental conditioning of nose poke for food reward, which promote either goal-directed (short-training) or habitual behavior (over-training). In the dorsolateral striatum (DLS), long-term depression (LTD) depends on the synaptic neuromodulator endocannabinoids (eCBs), released upon activation of group I metabotropic glutamate receptors (mGluR1/5) and activation of dopamine D2 receptors. In over-trained habitual mice, we found that eCB-mediated LTD, induced upon pharmacological activation of mGluR1/5, was specifically lost at cortical connections to the striatal medium spiny neurons of the striatopallidal pathway. This associated with reduced efficacy of the intracellular cascades downstream from mGluR1/5 activation, raising the possibility that training-induced metaplasticity of mGluR1/5 signaling and adaptations of eCB pathway may contribute to habit formation. At the behavioral level, to test whether mGluR1/5 receptors were actively recruited during instrumental learning, we used a selective mGlur5 antagonist, either administered systemically or selectively within the DLS during training. We found that *in-vivo* treatment with the antagonist restored aspects of goal-directed behavior in over-trained mice. All together, our data indicate that the functional interplay between mGluR5- and eCB signaling modulates the shift from goal-directed to habitual behavior under physiological conditions.

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Poster

036. Muscarinic Acetylcholine and Metabotropic Glutamate Receptors

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Topic: B.03. G-Protein Linked Receptors

Support: NIH Grant NS37436

Title: Activation of metabotropic glutamate receptor 1 overcomes NMDA receptor-mediated excitotoxicity

Authors: *H. A. HATHAWAY, S. PSHENICHKIN, J. T. WROBLEWSKI;
Pharmacol., Georgetown Univ., Washington, DC

Abstract: Glutamate is the predominant excitatory neurotransmitter in the mammalian brain. As such, glutamate-induced signal transduction is vital for the survival and health of neurons, especially during development. A frequently utilized model to study developmental elimination of neurons is primary cultures of cerebellar granule cells. In this system, high extracellular potassium (25 mM KCl, K25) maximizes cell viability, whereas reduction of potassium to physiological levels (5 mM KCl, K5) causes apoptosis by a mechanism that is analogous to removal of trophic support. Using this model of potassium deprivation in cerebellar granule cells, we have previously shown that addition of bath glutamate protects neurons from K5 toxicity, as measured by MTT assay to quantify cell viability. This neuroprotective action of glutamate is specifically mediated by activation of metabotropic glutamate 1 (mGlu1) receptors. Activation of mGlu1 receptors stimulates numerous signal transduction cascades, including canonical Gq-coupled hydrolysis of membrane phosphoinositides. We have found that mGlu1 receptor-meditated neuroprotection occurs via a Gq protein-independent mechanism. Additionally, some agonists of mGlu1 receptors (eg quisqualate) have maximum efficacy for stimulating phosphoinositide hydrolysis but no efficacy for protecting neurons from K5 toxicity, whereas glutamate has full efficacy for both of these mGlu1 receptor-mediated outcomes. Although glutamatergic signaling can be neuroprotective, under pathophysiological conditions (such as traumatic brain injury or ischemia) excess quantities of glutamate are released into the extracellular space and cause cell death. This excitotoxicity is mediated by NMDA receptor activation and subsequent increases in intracellular calcium. As cerebellar granule cells express both NMDA receptors and mGlu1 receptors, we hypothesized that neuroprotective mGlu1 receptor activation overcomes excitotoxic NMDA receptor activation in our model. To study this we measured viability of cerebellar granule cells in the presence of glutamate. Here we report that under these conditions a reduction of mGlu1 receptor activity, either by pharmacological blockade or reduced receptor expression, results in a decrease in cell viability that is blocked by antagonism of NMDA receptors.

Disclosures: **H.A. Hathaway:** None. **S. Pshenichkin:** None. **J.T. Wroblewski:** None.

Poster

036. Muscarinic Acetylcholine and Metabotropic Glutamate Receptors

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Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 36.19/C30

Topic: B.03. G-Protein Linked Receptors

Title: Epigenetic down-regulation of type-2 metabotropic glutamate receptors is linked to selective neuronal vulnerability following global transient brain ischemia

Authors: M. MOTOLESE[1], M. CANNELLA[1], F. MASTROIACOVO[1], A. GAGLIONE[1], B. RIOZZI[1], L. DI MENNA[1], G. BATTAGLIA[1], V. BRUNO[2,1], *F. NICOLETTI[3,1,4]; [1]Neurosci., I.R.C.C.S. Neuromed, Pozzilli, Italy; [2]Physiol. and Pharmacol., [3]Univ. Sapienza, Rome, Italy; [4]I.R.C.C.S. Neuromed, Sapienza University, Italy, and Univ. of Lille, France, LIA (International Associalted Laboratories), Roma, Italy

Abstract: Recent evidence suggests that epigenetic mechanisms play a critical role for risk, onset, and progression of ischemia. Epigenetics refers to functionally relevant modifications of the genome that do not involve changes in DNA sequence, and include chemical marks that regulate gene transcription. At molecular level epigenetic phenomena are mediated by a variety of mechanisms including DNA methylation and histone acetylation. In our study we aimed at identifying epigenetic marks of ischemic brain damage, and examining the possibility that a pathological gene programming induced by ischemia can be targeted by specific drugs. We used the 4-vessel occlusion (4VO) model of transient global ischemia in rats. Rats subjected to 4VO show selective degeneration of hippocampal CA1 pyramidal neurons, whereas neurons of the CA3 region are relatively spared. We examined the expression of DNA-methyltransferases (DNMTs) and histone deacetylases (HDACs), in CA1 and CA3 regions at different time that precede neuronal death following ischemia/reperfusion (6, 12 and 24 hours). We found that global ischemia caused a substantial increase in DNMT3a and HDAC2 expression in the vulnerable CA1 region at 12 hours after reperfusion. We then examined the expression profile of different metabotropic glutamate (mGlu) receptor subtypes, which have an established role in mechanisms of neurodegeneration/neuroprotection. We found that global ischemia caused a substantial and CA1 region-specific down-regulation of mGlu2 receptors, which are known to be regulated by HDAC2. Other mGlu receptor subtypes were unchanged. This suggests an hypothetical epigenetic cascade in which global ischemia up-regulates HDAC2 in vulnerable neurons, thereby suppressing the expression of mGlu2 receptors. These changes may be compensatory because pharmacological amplification of mGlu2 receptor function with a positive allosteric modulator enhanced neuronal death in the CA1 region. We are currently examining whether blockade of the residual mGlu2 receptors with a negative allosteric modulator protects vulnerable CA1 pyramidal neurons against ischemic damage.

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Poster

036. Muscarinic Acetylcholine and Metabotropic Glutamate Receptors

Location: Halls A-C

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Topic: B.03. G-Protein Linked Receptors

Title: mGlu1 receptors epigenetically restrain the expression of mGlu5 receptors in the cerebellum

Authors: *S. NOTARTOMASO[1], C. ZAPPULLA[1], G. MASCIO[1], M. MOTOLESE[1], M. CANNELLA[1], P. SCARSELLI[1], R. GRADINI[1], G. BATTAGLIA[1], V. BRUNO[1,2,3], F. NICOLETTI[1,2,3];
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Abstract: We moved from our recent finding that mGlu1 metabotropic glutamate receptors are down-regulated in Purkinje cells of mutant mice modeling type-1 spinocerebellar ataxia (SCA1). Surprisingly, the loss of mGlu1 receptors was associated with the appearance of mGlu5 receptors in Purkinje cells of these mice. Expression of mGlu5 receptors was prevented by treatment of SCA1 mice with the mGlu1 receptor enhancer, RO0711401 (Notartomaso et al., Mol. Brain, 2013). mGlu5 receptors are found in Purkinje cells in the first two weeks of postnatal life. Afterwards, their expression declines, as shown by immunohistochemistry, immunoblotting, and measurements of mRNA levels by quantitative PCR. At the same time, expression of mGlu1 receptors is increased in Purkinje cells. Cerebellar maturation from PND9 to PND18 was associated with an increased methylation of the Grm5 gene promoter and a reduced methylation of the Grm1 gene promoter. We measured polyphosphoinositide (PI) hydrolysis in cerebellar slices using DHPG as a mixed mGlu1/5 orthosteric agonist, and dissecting the mGlu1 and mGlu5 component of the stimulation with the aid of two selective negative allosteric modulators (MTEP for mGlu5 and JNJ6259658 for mGlu1 receptors). DHPG-stimulated PI hydrolysis was prominent at PND9, when both mGlu1 and mGlu5 receptors contributed to the action of the agonist. At PND16, the residual stimulation by DHPG was exclusively mediated by mGlu1 receptors. However, in mice pre-treated systemically with JNJ6259685 for 7 days, mGlu5 receptors were still functional at PND16. In adult mice, systemic treatment with JNJ6259685 restored the expression of mGlu5 receptors in Purkinje cells, and caused a substantial reduction in Grm5 methylation. These findings suggest that expression of mGlu5 receptors in Purkinje

cells is down-regulated by an epigenetic mechanism that is triggered by the activation of mGlu1 receptors. Perhaps this mechanism is relevant to processes of developmental plasticity that rely on the activation of mGlu1 receptors, such as the elimination of supernumerary climbing fibers.

Disclosures: **S. Notartomaso:** None. **C. Zappulla:** None. **G. Mascio:** None. **M. Motolese:** None. **M. Cannella:** None. **P. Scarselli:** None. **R. Gradini:** None. **G. Battaglia:** None. **V. Bruno:** None. **F. Nicoletti:** None.

Poster

036. Muscarinic Acetylcholine and Metabotropic Glutamate Receptors

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 36.21/C32

Topic: B.03. G-Protein Linked Receptors

Title: The role of metabotropic glutamate receptor signaling in sleep regulation in *Drosophila* melanogaster

Authors: ***S. LY**[1,2], N. NAIDOO[1], A. I. PACK[1];
[1]Ctr. for Sleep and Circadian Neurobio., [2]Neurosci. Grad. Group, Univ. of Pennsylvania, Philadelphia, PA

Abstract: Metabotropic glutamate receptors (mGluRs) modulate a wide range of processes in the central nervous system such as synaptic plasticity and neuronal signaling. In this study, we investigated the role of mGluR signaling in sleep and wake regulation in *Drosophila* melanogaster. While mammals express multiple forms of mGluRs, *Drosophila* melanogaster contains just one mGluR, known as DmGluRA, that is homologous to mammalian Group II mGluRs. We investigated the behavioral sleep effects of pharmacologically inhibiting DmGluRA by feeding female wCS10 flies food containing LY341495, a type II mGluR antagonist. LY341495-treated flies display total sleep reductions that are attributed to fewer and short sleep bouts during the day. Additionally, we examined the binding dynamics of DmGluRA and Homer, a sleep- and wake-regulating adaptor protein that links mGluRs to ionotropic glutamate signaling as well as to calcium signaling in the endoplasmic reticulum. Co-immunoprecipitation of DmGluRA and Homer confirm that DmGluRA and Homer physically interact in *Drosophila*. Furthermore, preliminary western blot analysis shows that levels of Homer/DmGluRA protein interaction are higher during the day than during the night. Our results indicate that mGluR signaling modulates daytime sleep in *Drosophila* and suggests that its involvement in sleep regulation may be mediated by binding to Homer proteins during the day.

These results have important implications for our understanding of the molecular mechanisms underlying sleep and wake and provide a link between sleep and other biological processes in the brain that depend on mGluR and Homer signaling.

Disclosures: **S. Ly:** None. **N. Naidoo:** None. **A.I. Pack:** None.

Poster

036. Muscarinic Acetylcholine and Metabotropic Glutamate Receptors

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 36.22/C33

Topic: B.03. G-Protein Linked Receptors

Support: Inserm ATIP AVENIR

UPMC PhD fellowship

Title: mGluR-dependent modulation of synaptic transmission in the lateral habenula

Authors: ***K. VALENTINOVA**[1,2], I. MOUTKINE[1,2], M. MAMELI[1,2];
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Abstract: The lateral habenula (LHb) is an excitatory structure controlling monoamine systems implicated in the encoding of motivational states. In this context, the excitatory inputs - from the globus pallidus - and inhibitory inputs - from the ventral tegmental area - onto LHb neurons drive avoidance, and reward-related behaviors respectively. Therefore fine tuning of excitatory and inhibitory transmission may control LHb neurons function and subsequent behaviors. However the cellular determinants modulating both excitatory and inhibitory synaptic inputs onto LHb neurons remain elusive. The group I metabotropic glutamate receptors (mGluRs) modulate synaptic transmission across the central nervous system. Further, mGluRs have been implicated in pathologies associated with LHb dysfunction, including depression, anxiety and addiction. Our hypothesis is that metabotropic glutamate receptors may represent a common substrate for the control of synaptic transmission onto LHb neurons. We employed patch-clamp recordings in brain slices from mice to test whether and how mGluRs regulate excitatory and inhibitory synaptic transmission onto LHb neurons. To assess whether mGluRs are present in the LHb we performed RT-PCR from microdissected LHb, which shows expression of both mGluR1 and mGluR5. Moreover, the selective agonist (RS)-3,5-dihydroxyphenylglycine (DHPG, 50 μM) induces an inward current, suggesting a functional expression of mGluRs in the LHb. We then

recorded evoked excitatory postsynaptic currents (EPSC) over time in voltage clamp mode from LHb neurons at -50 mV. We found that DHPG leads to a long-lasting depression of AMPA-mediated EPSCs (eLTD). Similarly, when recording evoked inhibitory PSC (IPSC), DHPG induces a long lasting reduction of GABAa currents (iLTD). We found different expression mechanisms underlying eLTD and iLTD. DHPG-induced eLTD occurs along with an increased paired-pulse ratio, indicative of a reduced neurotransmitter release. Further eLTD requires CB1 receptors (CB1R), as it is blocked by the CB1R antagonist Ness0327 (0.5 μM). Conversely, iLTD occurs in the presence of the CB1R antagonist, despite the functional expression of CB1Rs at GABAergic synapses. Indeed, the CB1R agonist WIN-55,212-2 (5 μM) decreased the IPSCs. These results suggest that the group I mGluRs reduce AMPA and GABAa-mediated synaptic transmission via different cellular mechanisms. In conclusion, mGluR modulation of synaptic transmission may represent a molecular mechanism relevant for LHb function in encoding motivation in physiological and pathological conditions.

Disclosures: **K. Valentinova:** None. **I. Moutkine:** None. **M. Mameli:** None.

Poster

036. Muscarinic Acetylcholine and Metabotropic Glutamate Receptors

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 36.23/C34

Topic: B.03. G-Protein Linked Receptors

Title: Production of xanthurenic acid from 3-hydroxykynurenine in rat and human brain *in vitro* and *in vivo*

Authors: *K. V. SATHYASAIKUMAR, M. TARARINA, H.-Q. WU, R. SCHWARCZ; Maryland Psychiatric Res. Center, Univ. of Maryland Sch. of Med., Baltimore, MD

Abstract: Xanthurenic acid (XA), a metabolite formed from 3-hydroxykynurenine (3-HK) in the kynurenine pathway, has been proposed to function as a modulator of glutamatergic neurotransmission in the central nervous system, since it inhibits the vesicular glutamate transporter (Neale et al., Neuropsychopharmacology, 2013) and is an agonist of Group II (mGlu 2 and mGlu 3) metabotropic glutamate receptors (Fazio et al., Curr. Neuropharmacol., 2012). The goal of the present study was to evaluate 3-HK as a substrate for the neosynthesis of XA and to identify the nature of the responsible enzyme(s) using rat and human brain tissue homogenates. In addition, we used rat cortical slices to study the mechanisms that modulate XA synthesis and examined the *de novo* synthesis of XA from 3-HK by microdialysis *in vivo*. XA

was identified and quantified by HPLC with electrochemical detection (Okech et al., BBRC, 2006). All experiments were designed in analogy to studies of the *de novo* synthesis of kynurenic acid (KYNA) from kynurenine (see Schwarcz et al., Nature Rev. Neurosci., 2012, for review). *In vitro*, production of XA from 3-HK was temperature-dependent and linear with time and substrate concentration. Synthesis in various rat brain regions was similar to the pattern seen in KYNA synthesis from kynurenine. XA production was enhanced in the quinolinate-lesioned striatum, indicating a non-neuronal localization of the synthesizing machinery. Use of BFF122 (Amori et al., JNC, 2009), a selective inhibitor of kynurenine aminotransferase II (KAT II), revealed that the majority of XA production in rat brain was catalyzed by KAT II (85% inhibition in tissue homogenate; 78% inhibition in slices). Similar results were obtained using human prefrontal cortex homogenates (74% inhibition), and confirmed using partially purified rat KAT II and recombinant human KAT II. Release of newly produced XA from cortical slices was influenced by ionic milieu, buffer composition and co-substrates. Thus, deletion of Na^+ increased XA release (+54%), while addition of 50 mM K^+ (-43%) or 50 µM veratridine (-72%) reduced XA release in cortical slices. Addition of the co-substrates pyruvate (+ 205%) or α-ketoglutarate (+109%) significantly increased XA release. *In vivo*, newly produced XA was recovered in striatal microdialysate samples after focal perfusion with 3-HK (10 µM). Basal values of XA in microdialysate were <4 nM. Jointly, these data confirm the cerebral production of XA from 3-HK both *in vitro* and *in vivo*. Further studies are needed to examine the role of this KAT II-catalyzed XA formation in the regulation of kynurenine pathway flux and function in physiology and pathology.

Disclosures: **K.V. Sathyasaikumar:** None. **M. Tararina:** None. **H. Wu:** None. **R. Schwarcz:** None.

Poster

036. Muscarinic Acetylcholine and Metabotropic Glutamate Receptors

Location: Halls A-C

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Program#/Poster#: 36.24/C35

Topic: B.03. G-Protein Linked Receptors

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Title: A photochromic allosteric modulator to control an endogenous G protein-coupled receptor with light

Authors: *S. PITTOLO[1], X. GÓMEZ-SANTACANA[2,3], K. ECKELT[4,5], X. ROVIRA[6,3,7,9], J. DALTON[10], C. GOUDET[6,7,9], J.-P. PIN[6,8,9], A. LLOBET[11], J. GIRALDO[3], A. LLEBARIA[2], P. GOROSTIZA[12,5];
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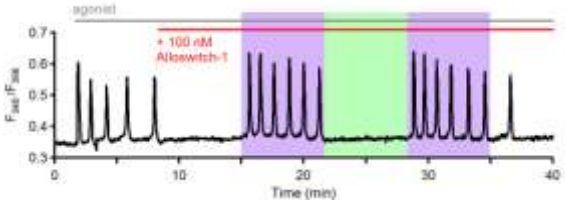
Abstract: Side effects of drugs are the major drawback for the use of any medicine in clinics, and depend mainly on small therapeutic windows and off-target actions. Obtaining a fine control over the temporal and spatial activity of a drug would allow precise dosing and localization of active drugs, respectively. These optimal therapeutic conditions cannot be achieved with any existing drug. Light can help solving the limitations of classical pharmacology. Optopharmacology is a recently developed technique that consists of attaching a light-sensitive moiety (usually azobenzene) to ligands that control endogenous proteins, so that drugs can be made available with the spatiotemporal precision of light. Still, optopharmacology was never applied before to the most interesting targets for drug development: allosteric modulators of G protein-coupled receptors (GPCRs). We report here the synthesis and characterization of the first light-sensitive allosteric modulator of the metabotropic glutamate receptor 5 (mGlu5), a class C GPCR, by a novel optopharmacological design. Instead of assembling azobenzene next to the ligand, like in previous optopharmacology, we integrated azobenzene inside a known allosteric ligand of mGlu receptors by simple substitution of few atoms in the parent compound. One of the obtained molecules (Alloswitch-1) was active with nanomolar potency and light wavelength-specificity as a negative allosteric modulator (NAM) of mGlu5. In cultured cells, Alloswitch-1 counteracts calcium oscillations induced by activation of both heterologous and endogenous mGlu5 receptors in the dark, and different light conditions (violet and green light) can be used to switch these oscillations on and off reversibly. In Xenopus tropicalis tadpoles, Alloswitch-1

controls the motility of animals in a light- and concentration-dependent manner. Alloswitch-1 is a novel and powerful tool for the optical control of an endogenous GPCR. Moreover, the uncommonly high success rate of our novel design strategy suggests that it can be extended to allosteric modulators of other GPCRs.



Disclosures: **S. Pittolo:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); patent application holder. **X. Gómez-Santacana:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); patent application holder. **K. Eckelt:** None. **X. Rovira:** None. **J. Dalton:** None. **C. Goudet:** None. **J. Pin:** None. **A. Llobet:** None. **J. Giraldo:** None. **A. Llebaria:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); patent application holder. **P. Gorostiza:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); patent application holder.

Poster

036. Muscarinic Acetylcholine and Metabotropic Glutamate Receptors

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 36.25/C36

Topic: B.03. G-Protein Linked Receptors

Support: NIH Grant DA010309

Title: Preso1 bidirectionally regulates group I mGluR phosphorylation

Authors: ***J.-H. HU**[1], P. F. WORLEY[2], D. A. HOFFMAN[1];
[1]NICHD, Bethesda, MD; [2]Johns Hopkins Univ., Baltimore, MD

Abstract: Group I metabotropic glutamate receptors (mGluR1/5) are enriched at excitatory synapses in the brain where they are important in neural plasticity and behavioral responses

including drug addiction, fear conditioning, inflammatory pain, and schizophrenia. Phosphorylation of mGluR1/5 at Homer binding site (TPPSPF), and subsequent Pin1 binding and isomerization of S-P bond are required for synaptic plasticity and cocaine motor sensitization. Therefore, it is a central goal to understand mechanisms that control dynamic mGluR5 phosphorylation. Here, we report that the scaffold protein Preso1 controls both phosphorylation and dephosphorylation of mGluR5. Previously, we reported that Preso1 scaffolds kinases that phosphorylate mGluR5 including CDK5 and Erk, and that Preso1 is required for activity-dependent mGluR5 phosphorylation. However, how mGluR5 is dephosphorylated remained elusive. Treatment of neurons with okadaic acid, a PP2A inhibitor, increases mGluR5 phosphorylation. PP2A dephosphorylates mGluR5 at the Homer binding site when expressed with mGluR5 in HEK293T cells. Furthermore, we identified the C-subunit of PP2A as a binding partner of Preso1 using Preso1 PDZ and FERM domains as bait in a yeast two hybridization screen. Binding is confirmed by co-IP when expressed in HEK293T cells. These data suggest that Preso1 not only serves as a scaffold protein for kinases but also for phosphatase. This dual functionality is similar to AKAPs. In support of the notion that Preso1 plays a role in recruiting PP2A to dephosphorylate mGluR5 in striatum, preliminary data shows that mGluR5 phosphorylation persists longer in striatum after cocaine administration in Preso1 KO mice compared to WT littermates. Moreover, behavioral analysis reveals that increased cocaine motor sensitization in Preso1 KO mice compared to WT littermates. Taken together, these biochemical and behavioral data suggest that Preso1 bidirectionally regulates mGluR5 phosphorylation by anchoring both kinases and phosphatase.

Disclosures: **J. Hu:** None. **P.F. Worley:** None. **D.A. Hoffman:** None.

Poster

036. Muscarinic Acetylcholine and Metabotropic Glutamate Receptors

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 36.26/C37

Topic: B.03. G-Protein Linked Receptors

Support: NIH Grant EY12354

NIH Grant EY014701

Title: Proper localization and function of trpm1 depends on LRIT3 expression in rod depolarizing bipolar cells

Authors: *R. G. GREGG[1], J. NOEL[2], K. M. HEATH[3], N. HASAN[3], T. RAY[4], M. A. MCCALL[5];
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Abstract: The retina processes light information through parallel ON and OFF pathways. Type 1 congenital stationary night blindness (CSNB1) is a human retinal disorder caused by disruption of ON pathway signaling within depolarizing bipolar cells (DBCs). In patients and mouse models, CSNB1 is characterized by night blindness and the absence of an ERG b-wave. Both patients and mice have normal photoreceptor function (ERG a-wave), but lack ON bipolar cell (BC) depolarization in response to light, due to absence of key components in the mGluR6 G-protein coupled cascade that ends with gating of the TRPM1 channel. CSNB1 mouse models have intact retinal morphology. Using a zinc-finger nuclease (ZFNs) strategy, we constructed a mouse lines with mutations in exon 2 of the leucine-rich repeat, immunoglobulin-like domain and transmembrane domain-containing protein 3 precursor (LRIT3). ZFNs injected into fertilized single cell embryos make double strand breaks in the target sequence, resulting in non-homologous end joining, and mutations, which can create null alleles. Using procedures approved by the University of Louisville Institutional Animal Care and Use Committee, we characterized LRIT3 mutant retinal function with ERG and rod DBC whole cell patch clamp as well as retinal morphology with immunohistochemistry. Similar to the published results in humans (Zeitz et al., 2013) with mutations in LRIT3, the dark- and light-adapted ERGs from the LRIT3$^{-/-}$ mice have a normal a-wave, although their b-wave is absent, a phenotype shared with other mouse models of CSNB1. Currents evoked in LRIT3$^{-/-}$ rod DBCs using either CPPG to gate the TRPM1 channel through the mGluR6 receptor or capsaicin to directly gate the TRPM1 channel produced small residual currents, similar to those found in TRPM1$^{-/-}$ rod DBCs and significantly smaller than WT. Consistent with these physiological differences between LRIT3$^{-/-}$ and WT mice, TRPM1 expression is absent from the LRIT3$^{-/-}$ DBC terminals. We conclude that LRIT3 is necessary for TRPM1 localization to DBC dendritic terminals and the absence of TRPM1 in LRIT3$^{-/-}$ retina leads to their CSNB1 phenotype. Zeitz C., et al., Am J Hum Genet. 2013 Jan 10;92(1):67-75.

Disclosures: **R.G. Gregg:** None. **J. Noel:** None. **K.M. Heath:** None. **N. Hasan:** None. **T. Ray:** None. **M.A. McCall:** None.

Poster

037. Postsynaptic Structure I

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 37.01/C38

Topic: B.07. Synaptic Transmission

Support: NIH/NIMH K99-MH101234

NIH/NIA R01-AG035071

Title: Significant differences in the structure and function of excitatory synapses in the rhesus monkey lateral prefrontal versus primary visual cortex

Authors: *J. I. LUEBKE, M. MEDALLA;
Anat. & Neurobio., Boston Univ. Sch. of Med., Boston, MA

Abstract: Excitatory synaptic response properties of layer 3 pyramidal neurons in the rhesus monkey lateral prefrontal cortex (LPFC) differ markedly from those in the primary visual cortex (V1). Spontaneous excitatory postsynaptic currents (EPSCs), exhibit significantly higher frequency, greater amplitude and slower rise and decay times in LPFC neurons, which computational models predict are due to higher gAMPA in LPFC than in V1 neurons (Amatrudo et al., 2012). Here, we sought to determine whether these distinct synaptic responses are due to differences in firing rates of presynaptic neurons, in postsynaptic entities, and/or in presynaptic entities. When action potentials were blocked with TTx, whole cell voltage clamp recordings revealed that, as with sEPSCs, TTx-insensitive miniature EPSCs exhibit significantly higher frequency, greater amplitude and slower kinetics in LPFC compared to V1 neurons demonstrating that differences in synaptic responses are not due to differences in the firing rates of presynaptic neurons. We next examined the size and distribution of spines across LPFC versus V1 neurons with confocal microscopy. As previously shown, LPFC neurons possessed significantly higher densities of spines of all subtypes compared to V1 neurons. K-means cluster analysis of spine head widths was used to separate the total population into small (< 0.52 um) and large spines. The mean spine width of large spines was significantly larger across the entire dendritic arbors of LPFC vs. V1 neurons. Electron microscopic quantification of asymmetric synapses in layers 2-3 neuropil demonstrated that while there was no difference in synapse density in LPFC and V1, there was a higher proportion of perforated axospinous synapses in LPFC (34 ± 1%) compared to V1 (20 ± 5%). The mean postsynaptic density (PSD) surface area of axospinous synapses was significantly greater in LPFC (0.11 ±0.01 μm2) compared to V1 (0.08 ±0.01 μm2), and perforated synapses were about twice as large. Consistent with the differences in spine and synapse size, the mean volume of axonal boutons in L2-3 of LPFC was significantly larger than of V1, due specifically to the population of boutons forming perforated synapses. In the LPFC there were approximately 1.6x more vesicles per bouton than there were in V1 boutons and the mean number of synaptic vesicles per bouton forming a perforated synapse was almost twice as high (LPFC, 746 ± 62 vs. V1, 438 ± 44). Taken together, these data are consistent with the idea that significantly more frequent and higher amplitude synaptic

currents in LPFC compared to V1 neurons are due, at least in part, to larger and more powerful synapses in LPFC.

Disclosures: **J.I. Luebke:** None. **M. Medalla:** None.

Poster

037. Postsynaptic Structure I

Location: Halls A-C

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Topic: B.07. Synaptic Transmission

Support: NIH/NIMH K99-MH101234

NIH/NIA R01-AG035071

Title: Distribution of dendritic spines and inhibitory inputs on layer 2 and layer 3 pyramidal neurons of the rhesus monkey anterior cingulate cortex

Authors: *M. MEDALLA, J. P. GILMAN, J. WANG, J. I. LUEBKE;
Anat. & Neurobio., Boston Univ. Sch. of Med., BOSTON, MA

Abstract: The role of the primate anterior cingulate cortex (ACC) in cognition remains poorly understood, but depends on pyramidal neurons that participate in diverse cortico-cortical and cortico-subcortical networks. The functional output of pyramidal neurons is largely determined by their intrinsic dendritic structure and excitatory and inhibitory synaptic connections, which are laminar specific. We compared the morphological features of layer 2 (L2) and layer 3 (L3) pyramidal neurons in the monkey ACC using intracellular filling, immunohistochemistry and confocal microscopy. The distribution of dendritic spines- the principal recipients of glutamatergic inputs- and inhibitory VGAT+ terminals apposed to distinct neuronal compartments was quantified. Dendritic arbors of L3 were larger and more complex than L2 neurons, exhibiting longer total dendritic lengths, more branch points, and spanning larger convex hull volumes and laminar extents. However the two populations do not differ significantly with regard to the distribution of dendritic spines or putative inhibitory inputs. Dendritic spine density along apical and basal dendritic arbors of both L2 and L3 neurons was lowest in the proximal third, highest in middle third, and progressively declined across the distal third of the arbors. Furthermore, the proportions of spine subtypes did not differ, being comprised of ~80% thin; ~10% mushroom; and ~8% stubby. The density of inhibitory VGAT+

appositions on apical and basal arbors was also similar between L2 and L3 neurons, which was highest in the proximal third and progressively declined distally. Majority (~72%) of these dendritic VGAT+ appositions were found along dendritic shafts and the rest were found on spines. VGAT+ puncta on spines were observed most frequently on thin spines, the most prevalent subtype. However thin spines with VGAT+ appositions comprise only a small (~14%) proportion of the total population of thin spines. In contrast, mushroom spines were selectively targeted by inhibitory inputs since 27-44% of mushroom spines (depending on layer and arbor) had VGAT+ appositions. In addition, VGAT+ appositions were present along axon initial segments (~30 μm from soma) in higher densities than along dendrites (L2: 0.7 ± 0.01 apps/μm axon vs. 0.4 ± 0.07/μm dendrite; L3: 0.8 ± 0.04 vs. 0.4 ± 0.05/μm). Moreover, axonal VGAT+ apposition density was significantly higher for L3 versus L2 neurons. In conclusion, this study provides detailed information on the topology of synaptic inputs to principal neurons in the primate ACC for the first time; such information is essential for a basic understanding of network function in this important brain area.

Disclosures: **M. Medalla:** None. **J.P. Gilman:** None. **J. Wang:** None. **J.I. Luebke:** None.

Poster

037. Postsynaptic Structure I

Location: Halls A-C

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Program#/Poster#: 37.03/C40

Topic: B.08. Synaptic Plasticity

Support: Thorberg's Foundation

American Heart Association 12GRNT16570006

NIH NS083858

Title: Mechanisms underlying spreading depolarization-induced dendritic beading

Authors: A. B. STEFFENSEN[1], J. SWORD[2], D. CROOM[2], S. A. KIROV[2,3], *N. MACAULAY[1];
[1]Univ. of Copenhagen, Copenhagen, Denmark; [2]Brain and Behavior Discovery Inst., [3]Dept. of Neurosurg., Georgia Regents Univ., Augusta, GA

Abstract: Spreading depolarizations (SDs) are waves of sustained neuronal and glial depolarization that propagate a massive disruption of ion gradients through the brain. SD is

associated with migraine aura and it was recently recognized as a novel mechanism of injury in stroke and TBI patients. SD leads to neuronal swelling and dendritic beading as assessed in real time with 2-photon laser scanning microscopy (2PLSM). Pyramidal neurons do not express aquaporins and thus display low inherent water permeability. Yet, SD rapidly induces focal swelling (beading) along the dendritic shaft. The molecular mechanism by which dendrites bead during SD remains elusive. To address this issue, we induced SD in mouse hippocampal slices by focal KCl-microinjection and visualized the ensuing dendritic beading by 2PLSM. We confirmed that dendritic beading failed to arise during large (100 mOsm) hyposmotic challenges, underscoring that neuronal swelling does not occur as a simple osmotic event. SD-induced dendritic beading was not prevented by pharmacological interference with formation of actin and stability of microtubules thus dendritic beading may entirely result from excessive water influx. Dendritic beading was strictly dependent on the presence of Cl- in the ACSF and accordingly, combined blockade of two Cl- -coupled transporters (K+/Cl- and bicarbonate cotransporters) in addition to a lactate transporter, led to a significant reduction in dendritic beading without interfering with SD. Furthermore, our *in vivo* 2PLSM data showed a strong inhibition of dendritic beading upon pharmacological blockage of these three cotransporters. We therefore propose that SD-induced dendritic beading takes place as a consequence of the altered driving forces for these cotransporters. As some of these transport mechanisms share the ability to transport water during their translocation mechanism, they may thereby generate dendritic beading by a mechanism independent of osmotic forces.

Disclosures: **A.B. Steffensen:** None. **J. Sword:** None. **D. Croom:** None. **N. MacAulay:** None. **S.A. Kirov:** None.

Poster

037. Postsynaptic Structure I

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Program#/Poster#: 37.04/C41

Topic: B.07. Synaptic Transmission

Support: NIH R01 MH 080046

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Netherlands Organization for Scientific Research (NWO-ALW-VENI)

FEBS Return-to-Europe Fellowship

Title: Triple miRNA Shank knockdown shows that Shank-cortactin interactions control actin dynamics to maintain flexibility of neuronal spines and synapses

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Abstract: The spine actin cytoskeleton forms a complex filamentous network required for maintenance of synaptic architecture and plasticity of excitatory transmission. However, we have little understanding of the molecular intermediates that permit spatially restricted control of actin polymerization over synaptic structure and function. The family of Shank scaffolding molecules (comprising Shank1, 2 and 3) are among the core components of the postsynaptic density (PSD). Shank proteins have multiple protein interaction domains that link surface glutamate receptors to other scaffolding molecules within the PSD, as well as to the actin cytoskeleton via diverse actin-binding proteins. Furthermore, Shank proteins are generally found in the deeper layers of the PSD facing the spine interior, and are thus ideally positioned to control actin cytoskeleton-PSD interactions. However, determining the function of Shank proteins in neurons has been complicated because the different Shank isoforms share a very high degree of sequence and domain homology. Therefore, to control spine Shank content while preventing potential compensatory effects, we developed a miRNA-based knockdown strategy to reduce the expression simultaneously of all synaptically targeted Shank isoforms in hippocampal neurons. Using this approach, we achieved a strong (>75%) reduction in total Shank protein levels at individual spines, prompting a ~40% decrease in mushroom spine density. Furthermore, miRNA-based Shank knockdown reduced spine actin levels and increased sensitivity to the actin depolymerizing agent Latrunculin A, an effect restored by re-expression of full-length miRNA-resistant human SHANK2. Interestingly, a SHANK2 mutant lacking the proline-rich cortactin binding motif (SHANK2-ΔPRO) was unable to rescue these defects, suggesting that Shank-cortactin interactions are required for the maintenance and stability of the spine actin cytoskeleton. Furthermore, Shank knockdown reduced cortactin levels in spines and increased the mobility of spine cortactin as measured by single-molecule tracking PALM, suggesting that Shank proteins recruit and stabilize cortactin at the synapse. Consistent with this model, we found that Shank knockdown significantly reduced spontaneous remodeling of synapse morphology, a phenomenon dependent on actin dynamics, and this could not be rescued by the SHANK2-ΔPRO mutant. We conclude that beyond their role as stable scaffolding molecules within the PSD, Shank proteins are key intermediates between the synapse and the spine interior that, via cortactin, permit the actin cytoskeleton to dynamically regulate synapse morphology and function.

Disclosures: **H.D. Mac Gillavry:** None. **J.M. Kerr:** None. **N.A. Frost:** None. **T.A. Blanpied:** None.

Poster

037. Postsynaptic Structure I

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 37.05/C42

Topic: B.07. Synaptic Transmission

Title: Syntaxin 4 is a postsynaptic t-SNARE required for synaptic growth and plasticity in *Drosophila*

Authors: ***K. P. HARRIS**[1], Y. AKBERGENOVA[1], R. W. CHO[1], Z. D. PICCIOLI[1], N. PERRIMON[2], J. LITTLETON[1];
[1]Biology; Brain and Cognitive Sci., MIT, Cambridge, MA; [2]Dept. of Genet., Harvard Med. Sch., Boston, MA

Abstract: Postsynaptic cells can induce synaptic plasticity through the release of retrograde signals in response to presynaptic activity. We have previously identified a calcium-dependent retrograde signaling pathway that is mediated by postsynaptic Synaptotagmin 4 (Syt4) at *Drosophila* neuromuscular junctions (NMJs). We hypothesize that Syt4 acts as a Ca2+ sensor to control the release of retrograde signals, similar to the role of Syt1 in presynaptic vesicle fusion. However, little is known about how postsynaptic exocytosis is regulated and what cargo molecules are trafficked in the Syt4 pathway. Further analysis of Syt4-mediated signaling will enhance our understanding of how synaptic growth and plasticity are regulated. To identify genes involved in Syt4 trafficking, we assembled a UAS-RNAi transgenic collection targeting 450 gene products that are resident at synapses and/or involved in membrane trafficking. Syt4 decorates the postsynaptic membrane of the NMJ, overlapping with glutamate receptor fields opposite active zones. Candidate UAS-RNAi constructs were co-expressed with fluorescently tagged Syt4 and animals were examined for changes in Syt4 distribution. We identified several candidates that regulate Syt4 distribution at the NMJ, including the t-SNARE Syntaxin 4 (Syx4). RNAi knockdown of Syx4 results in a reduction in Syt4 levels at the postsynaptic membrane and abnormal accumulation of Syt4 in the cytoplasm. To further explore the function of Syx4, we generated *loss-of-function* mutants. These animals exhibit a reduction in the number of boutons at the NMJ and the number of active zones per bouton, indicating impairment of synaptic structural plasticity. Syx4 protein is enriched postsynaptically at the NMJ, and postsynaptic

expression of Syx4 is sufficient to fully rescue Syx4 mutant defects. These observations are consistent with a role for Syx4 as a t-SNARE for postsynaptic exocytosis. Current studies are aimed at identifying additional retrograde signaling pathways that may be perturbed upon loss of Syx4 function. We observe a reduction in the delivery of Neuroligin (Nlg) to the postsynaptic membrane in Syx4 mutants and strong genetic interactions between Nlg, Neurexin (Nrx) and Syx4, consistent with Syx4 playing a role in regulating the important Nlg-Nrx trans-synaptic adhesion complex. We will continue to investigate the mechanism by which Nlg, Nrx and Syx4 interact, as well as other signaling pathways that may be disrupted in Syx4 mutants. We anticipate this approach will expand our understanding of postsynaptic regulation of synaptic plasticity.

Disclosures: **K.P. Harris:** None. **Z.D. Piccioli:** None. **Y. Akbergenova:** None. **J. Littleton:** None. **N. Perrimon:** None. **R.W. Cho:** None.

Poster

037. Postsynaptic Structure I

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Topic: B.07. Synaptic Transmission

Support: NIDA T32-DA07290

Simons Foundation Autism Research Initiative (206919)

NIH HD052731

Title: The cell-autonomous role of MEF2C in postnatal neocortical circuit and synapse development *in vivo*

Authors: *K. E. RAJKOVICH, K. W. LOERWALD, J. R. GIBSON, K. M. HUBER; Neurosci., UT-Southwestern Med. Ctr., Dallas, TX

Abstract: Neocortical circuits are sculpted by genetic programs and neuronal activity during development, and aberrant formation of these circuits is associated with neurodevelopmental disorders such as autism. Proper neocortical circuit development requires an appropriate balance of synapse proliferation and elimination: processes regulated by circuit activity and experience. The MEF2 family of activity-dependent transcription factors regulates excitatory synapse number and function in other brain regions. Although there are four MEF2 genes (*Mef2a-d*),

Mef2c accounts for ~75% of MEF2 expression in neocortex and is an autism-linked gene. Many studies show MEF2 genes to regulate synaptic structure and function in several brain areas, but the nature of this regulation is controversial. Previous studies from others and our group suggest that MEF2 negatively regulates synapse number. Therefore, we initially hypothesized that *Mef2c* deletion in neocortex should increase synapse number and function in neocortex. However, these studies employed *in vitro* and global deletion strategies where primary functions of MEF2 may be obscured by secondary, indirect changes. We examined the postsynaptic, cell-autonomous role of endogenous MEF2 genes in regulating synaptic pathways in acutely prepared cortical slices. Sparse AAV-Cre-mediated deletion of *Mef2c* within the postnatal mouse somatosensory (barrel) cortex allows a within-animal comparison of $Mef2c^{flx/flx}$ and *Mef2c*-deleted neurons. We use this preparation to perform intracellular patch clamping and morphological imaging experiments in L2/3 pyramidal neurons to determine the effects of postnatal *Mef2c* deletion on synaptic structure and function. *Mef2c* deletion downregulates evoked synaptic transmission over several synaptic pathways in L2/3 pyramidal neurons and requires experience-dependent neural activity to drive this effect, as assessed by laser-scanning photostimulation (LSPS) with glutamate uncaging. *Mef2c* deletion does not affect short-term plasticity, suggesting a postsynaptic locus for *Mef2c*-dependent regulation of synaptic transmission. Interestingly, *Mef2c* deletion enhances spontaneous synaptic transmission. Finally, *Mef2a/d* deletion does not affect these processes. Our data reveal that *Mef2c*-mediated synaptic regulation in the neocortex is complex. Here we study the cell-autonomous, postsynaptic role for endogenous *Mef2c* in the mammalian postnatal neocortex: a brain region where little is known about how MEF2 isoforms regulate neural circuit development *in vivo*.

Disclosures: **K.E. Rajkovich:** None. **K.W. Loerwald:** None. **J.R. Gibson:** None. **K.M. Huber:** None.

Poster

037. Postsynaptic Structure I

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 37.07/C44

Topic: B.08. Synaptic Plasticity

Title: Transmembrane-agrin overexpression in the adult murine cortex and hippocampus results in decreased dendritic spine density and altered spine morphology

Authors: *A. SCHICK[1], S. PFEIFFER[2], J. SCHICK[2], S. KRÖGER[1];
[1]LMU Dept Physiol., Munich, Germany; [2]Helmholtz Inst., Munich, Germany

Abstract: The heparan sulfate proteoglycan agrin is a well-characterized regulator of synaptogenesis at the neuromuscular junction. Its role in the developing and adult central nervous system is less well understood. Evidence however is accumulating that agrin also plays a role as synapse organizer in the CNS. Previously we demonstrated that overexpression or aggregation of the transmembrane form of agrin (TM-agrin), the predominant isoform of agrin in the CNS, results in the formation of numerous filopodia-like processes on axons and dendrites *in vitro*. We also showed that a partitioning of TM-agrin into lipid rafts, the activation of the Src family kinase Fyn and subsequently the activation of MAPK are prerequisites for the formation of these processes. The TM-agrin-induced processes appeared very similar to those extended by dendrites as precursors for spine synapses. We therefore investigated the role of TM-agrin-induced processes and their role in synapse development and plasticity in the CNS *in vivo*. To this end we generated knock-in mice that conditionally overexpress TM-agrin in the cortex and hippocampus, driven by a CamKII-CreERT2 promoter. Initial examination of adult TM-agrin overexpressing mice showed that they are healthy, normal in size, and do not show any obvious behavioral abnormalities despite a more than 10-fold increase in TM-agrin protein in whole brain membrane preparations. Upon examination of neuronal morphology, we observed a 33% decrease in spine density on CA1 pyramidal neurons. However, the decrease in spine number per dendrite length did not equally affect all types of spines. Instead stubby spines, which represent a subpopulation of transitory immature spines, showed an increase in 13% on TM-agrin overexpressing pyramidal neuron dendrites. Likewise, in the cortex of these mice we observed a decrease in the number of PSD95 immunoreactive punctae of approximately 20%. Taken together, these results show that overexpression of transmembrane-agrin in adult mice has an effect on spine density in the cortex and hippocampus. This further supports the hypothesis that agrin is an important regulator of synapse type and density in the adult CNS.

Disclosures: **A. Schick:** None. **S. Kröger:** None. **S. Pfeiffer:** None. **J. Schick:** None.

Poster

037. Postsynaptic Structure I

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 37.08/C45

Topic: B.07. Synaptic Transmission

Title: Functional study of the synaptic scaffold protein Shank2 and a mouse model for Autism Spectrum Disorders

Authors: *A. L. PAPPAS[1], R. RODRIGUIZ[1], W. WETSEL[1], R. WEINBERG[3], A. BEY[1], Y.-H. JIANG[2];
[2]Pediatrics, [1]Duke Univ., Durham, NC; [3]Univ. of North Carolina, Chapel Hill, NC

Abstract: Background: ASD is a neurodevelopmental disorder that has a complex etiology. Studies show that abnormal synaptic functions in the brain may be involved in the pathogenesis. Several synaptic proteins including Neuroligin3 and 4, CNTNAP2 and the SHANK family (SH3 and multiple ankyrin repeat domain) have been implicated in ASD from genetic studies. These findings support an emerging synaptic hypothesis in the pathophysiology of ASD. The SHANK proteins act as synaptic scaffolding, organizing the cytoskeletal and signaling complex at the postsynaptic density of excitatory synapses. SHANK2 has been identified as a strong causative gene in ASD. Small de novo microdeletions along with nonsense and missense point mutations have been identified within the *SHANK2* gene in individuals with ASD and intellectual disability (Berkel et al 2010).These results indicated a role of SHANK2 haploinsufficiency in the pathophysiology of ASD and intellectual disability, and provide an excellent opportunity to model SHANK2 causing ASD using a transgenic mouse model. **Objective:** The overall goal of my research project is to gain insights into the function of Shank2 and the pathophysiology of ASD using a model organism. I will generate and characterize Shank2 mutant mice. **Results:** 1) *In vitro* studies indicate that a human SHANK2 mutation may result in defects in protein trafficking. 2) Using SHANK2 protein domains as bait for yeast-two-hybrid screen, I identified an interacting protein containing a conserved ubiquitin ligase domain, MindBomb2 (MIB2). The ubiquitination of Shank family proteins has been suggested previously. The preliminary data support the co-localization and interaction of tagged Shank2 and MIB2 in HEK293 cells. *In vitro* data suggests MIB2 promotes the ubiquitination and degradation of Shank2. Data from MIB2 mutant animals indicate an increase in SHANK2 protein levels. 3) Using a gene targeting approach, I have obtained Shank2 exon 24 deletion mice (Δe24). I have analyzed the DNA and RNA of Shank2 and confirmed the complete excision of exon 24. Western blot shows an absence of the Shank2 protein in the Δe24 mice. I have begun biochemical analysis of the proteins in the post synaptic density and an extensive analysis of behavior, electrophysiology, and neuronal morphology is ongoing. **Conclusions:** 1) I have identified a ubiquitin ligase that potentially plays a role in SHANK2 regulation. 2) I generated a mouse with a deletion mimicking mutations seen in patients with ASD. 3) I identified behavioral phenotypes in Shank2 Δe24-/- mice that are significantly different that the WT behaviors and have begun pharmacological treatment.

Disclosures: A.L. Pappas: None. **Y. Jiang:** None. **R. Weinberg:** None. **R. Rodriguiz:** None. **W. Wetsel:** None. **A. Bey:** None.

Poster

037. Postsynaptic Structure I

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

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Topic: B.02. Ligand-Gated Ion Channels

Support: NIMH Grant MH086425

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Jefferson-Weizmann Foundation

Title: Extracellular phosphorylation regulates EphB trafficking and EphB-NMDAR interaction

Authors: N. XIA[1], *K. HANAMURA[1], S. I. SHEFFLER-COLLINS[2], M. B. DALVA[1]; [1]Neurosci., Thomas Jefferson Univ., Philadelphia, PA; [2]Neurosci. Grad. Group, Univ. of Pennsylvania Sch. of Med., Philadelphia, PA

Abstract: EphBs are trans-membrane receptor tyrosine kinases. When EphBs are activated by ephrin-B ligands and remain on the cell surface, they can recruit and maintain NMDARs to synaptic sites. In contrast, endocytosed EphBs generate repulsive responses. However, whether the localization of EphBs and interaction with the NMDAR are regulated by the same mechanism is unknown. Protein phosphorylation is a fundamental mechanism regulating cellular function. While the intracellular phosphorylation has been extensively studied, the function of extracellular phosphorylation is less well understood. We find that phosphorylation of a tyrosine residue in the extracellular fibronectin type III repeat domain of EphB2 receptor regulates both receptor trafficking and its ability to interact with the NMDAR. Using mass spectrometry, site-directed mutagenesis and phospho-specific antibodies, we identify a specific phosphorylation site on EphB2 that undergoes phosphorylation in a ligand-dependent fashion. The phosphorylation of EphB2 occurs on the cell surface as surface staining of live cultured neurons shows that amount surface phosphorylated EphB increases upon ligand stimulation. In contrast, pretreatment with a membrane impermeable kinase inhibitor blocks the increase in surface EphB phosphorylation. We next examine the functional significance of EphB2 extracellular phosphorylation. In the absence of NMDAR, EphB2 extracellular domain phosphorylation results in EphB2 internalization and degradation, indicating that ecotodomain phosphorylation may regulate trafficking and membrane retention of the receptor. In the presence of the NMDAR, EphB2 extracellular domain phosphorylation drives the EphB2-NMDAR interaction

both in HEK293T cells and neurons. Finally to begin to test whether extracellular phosphorylation might be a general mechanism for protein-protein interaction, we identify and test putative NMDAR interacting proteins. Using sequence alignment and homology, we predict that EphA8 should also interact with the NMDAR. Experiments in HEK293T cells validate that EphA8 can interact with the NMDAR. Taken together, our results suggest that extracellular phosphorylation might be an underappreciated mechanism in regulation of protein interaction and protein trafficking.

Disclosures: **N. Xia:** None. **K. Hanamura:** None. **S.I. Sheffler-Collins:** None. **M.B. Dalva:** None.

Poster

037. Postsynaptic Structure I

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

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Topic: B.07. Synaptic Transmission

Support: Simons Center for the Social Brain at MIT

NIH Grant MH080310

Title: Distinct roles of Shank family proteins in regulating glutamatergic synaptic transmission

Authors: R. SHI, P. REDMAN, Y. LIU, M. LIU, K. JONES, *W. XU;
MIT, Cambridge, MA

Abstract: Scaffold proteins in the postsynaptic density (PSD) of excitatory glutamatergic synapses have been shown to be important in regulating synaptic transmission. Defects in many scaffold proteins, including the Shank family of proteins, have been associated with neurological, neurodevelopmental, and neuropsychiatric diseases, but how these proteins regulate glutamate receptor-mediated synaptic transmission remains elusive. Here, we investigate the role of the Shank proteins in regulating synaptic transmission, and seek to determine any functional differences among Shank family members. Using an shRNA-mediated knockdown of Shank proteins, combined with dual whole-cell patch clamp in hippocampal slice culture, we show that knockdown of Shank1 or Shank2 reduced AMPAR-mediated evoked excitatory postsynaptic currents (eEPSCs). Our experiments further suggest that this reduction in AMPAR eEPSCs was likely mediated by a reduction in the number but not the strength of

AMPAR-containing synapses. Simultaneous knockdown of both Shank1 and Shank2 also decreased NMDAR eEPSCs, indicating further synaptic defect. However, knockdown of Shank3 showed no effect on synaptic transmission in our system, potentially due to a late developmental onset of Shank3 expression. Taken together, our results suggest that distinct Shank family members contribute differently to maintain glutamatergic synaptic function.

Disclosures: **R. Shi:** None. **P. Redman:** None. **Y. Liu:** None. **M. Liu:** None. **K. Jones:** None. **W. Xu:** None.

Poster

037. Postsynaptic Structure I

Location: Halls A-C

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Topic: B.08. Synaptic Plasticity

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Hong Kong Research Grants Council Theme-based Research Scheme (T13-607/12R)

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Title: S-nitrosylation of Cdk5 and its activator p35 in neuronal morphogenesis and synaptic function

Authors: ***P. ZHANG**[1,2,3], W.-Y. FU[1,2,3], A. FU[1,2,3], N. IP*[1,2,3];
[1]Div. of Life Sci., HKUST, HONG KONG, China; [2]Mol. Neurosci. Center, HKUST, Hong kong, China; [3]State Key Lab. of Mol. Neuroscience, HKUST, Hong kong, China

Abstract: Cyclin-dependent kinase 5 (Cdk5), a proline-directed serine/threonine kinase, plays important roles in neural development such as neuronal migration, neurite outgrowth and synapse formation. Unlike other cyclin-dependent kinase family members, which are regulated by cyclins, Cdk5 is activated through its association with the neuronal-specific activators, p35 and p39. We have previously demonstrated that Cdk5 can undergo S-nitrosylation at Cys83 which reduced its kinase activity and regulated the morphology of dendritic spines, where most excitatory synapses reside. We report here that p35 can also be S-nitrosylated *in vitro*. S-nitrosylation of Cdk5 and p35 could both be detected in mouse brains, and the specific modification was abolished in mice lacking neuronal nitric oxide synthase (nNOS). Importantly,

enhanced Cdk5 activity together with increased phosphorylation of various specific Cdk5 substrates was observed in the brains of nNOS-knockout mice, suggesting that S-nitrosylation is responsible for negatively regulating Cdk5 activity *in vivo*. Furthermore, we showed that blockade of NO production in cultured hippocampal neurons resulted in synaptic failures. More importantly, inhibition of Cdk5 activity partially rescued the synaptic deficits upon NO production blockade. Taken together, our findings reveal a new regulatory mechanism for Cdk5 that is essential for neuronal morphogenesis and synaptic functioning.

Disclosures: **P. Zhang:** None. **W. Fu:** None. **A. Fu:** None. **N. Ip*:** None.

Poster

037. Postsynaptic Structure I

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 37.12/C49

Topic: B.02. Ligand-Gated Ion Channels

Title: Pharmacological characterization of novel neuroactive steroid modulators of NMDA receptors

Authors: M. A. ACKLEY, G. M. BELFORT, G. MARTINEZ-BOTELLA, F. G. SALITURO, A. J. ROBICHAUD, *J. J. DOHERTY;
Sage Therapeut., Cambridge, MA

Abstract: NMDA receptors are intimately involved in neuroplasticity throughout the brain and as such, represent targets of interest for drug development for neuropsychiatric diseases. Positive modulators of NMDA receptors are of interest for their potential to alleviate cognitive deficits in diseases such as schizophrenia, whilst recent clinical evidence with Ketamine suggests a novel role for inhibitors of NMDA function for treatment resistant depression. We recently reported that the endogenous neurosteroid, 24(S)-hydroxycholesterol directly modulates the NMDA receptor and positively impacts plasticity in the hippocampus (Paul et al, 2013). Subsequently, we have developed a range of both positive and negative allosteric modulators of NMDA receptors based on this endogenous molecule. We have characterized the electrophysiological properties of these novel positive and negative allosteric modulators in heterologous cell lines expressing human GluN1/GluN2A subunits as well as confirming activity at native rodent NMDA receptors using primary hippocampal neuron cultures We have also undertaken a series of mechanistic experiments in the hippocampal neurons to determine the potential for use dependence, the rate of modulatory onset and the mechanism by which these compounds affect

the Glutamate concentration-response curve. Furthermore, we characterized the subunit selectivity of a subset of modulators in *Xenopus laevis* oocytes that expressed human GluN1 along with GluN2A, 2B, 2C or 2D. The data presented demonstrate that our neuroactive steroid chemistry platform supports the development of both positive and negative allosteric modulators displaying a range of activities at NMDA receptors. Compounds within this class may represent novel therapeutics for a range of neuropsychiatric diseases characterized by NMDA receptor hyper- or hypo-function.

Disclosures: **M.A. Ackley:** A. Employment/Salary (full or part-time):; Sage Therapeutics. **G.M. Belfort:** A. Employment/Salary (full or part-time):; Sage Therapeutics. **G. Martinez-Botella:** A. Employment/Salary (full or part-time):; Sage Therapeutics. **F.G. Salituro:** A. Employment/Salary (full or part-time):; Sage Therapeutics. **A.J. Robichaud:** A. Employment/Salary (full or part-time):; Sage Therapeutics. **J.J. Doherty:** A. Employment/Salary (full or part-time):; Sage Therapeutics.

Poster

038. Electrical Synapse and Gap Junction

Location: Halls A-C

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Topic: B.07. Synaptic Transmission

Support: Civilingeniør Frode V. Nyegaard og Hustru's Fond

Aase og Ejnar Danielsens Fond

Familien Hede Nielsens Fond

Title: No effect of DHA on synaptic transmission and plasticity in mice

Authors: *G. N. NIKOLAJSEN, M. J. WEST, M. S. JENSEN;
Biomedicine, Aarhus Univ., Aarhus C, Denmark

Abstract: Epidemiological and clinical trials suggest that insufficient consumption of the omega-3 fatty acid, docosahexaenoic acid (DHA), during pregnancy, is associated with poor cognitive development. In this regard, it has been suggested that DHA deficiency may affect normal synaptic plasticity. Previous studies of the effects of DHA on synaptic functions have been performed in rat hippocampal slices. It was reported, that rats kept on a DHA deficient diet had a significant lower level of long term potentiation (LTP). In this study, we investigated

DHA's influence on synaptic transmission and plasticity in wild type (Wt) mice and a transgenic (Tg) mouse model of Alzheimer Disease (AD) like amyloidosis. Female Wt and APPswe/PS1deltaE9 mice were given an enriched or DHA free diet during breeding. This Tg mouse shows an age related increase in Abeta42 (Aβ42) levels from 4 months of age but no changes of synaptic transmission when fed a normal diet. Only female offspring were used for this study, which were kept on the same diet as their parent through life. This resulted in 4 different experimental groups at 7 months of age (: DHA-Wt, DHA-Tg, non-DHA-Wt and non-DHA-Tg mice with 8 subjects within each group. In the present study, synaptic transmission was evaluated by recording field excitatory postsynaptic potentials (f-EPSP) and input/output responses to Schaffer collateral stimulation. Synaptic plasticity was evaluated by LTP recordings induced by theta burst stimulation. The results showed that a DHA deficit or enriched diet from embryo stage had no significant effect on LTP in these 7 months old female mice, regardless of genes. These findings suggest that DHA consumption in mice do not have the same impact on the synaptic plasticity as in rats. Moreover, the expression of amyloid does not seem make the mouse more responsive to a DHA deficient diet.

Disclosures: **G.N. Nikolajsen:** None. **M.J. West:** None. **M.S. Jensen:** None.

Poster

038. Electrical Synapse and Gap Junction

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 38.02/C51

Topic: B.07. Synaptic Transmission

Title: Innexins function as plasmamembrane unpaired channels in native *C. elegans* touch neurons

Authors: *R. SANGALETTI, G. DAHL, L. BIANCHI;
Physiol. and Biophysics, Univ. of Miami, Miller Sch. of Med., Miami, FL

Abstract: Cell-to-cell communication is mediated by intercellular protein complexes named gap junctions. In vertebrates, gap junctions are formed by connexon hemichannels, each consisting of homo or heteromers of connexin proteins. In invertebrates gap junctions are formed by evolutionarly-unrelated proteins called innexins, which share with connexins general membrane topology. At the sequence level, innexins share homology with another family of proteins in vertebrates, called pannexins. However, pannexins have been shown to form unpaired plasma membrane channels that mediate release of ATP and ions into the extracellular space, rather than

forming gap junctions. Since, innexins and pannexins share sequence homology, it was postulated that innexins may also function as plasmamembrane channels. However, no activity of innexins as plasma membrane channels has been documentated to date in native *C. elegans* cells. We show here that, *C. elegans* gentle body touch neurons express a channel sensitive to mechanical stimuli. This channel has a large conductance (~1 nS), is voltage-independent, non-selective, is blocked by innexin/pannexin inhibitors and is permeable to fluorescent dyes such as Ethidium Bromide. Moreover, we show that this channel functions also in a K+-selective, voltage-dependent subconductance state. Based on its functional and pharmacological features, we conclude that the mechanosensitive channel expressed in *C. elegans* touch neurons is an innexin. We thus propose that innexins form unpaired plasmamembrane channels in *C. elegans* touch neurons. Single cell RT-PCR and further *in vivo* analysis will be performed to identify the gene/s responsible for the channel activity detected in touch neurons. The identification of unpaired innexins in *C. elegans* opens a new venue for exploiting the genetic tractability of this powerful model system to further our understanding of the still elusive physiological role of the homologous vertebrate proteins pannexins in the context of a whole organism.

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Poster

038. Electrical Synapse and Gap Junction

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 38.03/C52

Topic: B.07. Synaptic Transmission

Title: Effects of NO-dependent signalling on electrical synaptic transmission in spinal cord neurones and HeLa cells stably expressing Cx36

Authors: A. D. WHYMENT[1], *N. E. DALE[2], D. SPANSWICK[3,4,1];
[1]NeuroSolutions Ltd, Coventry, United Kingdom; [2]Univ. Warwick, Coventry, United Kingdom; [3]Dept. of Physiol., Monash Univ., Melbourne, Australia; [4]Med. Sch., Univ. of Warwick, Coventry, United Kingdom

Abstract: Our knowledge of the role of chemical synaptic transmission and nitric oxide (NO) is extensive, however, the same cannot be said of electrical synapses although recent research has begun to recognise this mode of communication is more vastly more widespread than originally thought. A potential relationship between NO and gap-junctions in the CNS and elsewhere has been suggested for decades by the significant correlation of gap-junction mRNA and NO

synthase distribution. HeLa cells stably expressing Cx35, the mammalian homologue of Cx36, show NO-donors potently uncouple these cells. Recently, the importance of nitrite and nitrate has transformed with the realisation that these putative inert anions are stored in tissues and physiologically recycled in blood and tissues to produce NO and bioactive nitrogen oxides. Here we have used whole-cell patch-clamp recording techniques to investigate the effects of NO-dependent signalling on electrical synaptic transmission in spinal cord neurones and HeLa cells stably expressing Cx36. Simultaneous whole-cell recordings were made from 5 pairs of electrotonically coupled sympathetic preganglionic neurones (SPNs). The mean coupling coefficients (CC) and junctional conductances (Gj) were 0.22 ± 0.02 and 0.91 ± 0.06 nS, respectively. In the presence of SNOG (200 μM n = 5), mean CC and Gj were reduced to 0.16 ± 0.03 and 0.66 ± 0.15 nS, respectively. Application of NO donors activated three species of ion channels; most importantly an intermediate non-selective 140 pS channel which we believe to be a gap junction hemi-channel. Thus, the effects of NO and its precursors were tested on Cx36 hemi-channels expressed in HeLa cells. SNOG (200 μM, n = 8) reduced peak current amplitude to 66.1 ± 9.4% of control levels. Nitrite (200 μM, n = 8) reduced peak current amplitude to 58.2 ± 7.4% of control values. The potassium channel blocker barium (200 μM, n=5) slightly reduced peak current to 93.4 ± 12.4% of control values and reduced the effect of nitrite to 79.6 ± 12.4% (n=6). These data support the notion that NO acts to inhibit electrotonic coupling within SPNs and has a direct effect on Cx36 hemi-channels and suggest a pivotal role for nitrite in its own right as a signalling anion and not simply an alternative source for NO. This has important implications for the control of autonomic function, including heart rate, blood pressure and energy homeostasis.

Disclosures: **A.D. Whyment:** A. Employment/Salary (full or part-time):; NeuroSolutions Ltd.. **N.E. Dale:** None. **D. Spanswick:** A. Employment/Salary (full or part-time):; NeuroSolutions Ltd..

Poster

038. Electrical Synapse and Gap Junction

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 38.04/C53

Topic: B.07. Synaptic Transmission

Support: NIH, RO1 NS050434

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NIH, F31 NS083247

NIH, F32 NS084763

DARPA-BAA-09-27

Title: Roles of electrical synapses in gamma-band activity of sensory and association cortex

Authors: *B. W. CONNORS, G. T. NESKE, A. U. SUGDEN, S. R. CRANDALL, S. J. CRUIKSHANK;
Dept of Neurosci., Brown Univ., PROVIDENCE, RI

Abstract: Electrical synapses formed by connexin36 (Cx36)-containing gap junctions are extensive in the cerebral cortex, connecting many neighboring inhibitory interneurons of the same subtype; they are effective, passing a significant fraction of their voltage signals; and they are ubiquitous across species. Various theoretical and experimental investigations suggest that electrical synapses promote spike synchrony during current injection into interneuron pairs, and during pharmacological activation of interneuron subtypes *in vitro*. One natural form of synchronous activity in cerebral cortex is the gamma oscillation, in which the local field potential (LFP) and inhibitory synaptic inputs are characterized by a high power in the 30-80 Hz range. The activity of fast-spiking (FS) interneurons has long been considered critical to the generation of this oscillation, and gamma-band power in the hippocampus is reduced in Cx36 knockout (KO) mice. These results and the electrical coupling of FS cells suggest that electrical synapses may play an important role in gamma-band activity in the neocortex. We have measured the effects of Cx36 on the roles of electrical synapses in complex forms of cortical activity where recurrent excitatory and inhibitory synaptic conductances are activated. Acute slices of wild-type (WT) and Cx36 KO mice were compared using different cortical areas and gamma-inducing protocols. In barrel cortex (a primary somatosensory area), gamma-frequency activity was generated during the “Up” periods of slow oscillations. In dorsal postrhinal cortex (dPOR) (a visuospatial association area) gamma-frequency activity was induced by asynchronous optogenetic activation of excitatory cells. In layers 4 and 5 of barrel cortex, there was a small but significant decrease in the gamma power of inhibitory postsynaptic currents (IPSCs) recorded in pyramidal cells of the Cx36 KO compared to WT. Phase-locking of pyramidal cell spikes and the gamma-band LFP, phase-locking of IPSCs and the LFP, and correlations between IPSCs of pyramidal cell pairs were equally strong in Cx36 KO and WT; IPSC correlations were temporally sharper in WT neurons, however. During optically evoked gamma-band activity in dPOR, there were no Cx36-dependent differences in the power, frequency, or correlations of IPSCs recorded in pyramidal cell pairs. We will further investigate the Cx36-dependence of optically evoked gamma-band activity in barrel cortex. Our results to date suggest that electrical synapses contribute to certain temporal features of gamma activity, but also that robust gamma oscillations can persist in the absence of electrical synapses.

Disclosures: **B.W. Connors:** None. **G.T. Neske:** None. **A.U. Sugden:** None. **S.R. Crandall:** None. **S.J. Cruikshank:** None.

Poster

038. Electrical Synapse and Gap Junction

Location: Halls A-C

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Topic: B.07. Synaptic Transmission

Support: Brain and Behavior Foundation, Young Investigator Grant 21343

Title: Efficacy and modulation of spike timing at asymmetrical electrical synapses in the thalamic reticular nucleus

Authors: J. SEVETSON, *J. S. HAAS;
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Abstract: Neurons of the thalamic reticular nucleus (TRN) provide the main source of inhibition to thalamocortical relay cells and are interconnected by electrical synapses based on connexin36 gap junctions. While the effects of electrical synapses in synchronizing coupled neurons have been described, their efficacy as synapses and modulatory effects on spike timing in coupled neighbors have been underappreciated. Here, using dual-cell recordings, we show that spiking activity in one TRN cell modulates perithreshold spike timing in a coupled neighbor by 10s of ms. Further, we characterize asymmetry as a fundamental property of electrical synapses, and demonstrate that asymmetry also impacts modulation of spike times. Finally, we evaluate the systematic effects of electrical synaptic asymmetry in a two-cell network model with an asymmetrical electrical synapse, which we provide with inputs of varied amplitude and timing. Together, these results demonstrate that asymmetry of electrical synapses alters the dynamic range and computational role that these synapses play within a neuronal network. In the TRN, this directionality of electrical synaptic signaling could be an organizing principle for tuning its responses to arising sensory inputs.

Disclosures: **J. Sevetson:** None. **J.S. Haas:** None.

Poster

038. Electrical Synapse and Gap Junction

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Topic: B.07. Synaptic Transmission

Support: NIH F31DC012222

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Title: A synaptic excitatory/inhibitory sequence mediated by voltage-gated channels and gap junctions

Authors: P. F. APOSTOLIDES, *L. O. TRUSSELL;
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Abstract: Gap junctions act as lowpass filters of electrical signals and are theoretically better suited to transmit slow voltage changes such as subthreshold synaptic events rather than fast, supra-threshold spikes. However, most previous studies investigating electrical coupling have focused primarily on transmission of action potentials. It is thus unclear whether the propagation of non-spike mediated events such as excitatory postsynaptic potentials (EPSPs) through gap junctions represents a physiologically relevant signal in electrical networks. We examined this problem in the dorsal cochlear nucleus using patch-clamp recordings from brain slices taken from P15-25 mice. Excitatory synapses made by parallel fibers onto the fusiform principal cells activated a TTX-sensitive Na+ conductance which dramatically prolonged the EPSP. Moreover, during the prolonged depolarization a resting HCN conductance was deactivated, leading to a delayed AHP associated with the EPSP. In this way, intrinsic currents markedly reshaped subthreshold EPSPs. Most interestingly, these subthreshold voltage changes resulting from activation/deactivation of Na+ and HCN channels then generated a slow (>100 ms) excitation/inhibition sequence in the GABAergic stellate cells that we previously showed to be electrically-coupled to fusiform cells (Apostolides & Trussell 2013). This electrical transmission accounted for the majority of glutamatergic signaling to stellate cells, such that a subthreshold EPSP in a single fusiform cell can drive spikes in coupled interneurons. Thus, the interaction between a principal cell's synaptic and voltage-gated channels determines the spike activity of an inhibitory network without firing a single action potential in the principal cell.

Disclosures: **P.F. Apostolides:** None. **L.O. Trussell:** None.

Poster

038. Electrical Synapse and Gap Junction

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 38.07/C56

Topic: B.07. Synaptic Transmission

Support: Brain and Behavior Foundation Young Investigator Award

Title: Common and uncommon mechanisms of LTD at electrical synapses in the TRN

Authors: ***J. SEVETSON**, J. BRAGUE, J. HAAS;
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Abstract: Neurons of the thalamic reticular nucleus (TRN), which provide the main source of inhibition to thalamocortical relay cells, express a high density of connexin36-based gap junctions. Long-term depression (LTD) of electrical synapses in the TRN has been demonstrated following paired activity (Haas et al., Science 2011) and after activation of mGluR receptors by afferent stimulation (Landisman and Connors, Science 2005), but the interactions and downstream mechanisms of these two induction paradigms remain unknown. Using dual whole-cell recordings *in vitro*, we demonstrate occlusion of LTD following the sequential applications of paired bursting and of the mGluR agonist ACPD in pairs of coupled TRN neurons. Further, application of the specific T-type calcium channel antagonist TTA-A2 blocked bursting-induced LTD but did not block mGluR agonist-mediated LTD. Together, these data provide support for a signaling model of induction with separable components, whereby activity-induced depression is mediated by calcium entry while afferent activity-induced depression is independent of calcium entry. We hypothesize that the two induction mechanisms converge at a shared downstream pathway, and we are currently investigating the components of that pathway. Thus, our results provide a crucial link between electrical synaptic plasticity and the well-characterized mechanisms underlying plasticity at chemical synapses.

Disclosures: **J. Sevetson:** None. **J. Brague:** None. **J. Haas:** None.

Poster

038. Electrical Synapse and Gap Junction

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Topic: B.07. Synaptic Transmission

Support: Conacyt CB-2010-01-0154645

Title: Expression profile of connexin 26, -30, -31.1, -36, -43 and -45 in mouse SN and VTA

Authors: *A. HERNANDEZ SANCHEZ, J. A. MENDEZ;
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Abstract: Dopamine (DA) neurons of the substantia nigra (SN) and ventral tegmental area (VTA) participate in hedonism, memory and reward processes. However, within both SN and VTA there are other neuronal populations: GABA, DA/Glutamate (Glu) co-releasing and pure (Glu) neurons. It has been previously shown that DA neurons have the ability to communicate between them through electrical synapses. However, the connexin repertoire of DA neurons in mice is not known. Remarkably, identical electrical activity during appetitive and aversive learning has been shown in both DA and non-DA neurons in the VTA. One possibility to explain this would be electrical coupling through gap junctions. As first action to explore that possibility we determined the expression of 6 connexins (Cx26, Cx30, Cx31.1, Cx36, Cx43 and Cx45) by multiplex RT-PCR in both SN and VTA from new born (p0) up to postnatal day 120 (p120). Using the expression levels of each connexin at p90 as control, we found the highest expression level of Cx26, Cx30 and Cx43 in both SN and VTA at p120: 3.2, 2.5 and 3 times in SN and 6.1, 4.3 and 45.7 times in ATV for Cx26, Cx30 and Cx43 respectively. In addition, Cx36 showed its maximal expression level in p0 mice in SN (27.2 times) whereas in VTA the peak was at p15 (3.87 times). The expression levels of Cx31.1 as well as of Cx45 did not change significantly through all ages tested. Interestingly, we found that the expression of Cx26 at p0 and of Cx45 at p120 was null in VTA whereas in SN was highly expressed, perhaps this differential expression pattern can be used as a tool to readily establish the boundaries between SN and VTA in immunohystochemical experiments. To extend these observations, we are currently using single cell multiplex RT-PCR in acutely dissociated cells to determine the expression profile of these connexins in specific neuronal cell types in the adult mice.

Disclosures: **A. Hernandez Sanchez:** None. **J.A. Mendez:** None.

Poster

039. Signal Propagation in Neural Networks

Location: Halls A-C

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Topic: B.09. Network Interactions

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Title: Organotypically cultured hippocampal networks emit spontaneous neuronal activity similar to *in vivo* ongoing activity

Authors: *K. OKAMOTO[1], T. ISHIKAWA[1], R. ABE[1], D. ISHIKAWA[1], C. KOBAYASHI[1], M. MIZUNUMA[1], H. NORIMOTO[1], N. MATSUKI[1], Y. IKEGAYA[1,2];
[1]The Univ. of Tokyo, Bunkyo-Ku, Japan; [2]Ctr. for Information and Neural Networks, Osaka, Japan

Abstract: Spontaneous neuronal activity prevails in virtual all brain regions *in vivo* and even in brain slices *in vitro*. But its function, influence, or spatiotemporal patterns are not fully understood. Acute brain slice preparations exhibit a fewer level of spontaneous activity because of massive cut of neurites during slicing. Organotypic slice cultures self-restore neurites and synaptic connections and remodel the network complexity through the intrinsic rules of neural plasticity. Therefore, cultured slices could offer a better opportunity for investigating some aspect of spontaneous activity. In hippocampal networks, we compared spontaneous activity patterns among acute slices, cultured slices, and *in vivo* networks. Using functional multineuron calcium imaging and cell-attached patch-clamp recordings, we found that the mean firing rates of individual neurons of slice cultures did not differ from those of the *in vivo* hippocampus and were higher than those of acute slice neurons. Using whole-cell voltage-clamp techniques, we isolated spontaneous excitatory and inhibitory postsynaptic conductances (sEPSGs, sIPSGs) and measured five parameters, i.e., mean, coefficient of variance, skewness, kurtosis, and synaptic event frequency. The sEPSG parameters of slice cultures were similar to those of *in vivo* recordings, whereas the sIPSG parameters were not. We then used Soft Confidence Weighted (SCW), a machine learning technique, and tried to classify our datasets. We exposed SCW to the parameters of EPSGs or IPSGs of all datasets of *in vivo* and acute networks and then asked it which the parameters of a given cultured slice are similar to those of either *in vivo* neurons or acute slices. In both EPSGs and IPSGs, SCW judged almost all slice culture datasets as '*in vivo*-

like'. These results suggest that hippocampal slice cultures resemble the *in vivo* hippocampus, rather than acute slices, in terms of spontaneous activity.

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Poster

039. Signal Propagation in Neural Networks

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Topic: B.09. Network Interactions

Support: NIMH Intramural Research Program

Maryland Biophysics Graduate Program

Title: Non-parabolic unfolding of neuronal avalanches suggests preferred spatial pathways in cortical dynamics

Authors: *S. R. MILLER[1,2], S. YU[2], D. PLENZ[2];
[1]Univ. of Maryland Col. Park, College Park, MD; [2]Section on Critical Brain Dynamics, Natl. Inst. of Mental Hlth., Bethesda, MD

Abstract: Agreement between the theory of criticality and experimental neuroscience has been established in terms of observed scale-invariance for neuronal avalanches, which constitute spontaneous activity cascades in the cortex during rest. Similarly, pharmacologically-validated optimization of certain aspects of information processing during avalanche dynamics, such as dynamic range, information capacity, and the transient formation of phase-coupled neuronal groups, are in line with a balanced critical regime of neuronal activity. Given that evidence for emergent critical dynamics in the mammalian cortex is gaining increasing traction in the research community, it is worthwhile to consider how the brain might leverage neuronal avalanches to store, transmit, and process information. A major information aspect introduced by neuronal avalanches is related to their instantaneous spatial spread, e.g. the number of sites visited per time step as an avalanche unfolds. From graph theoretical studies, we expect critical dynamics on a neural network with random small-world connectivity to generate avalanches with a characteristic parabolic spread; avalanches will typically initiate at a single site, engage more and

more sites as they unfold, and eventually contract spatially and terminate. However, experimental findings presented here illustrate that neuronal avalanches recorded in cortical layers 2/3 of awake Macaque monkeys (n=2) do not appear to follow a parabolic function. Instead, neuronal avalanches recorded *in vivo* have a flattened spread such that (1) they visit significantly fewer sites during their unfolding than expected from a homogeneous network architecture, and (2) they display considerable variability around a characteristic mean function that is independent of avalanche life time. These stochastic features were especially evident for avalanches with long life times. These results suggest avalanches unfold along preferred pathways, indicating a heterogeneous functional architecture of the cortex.

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Poster

039. Signal Propagation in Neural Networks

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Topic: B.09. Network Interactions

Support: 3DNeuroN project in the European Union's Seventh Framework Programme, Future and Emerging Technologies, grant agreement no. 296590

Title: Signal propagation in small neuron networks with memory function - Characterization and modeling

Authors: ***L. DEMKO**, H. DERMUTZ, C. FORRO, S. WEYDERT, T. ZAMBELLI, J. VOROS;
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Abstract: Understanding how the human brain stores and processes information is undoubtedly one of the grand challenges of this century. Despite of the vast amount of technical possibilities we still have very little understanding (and especially consensus) about e.g. learning, which might be partially due to the lack of well-defined, small "study networks" of real neurons that can be reproducibly and quantitatively analyzed at a few or single neuron level over extended periods of time. Apart from the experimental approach, modeling has always played a key role in understanding neural coding and signal transmission. However, in spite of the large variety of existing models there is still very little correlation with experimental results, and model based predictions for large networks are far from reality. In order to gain insight into the information

processing and neurocomputation of small networks, we study and model small neuronal systems with simple but controlled topology, how the activity changes as a function of it, study the effect of directional connections, and how these networks react to different stimuli. First we start with the experimental characterization of signal propagation between small groups of neurons with or without directional connections, by measuring the delay time between the neuronal activities of the different groups. When changing the network topology from simple linear feed-forward type to circular ones including loops, we expect to observe activity dependent systematic changes in the delay times due to long-term potentiation (memory effect). As for the theoretical aspect, we try to make one step above the classical models that operate with different synaptic strengths and transfer probabilities, and construct a simplified model based on our observations of rather deterministic firing patterns in the above mentioned small networks. The model assumes that the flow of information (activity) between groups of neurons can be described by delay times representing the time needed for the information traveling from one group of neurons to the other. This delay time what is in the focus of our approach is a property that is easily measurable by common techniques such as patch clamping or multi-electrode arrays as a part of the characterization step, but it is also possible to study its time dependence during and after the different stimulation protocols, providing a genuine way to bridge the gap between experiments and theory. Our model enables us to design simple networks with memory effect, a feature being the ultimate target of our experimental investigations with small neuronal networks.

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Poster

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NHMRC Grant 1005427

Title: Dynamic coherent structures in slow-wave activity of primate visual cortex

Authors: *R. TOWNSEND[1], S. SOLOMON[2], P. R. MARTIN[3,2], S. G. SOLOMON[4], P. GONG[1];
[1]Sch. of Physics, [2]Sch. of Med. Sci., [3]Save Sight Inst., The Univ. of Sydney, Sydney, Australia; [4]Exptl. Psychology, Univ. Col. London, London, United Kingdom

Abstract: Many complex physical and biological systems self-organize into dynamic spatiotemporal patterns known as coherent structures. In neural systems, only synchronized nerve cell activity and, more recently, simple planar travelling waves are typically considered. Here, we characterize complex coherent structures in slow-wave (<10 Hz) neural activity, which is considered important for key brain functions including memory consolidation and sleep. We made multi-electrode local field potential (LFP) recordings from the middle temporal area of visual cortex in 6 sufentanil anaesthetized marmoset monkeys, during presentation of a spatially uniform gray field (60 Cd m^2) presented on a video monitor. By adapting techniques established for the analysis of turbulence in fluids, we describe a rich repertoire of patterns present at sub-millimeter spatial scales, and sub-second temporal scales. The most commonly observed patterns were planar travelling waves (30% of measurement time) and widespread synchronous activity (17% measurement time). In addition, 11% of measurement time was characterized by more complex coherent structures. These include waves that radiate from, converge to, or spiral around phase singularities within the local nerve cell network, typically persisting for 10-100 ms before dissipating or drifting out of the recording area. Because our approach provides a physical framework for analyzing these patterns, we are able to quantify their dynamic properties including durations, rates, speeds, and spatiotemporal evolution. We show there are preferred paths of change from one coherent structure to another, indicating deterministic elements in pattern evolution. We also show that the presence of coherent structures is associated with a reduction in power of slow-wave activity and is reflected in simultaneously recorded action potentials. Slow-wave brain activity must thus be understood in context of complex and dynamic local processes, rather than simple synchronous activation of nerve populations within relevant brain regions.

Disclosures: **R. Townsend:** None. **S. Solomon:** None. **P.R. Martin:** None. **S.G. Solomon:** None. **P. Gong:** None.

Poster

039. Signal Propagation in Neural Networks

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Topic: B.09. Network Interactions

Support: DFG SFB 1089/B2

bonnFOR Instrument 1

Title: Dendritic calcium signaling in basket cells and its role during network activity

Authors: *S. DELATTRE, D. DIETRICH, E. A. MATTHEWS;
Dpt of Neurosurg., Bonn, Germany

Abstract: Inhibitory basket cells have a key role in synchronizing pyramidal cells during network activity. Basket cells are subdivided into two classes: parvalbumin (PV) containing basket cell and cholecystokinin (CCK) containing basket cell. These two cells types, however similar in morphology and location, have different roles, electrophysiological characteristics and molecular contents. Particularly, CCK basket cells have no reported calcium binding protein whereas PV is present in the other basket cell type. Because calcium has an important role in excitability and plasticity, we therefore investigated the different calcium buffering and diffusion environments in these basket cells, and the potential role that calcium plays in dendritic signaling. GFP positive interneurons in hippocampal slices from the mouse line Tg(Gad2-EGFP)DJ31Gsat were patched in current clamp mode and somatic action potentials (APs) were elicited with current injection. In the same set of cells, OGB6F dye concentration was varied ("added buffer" approach) and dendritic calcium transients were quantified based on confocal laser scanning microscopy. Endogenous buffering capacity was calculated from both the decay time constant and the inverse amplitude of the transients according to the single compartment model. Previous work has shown that PV basket cells display a buffering capacity of $\kappa \approx 210$ and contain a mobile endogenous buffer[1]. In the current study, CCK basket cells were found to have a buffering capacity of $\kappa = 150 \pm 25$ and no mobile endogenous buffers were detected as expected. Dendritic spread of calcium transients induced by back propagating APs was also investigated in the CCK basket cell. In CCK basket cells calcium transients penetrate up to 90 µm into their dendrites with stable amplitude (% Δ F/F) 22 ± 3 with 300µM of calcium dye OGB6F. This behavior could support spike timing dependent plasticity and might influence network activity. We furthermore investigated the calcium transients during oscillatory activity in a submerged slice preparation. We hypothesize that calcium signaling in dendrites of CCK basket cell mimics the oscillatory activity of the slice. 1 Aponte, Y., Bischofberger, J. & Jonas, P. Efficient Ca2+ buffering in fast-spiking basket cells of rat hippocampus. *The Journal of physiology* **586**, 2061-2075, doi:10.1113/jphysiol.2007.147298 (2008).

Disclosures: **S. Delattre:** None. **D. Dietrich:** None. **E.A. Matthews:** None.

Poster

039. Signal Propagation in Neural Networks

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Title: Sensorimotor function is implemented by a coordinated interplay of brief neuronal activations that traverse space and time

Authors: *W. G. COON[1,2], A. GUNDUZ[3], P. BRUNNER[1,4], B. PESARAN[5], G. SCHALK[1,6,4]; [1]Neural Injury & Repair, Wadsworth Ctr., Albany, NY; [2]Biomed. Sci., State Univ. of New York, Albany, Albany, NY; [3]Biomed. Engin., Univ. of Florida, Gainesville, FL; [4]Neurol., Albany Med. Col., Albany, NY; [5]Ctr. for Neural Sci., NYU, New York, NY; [6]Biomed. Sci., State Univ. of New York, Albany, NY

Abstract: Behavior arises from neuronal population activity that occurs across distant areas of the brain. The fleeting nature of this activity has been recognized at least since Sherrington likened the brain to an “enchanted loom” bustling with “millions of flashing shuttles.” Charting the trajectory of these flashing shuttles, i.e., of neural activity traversing space and time, and examining its relation to behavior, is of central importance to advancing our understanding of brain function. Studies over the past decades have been successful in identifying the locations of neuronal populations involved in specific behaviors, or in characterizing the specific relationship of individual populations of neurons to task-related variables. They have only been marginally successful in characterizing the spatial and temporal sequence of distant populations of neurons that together implement a behavior such as the translation of a visual stimulus into a button press. Superficially, this issue appears to be largely due to limitations of traditional imaging techniques. More fundamentally, it is also caused by the significant inter-trial variance of the timing of neuronal activity, which greatly reduces the temporal resolution of our observations when standard analytic methods (ex. across-trial averaging) are employed. For these reasons, it remains largely unknown how neuronal activity traverses space and time, and how it relates to behavior or to other physiological mechanisms such as modulatory oscillatory activity. In our

study, we set out to begin to address this critical problem. To do this, we recorded electrocorticographic (ECoG) signals in four human subjects while they performed a visuomotor response task: subjects pressed a button as soon as they detected a change in a visual stimulus. We then applied a novel detection technique to identify the time of activity onset and offset in neuronal populations in single trials. The results show that population activity at each location was typically much briefer (mdn = 42.5ms) than implied by previous studies. Furthermore, they demonstrate that variance in the timing of population activity is strongly related to variance in the timing of the behavior ($r2 = 0.32$, $p < 0.001$). Finally, our results reveal an intriguing preference of the timing of the onset of cortical activity to the first half of the trough of underlying oscillatory activity in the alpha band (binomial test, $p < 0.001$). Our results document this effect across all subjects and across many brain regions. This result provides tantalizing evidence for a general physiological mechanism that governs the activation and/or synchronization of distant neural populations.

Disclosures: **W.G. Coon:** None. **A. Gunduz:** None. **P. Brunner:** None. **B. Pesaran:** None. **G. Schalk:** None.

Poster

039. Signal Propagation in Neural Networks

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UK MRC Fellowship G0900425 (MHH)

Title: Synaptic depression enables reliable sequential activity cascades in recurrent networks

Authors: *M. M. BIHUN, M. H. HENNIG;
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Abstract: A growing body of research reports the sequential activation of neural assemblies, suggesting that some brain computations are performed through sequence-based dynamics. Hippocampal replay, songbird, neural activity found in posterior parietal cortex and hippocampus during working memory tasks are just a few examples. It remains unclear how

those sequences are generated and a number of models have been proposed. Kremkow et al. (J. Neurosci 2010) demonstrated that timing differences between correlated excitation and inhibition - temporal gating - can control the propagation of spiking activity transients. We adapted this mechanism in the following scenario. We simulated a recurrent balanced network of 20,000 conductance-based leaky integrate-and-fire with sparse connectivity (5%) and embedded a chain of gates, exploiting the temporal gating principle. The chain consisted of 3 to 20 gates to test whether this framework can underlie the generation of sequential activity in the spiking networks. We found that this connectivity enables, in principle, reliable feed-forward propagation and transient amplification along such a chain. However, in a recurrent network the synchronous activity in a chain can also excite the rest of the network, which then leads to instabilities such as persistent oscillatory activity. If the total excitation is weaker to compensate for such effects, the network elevates its activity only during the signal propagation which is followed by the period of hyperpolarization of most of the neurons. On the network level it can be interpreted that the activation of a chain destabilizes and then transiently shuts down the whole network which then recovers to its normal, low firing rate activity. We next investigated the possible role of two mechanisms in stabilising network activity under these conditions. Spike Frequency Adaptation did not prevent the network from entering the oscillatory regime but allowed it to escape it and return to its normal state. Short-term plasticity in a form of synaptic depression allowed the network to remain in its stable state and shortened the hyperpolarization following chain activation. Next, we embedded multiple chains to test the robustness of this framework. If the chains are non-overlapping, the signal propagation is successful in all of the chains. When the chains have too many shared neurons, the signal gets disrupted and may lead to simultaneous activation of two or more chains. The next step is to find the conditions that allow for reliable signal propagation in such overlapping chains.

Disclosures: **M.M. Bihun:** None. **M.H. Hennig:** None.

Poster

039. Signal Propagation in Neural Networks

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 39.08/C65

Topic: B.09. Network Interactions

Support: NIMH intramural Research Program

Title: Rate and size modulation of neuronal avalanches during motor and cognitive tasks in macaque monkeys

Authors: *S. YU[1], S. CHOU[1], A. R. MITZ[2], R. SAUNDERS[2], D. PLENZ[1];
[1]Section on Critical Brain Dynamics, [2]Lab. of Neuropsychology, Natl. Inst. of Mental Hlth., Bethesda, MD

Abstract: All cortical functions, even simple ones, engage large, spatially distributed groups of neurons, yet the precise spatiotemporal organization of such group activity is unclear. During well-controlled behavioral studies, neurons often modulate their firing rates in relation to significant behavioral events, supporting the idea of a rate code, which may not require precise temporal coordination of neuronal activities. In contrast, firing of neuronal pairs often undergoes transient phase-locking in relation to behavioral events, supporting a precise temporal code. So far, it is unclear how both frameworks can be reconciled at the level of large neuronal groups. Here we address this issue by studying neuronal avalanches in behaving monkeys. Neuronal avalanches are cascades of neuronal activity in superficial layers of cortex with spatiotemporal organization precisely governed by power laws. We recorded neuronal avalanches in 2 monkeys engaged in 2 different tasks using chronically implanted 10 by10 microelectrode arrays (monkey 1: pre-motor cortex; monkey 2: dorsal-lateral prefrontal cortex). Monkey 1 self-initiated the touching of a sensor pad for rewards. Monkey 2 performed a visual-motor mapping task in which food rewards were retrieved from dispensers according to a visual cue. We recorded the local field potential (LFP, 1-100 Hz) and spiking activity (300-7500 Hz) for 0.5-1 h during which the monkeys performed 150-300 trials. In both monkeys, neuronal firing correlated with distinct behavioral aspects (e.g., hand movement, cue presentation, cue identity), demonstrating that the areas recorded from participate in task encoding. For avalanche analyses, negative deflections in the LFP on the array (nLFPs; -2.5SD) were concatenated into spatiotemporal patterns. We found that pattern rate and size were strongly modulated by behaviorally relevant events (e.g., cue presentation, movements). Despite non-stationary rates and sizes, the size distribution of patterns invariably obeyed a power law over the duration of the trial, demonstrating that the patterns emerged from avalanches. The power law was abolished using a shift-predictor, which randomizes individual recording sites across trials and, therefore, controls for nLFP rate modulation during trials but destroys fine temporal correlations between sites. These results, for the first time, demonstrate that neuronal dynamics organize as avalanches during well-defined behavioral episodes. It extends our previous work based on resting state activity to behavior activity and introduces a precise spatiotemporal coordination of neuronal population activity that underlies cortical functions.

Disclosures: **S. Yu:** None. **S. Chou:** None. **A.R. Mitz:** None. **R. Saunders:** None. **D. Plenz:** None.

Poster

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Program#/Poster#: 39.09/C66

Topic: B.09. Network Interactions

Title: Selective activation of columnar neural population by lateral inhibition in a realistic model of primary motor cortex

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Abstract: Spatially arranged columns in the neocortex has been considered to process information with interacting neighboring columns through horizontal connection. In order to understand how the horizontal interaction regulates the activity of columns in motor control, we developed a model of the primary motor cortex (M1) including realistic spatial distribution of the neural connectivity. The constructed model consisted of layer 2/3, 5A, 5B, and 6. We used integrate-and-fire neuron model for excitatory and inhibitory neurons in each layer. The connection probability among neurons across/within layers was based on experimental reports using laser scanning photo stimulation on M1 [1]. First, to investigate the contribution of horizontal connection in the model, we gave stimulation to neurons within a 90 micrometer diameter sphere centered in layer 2/3 and recorded the synaptic currents from the cells at different horizontal distances. Both excitatory and inhibitory synaptic currents decreased with the distance from the stimulus site, with less than 50 % at 400 micrometer, which was similar to the experimental result observed in the barrel cortex. We next investigated interaction among neural populations by stimulating two neighboring sites (diameter 90 micrometer) in layer 2/3 simultaneously. With different strengths of stimulus to two sites, inhibition from strongly stimulated site prevented the neurons in the other site from firing. The result suggests that the neural population in layer 2/3 comparable to column size may have the ability to generate lateral inhibition. Finally, assuming sensory input to layer 2/3, and top-down input to layer 5B, we tested simultaneous stimulation to layer-2/3 and layer-5B cells of two neighboring columnar populations, with different intensity for layer-2/3 subpopulations and the same subthreshold intensity for layer-5B subpopulations. Selective activation of the layer-2/3 subpopulation receiving strong stimulus occurred due to lateral inhibition, which resulted in selective activation of a layer-5B subpopulation including corticospinal neurons. These results suggest that the

horizontal connections in M1 may enable selection of motor outputs by lateral inhibition among columns. [1] Kätzel D. et al. 2011. Nature. Neuroscience. 14: 100-107.

Disclosures: **J. Igarashi:** None. **J. Moren:** None. **J. Yoshimoto:** None. **K. Doya:** None.

Poster

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Program#/Poster#: 39.10/C67

Topic: B.09. Network Interactions

Support: NIA P01 AG022550

NIA P01 AG027956

Title: Crosstalk between Nrf2 anti-oxidative signal pathway and mitochondria

Authors: ***J. SUN**, J. W. SIMPKINS;
Physiol. & Pharmacol., Univ. of West Virginia Robert C. Byrd Hlth. Sci. Ctr., Morgantown, WV

Abstract: The accumulation of reactive oxygen species (ROS) in the neurons contributes to the development of many neurodegenerative diseases. The elimination of oxidative stress partly depends on the transcription factor nuclear factor E2-related factor 2 (Nrf2)-antioxidant response element (ARE) signal pathway which induces the expression of various anti-oxidative protein. Nrf2 is a transcription factor and regulates ARE-containing gene expression which is a cis-acting regulatory element that controls the transcription of phase II detoxification enzymes and antioxidant protein levels, such as glutathione synthesis. Activation of Nrf2-ARE signaling system is dependent upon redox homeostasis in cells. Under normal conditions, Keap1, as an adaptor that binds to Nrf2, causes Nrf2 ubiquitination and its rapid degradation. Under oxidative stress, the binding between Nrf2 and Keap1 is disrupted; then Nrf2 translocates into the nucleus and binds to ARE that promote the transcription of ARE-driven genes. Tertiary-butylhydroquinone (tBHQ) is a well-known phenolic compound that causes Nrf2 nuclear localization and activation of Nrf2-ARE pathway. We investigated the effect of activation of the Nrf2-ARE pathway by tBHQ on mitochondrial function under oxidative stress *in vitro*. Glutamate toxicity in HT22 cells, a hippocampus neuronal cell line, is a commonly used *in vitro* oxidative stress model. We found that tBHQ exerted a complete protective effect against glutamate toxicity. This neuroprotection was effective even with an 8 hours treatment delay with

tBHQ. tBHQ treatment prevented the increase in ROS and mitochondrial membrane potential disruption caused by glutamate. Also, glutamate impaired mitochondrial respiration and ATP production; a functional deficiency that was inhibited by tBHQ. In addition, We demonstrated that tBHQ suppressed mitochondrial-mediated apoptosis which contributed to its neuroprotective effect. Apoptosis-inducing factor (AIF), located in mitochondria, translocates to the nucleus and induces cell death. To be released from mitochondria, AIF is cleaved by clapain and this process is suppressed by calpastatin. We found that tBHQ prevented glutamate-induced translocation of AIF. There was no significant change of calpain level. However, glutamate suppressed calpastatin activity which indirectly promoted calpain activity and led to AIF cleavage, effects that were prevented by tBHQ. In conclusion, tBHQ rescues cells from glutamate toxicity through maintaining mitochondrial function and inhibiting mitochondrial-mediated apoptosis.

Disclosures: **J. Sun:** None. **J.W. Simpkins:** None.

Poster

039. Signal Propagation in Neural Networks

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Program#/Poster#: 39.11/C68

Topic: B.09. Network Interactions

Title: Inference of neural pathways in a dynamical connectome of the *C. elegans* worm

Authors: *E. SHLIZERMAN;
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Abstract: The nervous system of the nematode Caenorhabditis elegans (*C. elegans*) is comprised of 302 neurons for which electro-physical connectivity map (i.e. connectome) is fully resolved. Although the static connectome is available, inference of dominant neural pathways that control sensorimotor responses is challenging since neurons are dynamical objects and interactions within the network are also dynamic. In our study, we construct a Probabilistic Graphical Model (PGM) for the *C. elegans* connectome that represents the 'effective connectivity' between the neurons (correlations) and takes into account the dynamics. The structure of the PGM is learned using Bayesian methods capable of learning the structure of an undirected graphical model from a collection of time series. The collections are obtained by a systematic excitation of neurons in a recently developed computational dynamical model for the *C. elegans* that simulates single neural responses and interactions between the neurons (synaptic and gap). Bayesian inference methods applied to the constructed PGM allow us to extract neural

pathways in the connectome of *C. elegans* responsible for experimentally well characterized movements of the worm such as forward and backward locomotion. In addition, we show that the framework allows for inference of pathways that correspond to movements that were not fully characterized in experiments and to perform 'reverse-engineering' studies in which a typical setup on the motor neurons layer is imposed and dominant pathways that propagate to the sensory layer through the interneurons layer are being identified.

Disclosures: E. Shlizerman: None.

Poster

039. Signal Propagation in Neural Networks

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Topic: B.09. Network Interactions

Support: EPA-G2012-STAR-F1

Title: Exosomes with cell signaling capabilities can be isolated from neuronal cells following *in vitro* differentiation under feeder and serum free conditions

Authors: *R. L. WEBB[1,2,3], H. M. REISS[2,3], E. T. JORDAN[2,3], F. GOODFELLOW[2,3], S. L. STICE[2,3];
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Abstract: Exosomes are small, concave secretory vesicles originating from the fusion of intracellular multivesicular bodies with the plasma membrane. These vesicles range in size from 50-100 nm, and contain cargoes implicated in intercellular signaling mechanisms, including lectins, lipids, proteins, and various species of regulatory RNAs. While the role of exosomes in cellular signaling has been best described in terms of antigen presentation for immune cells, it is becoming ever more apparent that exosomes are secreted by many types of cells, including cortical neurons, astrocytes, and oligodendrocytes of the central nervous system. We hypothesize that exosomes confer paracrine benefits on neural stem cells and play a critical role in both optimal *in vitro* neural cultures conditions and in therapeutic outcomes of neural stem cell treatments. The objective of the current study was to determine if it was possible to isolate exosomes from human stem cells of a neural lineage, specifically neural progenitor cells and / or differentiated post-mitotic neuronal cells. Exosomes were purified from neural progenitor cells (SOX 1+and 2+, OCT4-; hNP1™ ArunA Biomedical), derived from human pluripotent stem cell

lines or differentiated neuronal cells (β-III tubulin (Tuj1) +, MAP2+, Oct4-; hN2™ ArunA Biomedical). While preliminary data indicate that neural progenitor cells secrete fewer exosomes than SY5Y neuroblastoma cells or other cell types, we were able to purify exosomes using either polymer extraction methods or ultracentrifugation. Coomassie staining of purified fractions revealed a broad range of proteins expressed in the neural progenitor population compared to the differentiated neuronal cells, a finding that will be further investigated using silver staining and mass spectroscopy to develop a profile of proteins. Under *in vitro* conditions, treatment of cells with purified exosomes resulted in an approximate 3 fold increase in intracellular calcium following addition of human differentiated neuronal cell exosomes compared to those from SH SY5Y cells, as measured with Molecular Devices Calcium 6 FLiPR assay. This detects calcium flux as a result of activation of either ionotropic or metabotropic glutamate receptors, the receptor class that mediates the majority of excitatory neurotransmission, and a receptor class implicated in several pathological conditions. Future studies will evaluate the biochemical properties of these exosome populations in greater detail with the goal of determining the mechanism and potential benefits that exosomes confer on human neural cells.

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Poster

039. Signal Propagation in Neural Networks

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Topic: B.09. Network Interactions

Support: NSF Grant 1146708

Office of Navel Research (ONR) N0014-10-1-0072

NINDS grant NS45260

Title: Processing of theta input differs markedly at the individual stages of the piriform-hippocampal network

Authors: ***B. H. TRIEU**[1], E. A. KRAMAR[1], Y. JIA[1], W. WANG[1], C. D. COX[1], D. T. PHAM[1], C. M. GALL[1,2], G. LYNCH[1,3];

[1]Anat. and Neurobio., [2]Neurobio. and Behavior, [3]Psychiatry and Human Behavior, Univ. of CA - Irvine, Irvine, CA

Abstract: The series of steps leading from the lateral olfactory tract through piriform/entorhinal cortex and the primary intra-hippocampal circuit constitutes one of the best defined, many stage networks found in the forebrain. We have conducted a first test of whether individual components in the extended network perform different types of signal processing. Theta is a prominent rhythm in the piriform-hippocampal system and so we applied 20 second trains of 5Hz stimulation to each of five stages in the piriform/hippocampal system. Dramatic regional differences in synaptic responses were found. Two connections exhibited a brief facilitation (lateral olfactory tract to piriform, lateral perforant path), one steadily declined during the train (piriform associational), a third showed progressively greater facilitation (mossy fibers), and a fourth generated a biphasic pattern (CA3 to CA1). Transmitter mobilization and adenosine modulation were strikingly different between the relays and contributed to the observed variations in theta processing. Factors potentially involved in the neurobiological differentiation between network elements, including vesicle loading proteins and key elements in the 'tripartite synapse', were identified. Notably, input / output (number of stimulated axons / size of EPSP) relationships described a third discriminative dimension. This third vector likely reflects relative anatomical divergence of projections to the target fields and may contribute to regional differences in LTP. Simulations based on the above results describe a network whose initial stages (piriform cortex, dentate gyrus) respond to external information with a brief surge of activity which is followed by delayed elaboration in the output components (pyramidal cell fields of hippocampus). We propose that these differences in signal processing play important roles in pattern separation by the former structures and pattern completion by the latter.

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Poster

039. Signal Propagation in Neural Networks

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Support: ERC Advanced Investigator grant SERRACO

OTKA K109790

Title: The hippocampo-septal feedback state-dependently modulates the medial septum

Authors: *L. NIKITIDOU, E. PAPP, T. F. FREUND, V. VARGA;
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Abstract: Theta rhythm in the limbic system is a highly regular oscillation in the 4 -10 Hz frequency range strongly associated with information acquisition, memory formation and sensory-motor integration. The reciprocal inhibitory connection between the medial septum (MS) and the hippocampus comprises the backbone of the theta generating circuitry. GABAergic MS neurons are thought to generate the pacemaking input to the hippocampal inhibitory network. According to theoretical and *in vitro* studies, somatostatin (SOM)-containing hippocampo-septal (HS) neurons may synchronize the MS pacemaker cells. However, the hippocampus is capable of autonomously generating theta oscillations *in vitro*. Thereby, theta rhythmicity would emerge from the communication of the MS and hippocampal oscillating networks, but the role of the HS pathway in this dialogue is still unknown. Here, we aim to disentangle the action of the HS feedback, by recording the response of MS neurons to the selective manipulation of HS fibers, in urethane-anesthetized as well as in freely moving mice. To selectively manipulate HS fibers or terminals, SOM-expressing hippocampal neurons were in SOM-Cre transgenic mice transduced by a Cre-dependent adeno-associated viral vector encoding channelrhodopsin 2. Activation of either HS axon terminals in the MS or passing fibers in the fimbria vigorously inhibited theta-coupled MS neurons and resulted in a 5 - 100 ms post-pulse inhibitory period. HS-elicited inhibition was weaker during sensory evoked theta oscillations compared to non-theta states. Surprisingly, the majority of neurons were activated by 5 and 10 Hz stimulation despite exhibiting post-pulse inhibition. The response of the activated neurons was strongly state-dependent, as characterized by resistance to perturbation during theta oscillations as opposed to significant alteration of firing pattern in non-theta states. In a freely moving mouse HS fiber stimulation at high frequency (20 or 50 Hz) blocked the firing of MS units during non-theta or still theta whereas it failed to alter neuronal activity during movement theta epochs. Thus, the HS feedback modulates the medial septal network in a state-dependent manner.

Disclosures: **L. Nikitidou:** None. **E. Papp:** None. **T.F. Freund:** None. **V. Varga:** None.

Poster

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Program#/Poster#: 39.15/C72

Topic: B.09. Network Interactions

Support: HFSP Grant LT000132/2012

Title: Signal and noise correlations in a recurrent network of neurons in culture

Authors: *J. BARRAL[1], T. TCHUMATCHENKO[2], A. REYES[1];
[1]Ctr. for Neural Sci., New York Univ., New York, NY; [2]Max Planck Inst. for Brain Res., Frankfurt, Germany

Abstract: Neighboring neurons share common synaptic inputs, potentially leading to correlated spiking activity (so-called noise correlation). In addition, spiking activity may be correlated in the presence of a global drive to the network (so-called signal correlation). Both experimental results and theoretical analyses suggest that noise correlation may be attenuated actively via the arrival of strongly correlated inputs. This decorrelation mechanism is made possible by the cancellation of synchronous excitatory and inhibitory inputs at resting membrane potential. Here, we used a network of cortical neurons in culture to examine whether active decorrelation operates equally on signal and noise correlations and whether it depends on network state and neuronal distance. Cultures containing excitatory and inhibitory neurons were made from tissue excised from mouse cortex and transfected with channelrhodopsin. We developed an innovative optical device to stimulate optogenetically a large neuronal population with both spatial and temporal precision. Using an ultra-fast video-projector, we stimulated 10 to ~50 neurons with independent trains of light pulses that evoked action potentials with high temporal resolution. With this preparation, we systematically varied the spatial correlation of the stimulated neurons, their firing rates and the spatial extent of the activated network. We experimentally examined correlations between inhibitory and excitatory inputs in the recurrent network when a sub-population was activated by a given spatiotemporal pattern. We showed that active decorrelation operates in recurrent networks of neurons in culture. A systematic delay between excitation and inhibition guarantees that some correlated activity persists and can be modulated by an external input. In particular, the correlation between post-synaptic potentials increases with the firing rate and with the spatial correlation of the stimulated neurons. Comparison to simulations of integrate-and-fire neuron networks highlighted the generic properties of active decorrelation and its robustness. Although signal and noise are both actively decorrelated, signal correlation remains dominant, allowing information to be encoded by the correlated activity of neurons. Additionally, we observed that signal correlation remains high even for distant neurons while noise correlation decays rapidly, consistent with noise correlation arising from local shared inputs. Hence, signal-to-noise ratio increases with distance, suggesting that signal is better transmitted between spatially disparate neurons.

Disclosures: **J. Barral:** None. **T. Tchumatchenko:** None. **A. Reyes:** None.

Poster

039. Signal Propagation in Neural Networks

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Topic: B.09. Network Interactions

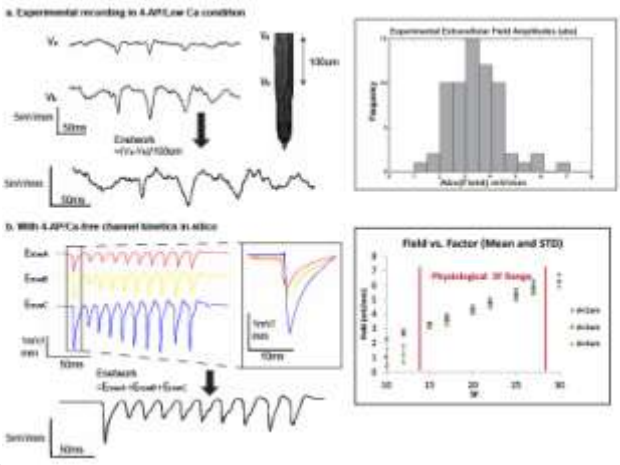
Support: NIH grant # 2R01NS060757-05A1

Title: Propagation of neuronal activity by electrical field

Authors: *C. QIU, M. ZHANG, D. DURAND;
Biomed. Engin., Case Western Reserve Univ., Cleveland, OH

Abstract: The propagation of 4-AP induced epileptiform neuronal activity in the hippocampus exhibits a speed of approximately 0.1m/s and could propagate without synaptic transmission or gap junctions. This non-synaptic propagation could not be explained by diffusion since it is too slow, suggesting that there exists another mechanism for such type of propagation. Electrical field effect is a highly possible candidate as it can modulate the spiking patterns and timings of single neuron or network firing activity. We developed a simulated CA1 pyramidal network model with cells connected solely through field effect and tested the hypothesis that electrical field alone can cause neuronal activity to travel with a speed of 0.1m/s. The results showed that with 4-AP/No [Ca] and normal aCSF cell kinetics, field effect could mediate transverse propagation with a speed of 0.12 ± 0.097 m/s and 0.11 ± 0.034 m/s, respectively, upon spiking initiation of the first row cells. The network activity under both conditions resulted in an endogenous network field amplitudes of ~2-~5mV/mm, matching the weak field measured in unfolded hippocampus or cortex in-vitro. Increasing the extracellular resistivity by 40% caused an exponential increase of speed and linear increase of network field amplitude; additionally, an inversely linear relationship was observed between the field amplitude and propagation delay time. Changing the membrane capacitance and resistance did not significantly change either the speed or the field amplitude. Finally, simulation results predicted that smaller extracellular space would generate higher propagation speeds. Experiments in hippocampal slices confirmed this prediction when the osmolarity was changed to affect the extracellular space. Taken together, these results show that despite its weak amplitude, the field effect can be solely responsible for neuronal activity propagation with a speed of 0.1m/s in both pathological and physiological

conditions. The main parameter affecting the speed of propagation is the amplitude of the field.



Disclosures: **C. Qiu:** None. **M. Zhang:** None. **D. Durand:** None.

Poster

039. Signal Propagation in Neural Networks

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Topic: B.09. Network Interactions

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Title: Electrical coupling and synchronized activity within iPSC-derived neuronal networks

Authors: M. W. TOIVONEN[1], Z. ZHU[1], X.-H. ZENG[1], J. TURECEK[2], V. Z. HAN[1], *J. P. WELSH[1];
[1]Ctr. for Integrative Brain Res., Seattle Children's Res. Inst., Seattle, WA; [2]Neurobio., Harvard Univ., Boston, MA

Abstract: We examined the synaptic properties of cultured neurons derived from induced pluripotent stem cells (iPSCs). Fibroblasts from adult C57BL/6 mouse tail were reprogrammed into iPSCs by forced expression of OCT4, SOX2, KLF4, and c-MYC and subsequently differentiated into neuronal networks by culturing with N2 medium (DMED/F12 with 1% NEAA, 2 mM L-Glutamine, 2% B27, 1% FBS). iPSC-derived neuronal networks were studied over days 12-20 of differentiation with immunostaining and dual whole-cell patch-clamp electrophysiology. iPSC-derived neurons uniformly expressed the neuronal marker MAP2 in

dendrites. In addition, iPSC-derived neurons expressed the neuronal gap junction protein connexin36 in nearly all somata and dendrites and GABA in 18% of the neuronal somata. As has been reported with single neuron recordings, iPSC-derived neurons often exhibited spontaneous spiking and spontaneous EPSPs and IPSPs. Dual recordings were obtained in neuron pairs having interwoven dendrites whose somata were separated by no more than 70 μm. Of 141 dual recordings, 21% showed synchronized spontaneous spiking and/or subthreshold activity that was blocked by 20 μM CNQX, indicating a necessary role for glutamatergic synaptic transmission. Cross-correlation of synchronized spiking showed high temporal variability, with spike bursts of one neuron randomly leading or lagging the other by 400 μs to 50 ms (Cv=0.58 ± 0.12 SEM). Direct chemical synaptic transmission was observed in 5% of neuron pairs in which depolarizing current injected into one neuron evoked a direct spike and a following spike in the neighboring neuron with high probability (75 ± 13%), stereotypic latency (9.95 ± 0.5 ms), and low temporal variability (Cv=0.15 ± 0.02). Of note, 3.6% of neuron pairs showed electrotonic coupling as measured by membrane potential hyperpolarization to -300 or -500 pA injected into the neighboring neuron. Coupling coefficients and gap junctional conductances ranged from moderate (3.4% and 130 pS) to very strong (38% and 6.5 nS) with means of 13.3 ± 4.3% and 1.5 ± 0.8 nS and medians of 7.3% and 300 pS, respectively. Electrical coupling among iPSC-derived neuron pairs had a median symmetry of 1.37, but could be perfectly symmetric (1.02) or strongly asymmetric (3.7). The study indicated that monosynaptic chemical and electrical synaptic transmission integrate in iPSC-derived neuronal cultures and contribute to activity synchronization.

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Poster

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Topic: B.09. Network Interactions

Support: EC BrainScaleS FP7-269921

EC Human Brain Project

CNRS

Title: High-frequency oscillations in human and monkey cortex during sleep

Authors: *M. LE VAN QUYEN[1], L. MULLER[2], B. TELENCZUK[3], N. DEHGHANI[5], S. CASH[6], E. HALGREN[7], N. HATSOPOULOS[8], A. DESTEXHE[4];
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Abstract: High-density 2D microelectrode arrays offer an unique way to simultaneously record spike and local field potential (LFP) activity along the cortical surface. Here, with 4 mm × 4 mm 100-electrode Utah arrays in human and monkey neocortex, we analysed spontaneous high-frequency LFP oscillations (>20 Hz) during wakefulness and the different stages of sleep. In both monkey and humans, narrow-band beta (20-35 Hz) and gamma range (40-80 Hz) were identified during slow-wave sleep and they were also present, but generally less pronounced, during waking and REM sleep. Very frequently, in all states, beta and gamma patterns appeared at about the same time on the array, forming large spatially coherent patterns separated by many seconds with less activity. We applied phase-based analysis methods to precisely quantify the propagation of these beta/gamma patterns. We found that that beta and gamma oscillations formed waves of activity that propagate over the array with a velocity of 20-30 cm/s, close to axonal propagation velocity. After separation of units between “regular-spiking” (RS) and “fast spiking” (FS) neurons based on spike waveform and duration from a large number of cells (up to 180), we found that a strong increase in firing of specific FS cells during beta/gamma patterns. Furthermore, specific correlated firings of spatially distant neurons (>1 mm) were detected during these high-frequency patterns, suggesting that fast oscillations mediate neuronal synchronizations between large-scale patches of cortex. Altogether, these findings confirm and extend earlier animal studies reporting high-frequency oscillations during sleep. In addition, we report that FS cells play a key role in the generation of high-frequency oscillation. Furthermore, beta/gamma waves have been found to organize and modulate cortical population activity on the mesoscopic scale. The partial overlap between the spatial distribution of high-frequency oscillations during sleep and wakefulness suggests that these waves are of similar nature and that, during sleep, they may restore wake-like patterns that support the consolidation of information related to the experiences of the previous awake period.

Disclosures: **M. Le Van Quyen:** None. **L. Muller:** None. **B. Telenczuk:** None. **N. Dehghani:** None. **S. Cash:** None. **E. Halgren:** None. **N. Hatsopoulos:** None. **A. Destexhe:** None.

Poster

039. Signal Propagation in Neural Networks

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 39.19/D4

Topic: B.09. Network Interactions

Support: CNRS

ANR grant ComplexV1

ENP fellowship (LM)

EU grant BrainScales

Human Brain Project (AD)

Title: Propagating waves from local field potentials in multi-electrode recordings in human and monkey cortex match the properties found in voltage-sensitive dye imaging

Authors: *A. DESTEXHE[1], L. MULLER[1], G. BENVENUTI[2,3], F. CHAVANE[2,3];
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Abstract: The existence of propagating waves, either spontaneous or stimulus-evoked, in neocortex during the awake state has been a subject of recent interest [1,2,3]. Here, following work done previously in voltage-sensitive dye imaging of the primary visual cortex in the awake monkey [3], we apply an analysis method for non-parametric, automated detection of propagating waves to multielectrode (Utah) array recordings taken from the neocortex of monkey (primary visual cortex), and compare to recordings in human (middle temporal gyrus; data from [4]). In the spontaneous activity during wakefulness, we detect transient, intermittent propagations occurring in the local field potential (LFP) across the biological frequency range. After selecting the propagation epochs within the highest confidence interval, we observe that the speed distribution falls naturally into the range of the horizontal fibers (0.1 - 0.5 m/s), suggesting a similar propagation substrate as that observed previously in voltage-sensitive dye imaging data [3]. With this in mind, we go on to compare the spatiotemporal dynamics on the array across various states of arousal. One question raised by our earlier VSD analysis is the extent to which the wave evoked by a small visual stimulus is supra- or sub-threshold. Specifically, because neurons during awake, "activated" cortical states sit a few millivolts below threshold [5,6], operating in a fluctuation-driven regime [7,8], the transient depolarization

detected in the VSD signal as the wave passes may change the background spiking probability in the local circuit. To address this, we analyzed the relationship between single- and multi-unit activity (SUA/MUA) and LFP. We study the spatiotemporal dynamics of this relationship to determine the coupling of spiking activity with transient propagating waves, and compare the results with computational models of spiking neurons. In conclusion, we show here that a combination of multi-electrode recordings, time-series analysis and computational modeling provides a powerful set of tools for quantifying the spatiotemporal dynamics of the awake state in human and monkey. 1. Ray S & Maunsell JH (2011) J Neurosci. 31: 12674-12682. 2. Nauhaus I, Busse L, Ringach DL & Carandini M (2012) J Neurosci 32: 3088-3094. 3. Muller LE, Reynaud A, Chavane F & Destexhe A (2014) Nature Commun. 5: 3675. 4. Peyrache A et al. (2012) Proc. Natl. Acad. Sci. USA 109: 1731-1736. 5. Destexhe A, Rudolph M & Pare D (2003) Nat Rev Neurosci 4: 739-751. 6. Poulet J & Petersen C (2008) Nature 454: 881-885. 7. Destexhe A, Rudolph M, Fellous J & Sejnowski T (2001) Neuroscience 107: 13-24. 8. Kuhn A, Aertsen A & Rotter S (2004) J Neurosci 24: 2345-2356.

Disclosures: **A. Destexhe:** None. **F. Chavane:** None. **L. Muller:** None. **G. Benvenuti:** None.

Poster

039. Signal Propagation in Neural Networks

Location: Halls A-C

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Program#/Poster#: 39.20/D5

Topic: B.09. Network Interactions

Support: EC BrainScaleS FP7-269921

EC Human Brain Project

CNRS

Title: Relations between units and LFPs inferred from multielectrode recordings in human and monkey

Authors: *B. TELENCZUK[1], N. DEHGHANI[2], M. LE VAN QUYEN[3], S. CASH[4], E. HALGREN[5], N. HATSOPOULOS[6], A. DESTEXHE[1];
[1]Unité de Neurosciences, Information & Complexité,, Ctr. Natl. De La Recherche Scientifique, Gif-sur-Yvette, France; [2]Wyss Inst., Boston, MA; [3]L'Institut du Cerveau et de la Moelle Épinière, Paris, France; [4]Massachusetts Gen. Hospital, Harvard Med. Sch., Department of

Neurology, MA; [5]Departments of Radiology and Neurosci., Univ. of California, San Diego, CA; [6]Univ. of Chicago, Chicago, IL

Abstract: We investigated the local field potential (LFP) contribution associated with a single spike in human and non-human-primate cortex. The data were recorded from the temporal cortex of patients who underwent a surgical treatment for the localisation of the epileptic foci [2] and from the motor cortex of macaque monkeys [3]. The LFP and spiking activity were recorded with a 10-by-10 array of intracortical electrodes (Utah array, interelectrode distance 400 μm). Spikes of single neurons were discriminated by semi-automatic clustering and the cell type was determined based on a spike waveform [2]. The average of LFP segments triggered on spontaneous spikes (LFP-STA) showed components that were non-causal, i.e. preceding spike occurrence, and non-local, i.e. appearing with no delay at large distances. We investigated whether this spatial and temporal spread could be explained by the morphology, the volume conduction and/or network interactions. We developed a simple statistical model of the LFP generated by a population of correlated Poisson-like neurons. The contribution of each spike to the LFP was described by a LFP kernel. In a population of independent neurons we could perfectly recover this contribution by means of the LFP-STA, but correlations made the LFP-STA non-causal and spatially non-local. Its width and amplitude depended on the jitter, correlation coefficient and the number of contributing neurons. Based on realistic values of the parameters we estimated that in presence of correlations the LFP-STA is about 100-1000 times larger the LFP-STA in an uncorrelated population. To test whether the correlations could explain the spatial and temporal broadening of the experimental LFP-STA we estimated the spatial co-variance matrix from the on-going LFP. By inverting the matrix we derived spatio-temporal filters, which disambiguated the local (<800 μm) LFP components triggered by a single neuron. The amplitude and spatial reach of these components were larger for putative inhibitory neurons compared to excitatory neurons. These differences might be are related to axon morphology and/or synaptic connectivity. We conclude that while the contribution of a single neuron is local and does not exceed the area within a radius of 800 μm, the LFP is spatially and temporally extended due to the neuronal correlations and volume conduction. The local LFP contributions are dominated by inhibitory neurons confirming results of *in vitro* experiments [1]. [1] M. Bazelot et al. J. Physiol. (Lond.), 588:2077, 2010. [2] A. Peyrache et al. Proc. Natl. Acad. Sci., 109:1731, 2012. [3] M.Saleh et al. J. Neurosci., 32:1220, 2012.

Disclosures: **B. Telenczuk:** None. **N. Dehghani:** None. **M. Le Van Quyen:** None. **S. Cash:** None. **E. Halgren:** None. **N. Hatsopoulos:** None. **A. Destexhe:** None.

Poster
[Unable to Attend]

039. Signal Propagation in Neural Networks

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 39.21/D6

Topic: B.09. Network Interactions

Title: Mechanisms of memory destruction in an *in vitro* model of interictal epileptiform discharges

Authors: *M. R. DRANIAS, A. M. J. VANDONGEN;
Neurosci. and Behavioral Disorders (NBD), Duke-NUS Grad. Med. Sch., Singapore, Singapore

Abstract: Brief synchronizing bursts of epileptiform activity known as interictal epileptiform discharges (IEDs) can cause transient cognitive impairments (TCI) in the absence of overt seizures. We show that stimulus information represented in networks of living neurons is also disrupted by brief synchronizing bursts of neural activity (SNBs), analogous to IEDs, and demonstrate some of the mechanisms of this disruption. Experiments were performed using primary cultures of E18 dissociated rat cortical neurons transfected with ChannelRhodopsin-2 and plated on multielectrode arrays. These optogenetically modified networks encode and maintain stimulus information for as long as one second and while stimulus information persists in memory, additional stimuli elicit an adapted response that reflects the identity of the prior stimulus. On each trial we presented either one stimulus or two sequential stimuli. On trials interrupted by SNBs, stimulus-specific information tends to be lost. To better understand this process, we investigated how the timing of SNBs impacts stimulus adaptation in the sequential stimulus task and the changes in entropy associated with SNBs. SNB timing was analyzed by breaking trials into encoding, delay and readout phases. The adapted response of the network to a second stimulus is lost regardless of timing. Examination of the pattern of network activity indicates SNBs do not interfere with stimulus processing by acting like white noise, rather some networks have a single stereotyped response and others several. An inverse relationship was found to hold between normalized entropy of an SNB and the overall mean firing rate of the network across a trial. This indicates the more excitable a network, the more stereotyped its response. These results are consistent with past experiments, predict how neural circuits *in vivo* will behave during IEDs, and provide a model useful for identifying drugs and human genetic mutations that modulate short-term memory and TCI.

Disclosures: **M.R. Dranias:** None. **A.M.J. VanDongen:** None.

Poster

039. Signal Propagation in Neural Networks

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 39.22/D7

Topic: B.09. Network Interactions

Support: HHMI

Title: A predictive filter for input comparison in the CA1 microcircuit

Authors: *A. D. MILSTEIN, J. C. MAGEE;
Magee Lab., Hhmi/Janelia Farm, Ashburn, VA

Abstract: Spatial and temporal features of synaptic inputs engage integration mechanisms on multiple scales, including presynaptic release sites, postsynaptic dendrites, and networks of inhibitory interneurons. Here we investigate how these mechanisms cooperate to filter synaptic input onto CA1 pyramidal cells. We find that inputs from CA3 onto proximal dendrites as well as inputs from entorhinal cortex layer III (ECIII) onto distal dendrites sum sublinearly or linearly at low firing rates due to feedforward inhibition, but sum supralinearly at high firing rates due to presynaptic facilitation, producing a high-pass filter. However, during punctuated bursts of inputs that mimic theta rhythmic activity, additional nonlinear integration mechanisms in dendrites are balanced by the recruitment of additional feedforward and feedback inhibition, resulting in suppression of dendritic complex spikes. We show that a particular subpopulation of inhibitory interneurons expressing neuropeptide Y (NPY) contributes prominently to this predictive filter by integrating both CA3 and ECIII input pathways and potently inhibiting CA1 pyramidal cell dendrites.

Disclosures: **A.D. Milstein:** None. **J.C. Magee:** None.

Poster

039. Signal Propagation in Neural Networks

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 39.23/D8

Topic: B.09. Network Interactions

Title: Lipopolysaccharide-induced calcium oscillations in neonatal rat nucleus tractus solitarius (NTS) *in vitro*

Authors: M. R. CUSTER, N. OSMAN, *C. G. WILSON;
Ctr. for Perinatal Biol., Loma Linda Univ., Loma Linda, CA

Abstract: Lipopolysaccharide (LPS) is commonly used to induce an inflammatory response in animal models. In premature infants, systemic infection (sepsis) causes tachypnea and an increase in the number of apneas over time. The mechanism by which sepsis alters breathing control centrally is unknown. Our laboratory has previously shown that LPS instilled into the airway of 10 day old rats causes changes in IL-1beta expression in brain stem regions associated with breathing control, including the rostral medulla, NTS, and the hypoglossal (XII) motonucleus. We hypothesized that expression and release of cytokines in the brain stem alters neuronal activity in these brain stem areas which, in turn, alters breathing rhythm. To test this hypothesis, we performed experiments using confocal imaging to evaluate changes in calcium flux in neurons within the NTS before and after exposure to LPS (75 micrograms/200 mL ACSF). We cut acute organotypic slices (300 microns) from brain stems of postnatal day 1 to 5 rat pups. These slices included the preBötzinger complex, XII motoneurons, and the NTS. Fluo-4-AM was used to label the slices, prepared in DMSO/Pluronic and incubated for 1 hour (14 micrograms dye/mL of ACSF) and then the slices were transferred to a Zeiss LSM-710 confocal microscope for imaging of calcium transients. NTS neurons showed an increase in rhythmic calcium oscillations within 10 min after LPS infusion. Baseline burst duration averaged 0.02 ± 0.03 (SD) per minute and increased to 0.56 ± 0.16 bursts per minute. After 30 min, burst frequency declined to 0.19 ± 0.21 per min and continued to fall to 0.01 ± 0.03 after 50 minutes of exposure. The changes in network activity we observed may be the underlying substrate for the changes in breathing pattern seen in sepsis. Changes in the NTS network may gate afferent input information carried via the vagus nerve to the CNS and gating of these afferent inputs may be critical to the breathing changes we have previously observed in our rat models.

Disclosures: **M.R. Custer:** None. **N. Osman:** None. **C.G. Wilson:** None.

Poster

039. Signal Propagation in Neural Networks

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 39.24/D9

Topic: B.09. Network Interactions

Support: The National R&D Program for Cancer Control, Ministry of Health & Welfare, Republic of Korea

Title: TIMP-1 modulates chemotaxis of human neural stem cells through CD63 and integrin signaling

Authors: H. SHIN, *M. LEE;
Brain Dis. Res. Ctr., Sch. Med. Ajou Univ., Suwon, Korea, Republic of

Abstract: **Hee Sun Shin**[1], Soo Youn Lee[1], Jung Mi Kim[1], Soo Young Cho[2, 3], Hyun Suk Kim[1], Jeong Yong Jeon[1], Rukhsana Kausar[1], Seon Yong Jeong[4], Young Seek Lee[5] and Myung Ae Lee[1] [1]Department of Brain Science, Ajou University School of Medicine, Suwon, Korea [2]Mammalian Genetics Unit, Medical Research Council Harwell, Harwell, Oxford shire OX11 0RD, U.K. [3]Laboratory of Developmental Biology and Genomics, College of Veterinary Medicine, and Interdisciplinary Program for Bioinformatics, Program for Cancer Biology and BIO-MAX Institute, Seoul National University, Seoul, Korea [4]Department of Medical Genetics, Ajou University School of Medicine, Suwon, Korea [5]Division of Molecular Life Science, Hanyang University, Ansan, Korea The hNSCs (human neural stem cells) have the interesting characteristic of migration towards an intracranial glioma. However, the molecules and mechanisms responsible for tumour tropism are unclear. In the present study, we used microarray and proteomics analyses to identify a novel chemoattractant molecule, TIMP-1 (tissue inhibitor of metalloproteinase-1), secreted from human brain tumour tissues. We demonstrate that TIMP-1 significantly enhances hNSC adhesion and migration in a cell culture system. These effects were critically dependent on CD63, as shRNA-mediated ablation of CD63 expression attenuated the response. TIMP-1 significantly increased the number of FAs (focal adhesions) and cytoskeletal reorganization for cell migration in hNSCs, whereas knockdown of CD63 resulted in decreased hNSC spreading, FAs and migration, even after TIMP-1 treatment. In addition, TIMP-1 binding to CD63 activated β1 integrin-mediated signalling through Akt and FAK phosphorylation, leading to pattern changes in distribution of vinculin and F-actin (filamentous actin). Furthermore, inactivation of β1 integrin by use of a blocking antibody or inhibition of PI3K (phosphoinositide 3-kinase) signalling impaired the migration of hNSCs towards TIMP-1. Collectively, our results underline TIMP-1 as a novel and effective key regulator of CD63 and β1 integrin-mediated signalling, which regulates hNSC adhesion and migration.

Disclosures: **H. Shin:** None. **M. Lee:** None.

Poster

039. Signal Propagation in Neural Networks

Location: Halls A-C

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Topic: B.09. Network Interactions

Support: Chinese 973 Program (2011CBA00400)

Projects of the Scientific Research Foundation

Title: Visualizing an emotional valence map in the limbic forebrain by TAI-FISH

Authors: *H. HU;
Inst. of Neuroscience, Chinese Acad. of Sci., Shanghai, China

Abstract: A fundamental problem in neuroscience is how emotional valences are represented in the brain. Thus far, we know relatively little about how appetitive and aversive systems interact, and to what extent information regarding these two opposite values segregate and converge. Here we introduce an experimental strategy to simultaneously visualize the neural correlates of two stimuli of contrasting emotional valence across the whole limbic forebrain at single cell resolution. We discovered characteristic patterns of interaction - segregated, convergent and intermingled - between the appetitive and aversive neural ensembles. In the nucleus accumbens, we identified a mosaic pattern of activation by multiple pairs of positive and negative emotional cues, and unraveled previously unappreciated functional heterogeneity within the D1- and D2-type medium-spiny neurons. These results provide new insights into the coding of emotional valence in the brain, and demonstrate proof-of-principle of a powerful methodology for the simultaneous functional mapping of two distinct behaviors.

Disclosures: **H. Hu:** None.

Poster

039. Signal Propagation in Neural Networks

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 39.26/D11

Topic: B.09. Network Interactions

Support: NSF IIS-1009542

Title: Efficient discovery of functional brain networks in large multisubject fMRI datasets

Authors: *J. R. MANNING[1], R. RANGANATH[2], K. NORMAN[2], D. BLEI[2];
[1]Princeton Neurosci. Inst., [2]Princeton Univ., Princeton, NJ

Abstract: Standard approaches to examining functional connectivity patterns in fMRI datasets entail correlating the time series of activations (across images) of each pair of voxels. Because the size of the full brain connectivity matrix grows with the square of the number of voxels, this approach carries a substantial computational burden. For example, the voxel-to-voxel connectivity matrix for a 50,000 voxel image occupies several GB of memory. Comparing many such matrices (e.g. across experimental conditions or participants) requires an alternative approach. We will present a technique, called Hierarchical Topographic Factor Analysis (HTFA), that leverages the strong spatial correlations that pervade fMRI data to reduce the full brain connectivity network to a much smaller K-node network (where the number of nodes, K, is chosen by the practitioner). Our Bayesian inference procedure scales well, allowing researchers to discover networks in huge fMRI datasets, with dozens of subjects and thousands of images, even on modest computer hardware (e.g. a laptop computer). Unlike approaches that require pre-selecting seed regions or regions of interest, the locations (and sizes) of the nodes HTFA uncovers are determined automatically from the full dataset. We will present representative applications of HTFA to several existing fMRI datasets, illustrating how HTFA can be used to identify task-based differences in functional connectivity.

Disclosures: **J.R. Manning:** None. **R. Ranganath:** None. **K. Norman:** None. **D. Blei:** None.

Poster

039. Signal Propagation in Neural Networks

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Topic: B.09. Network Interactions

Support: NIH Grant R37GM049202

NIH Grant R01GM066358

IEEE Computational Intelligence Society

Title: Emergence of consciousness as a phase transition in information percolation

Authors: D. MOWREY, D. W. ZHOU, P. TANG, *Y. XU;
Anesthesiol., Univ. Pittsburgh Sch. Med., Pittsburgh, PA

Abstract: Mammalian brains have evolved to achieve great functional diversity using a limited set of structural motifs. The ways in which highly complex cognitive functions emerge from a finite number of network connections are not fully understood. We present a theoretical model to describe the fundamental processes of information transmission in a hierarchical network, whose edges are stochastically defined by a single parameter, p, representing information percolation probability. The system expands vertically in a layered fractal structure, with each layer forming a Watts-Strogatz small-world network. By treating neural activity as a time series of sensory information percolating from an input node to multiple nodes in an output layer, we reproduced major clinical electroencephalographic (EEG) characteristics found in the loss of consciousness during general anesthesia. As the percolation edge probability increases, the rapid convergence of output and input signal distributions exhibits a steep phase transition, with a concomitant increase in both the spectral power and signal synchrony in the α, β, and γ frequency range and a rapid decrease in information entropy, signifying information integration and the emergence of meaningful correlation among output nodes. Our results demonstrate that by modeling consciousness as information percolation we can capture emergent phenomena clinically observed along the pathway between the anesthetized and conscious states. Robustness of information integrity is maintained even in a sparsely connected network and shows a sharp transition consistent with the precipitous loss of consciousness during general anesthesia.
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Disclosures: **D. Mowrey:** None. **D.W. Zhou:** None. **P. Tang:** None. **Y. Xu:** None.

Poster

040. Oscillations and Synchrony

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 40.01/D13

Topic: B.09. Network Interactions

Support: NIH Grant R01NS026143-28

Title: Tight synchrony between fast spiking inhibitory interneurons during endogenous 20-80 Hz cortical network activity

Authors: *D. B. SALKOFF[1], D. MCCORMICK[2];
[1]Interdepartmental Neurosci. Program, [2]Neurobio., Yale Med. Sch., New Haven, CT

Abstract: The activated cortical state is associated with the generation of 20-80 Hz activity which has been proposed to be generated either through interactions between pyramidal neurons and inhibitory interneurons, between inhibitory interneurons themselves, or both. These models are differentiated by the mechanisms driving and synchronizing inhibitory interneurons (e.g. EPSPs, IPSPs, gap junction potentials). Here we investigated this question through dual whole cell recording from pairs of fast spiking (FS) inhibitory interneurons during the generation of spontaneous UP states (which contain high power at 20-80 Hz) in the mouse entorhinal cortical slice *in vitro*. Using the active slice preparation, we found that FS cells <150 um apart have tightly-synchronized spiking, with a width at half-maximum of ~3 ms in the cross-correlogram. Excitatory currents were measured in pairs of cells by holding voltage at -80 mV, and the similarity of currents was quantified by calculating the correlation coefficient (all pairs 0.26 +/- 0.15). Common excitatory input was an excellent predictor of the degree of spike synchrony, capturing 82% of the variance. Electrical coupling between the recorded FS cells was only a weak predictor of spike synchrony. These results suggest that nearby FS interneurons are driven to discharge in a synchronized manner through the arrival of excitatory postsynaptic potentials from one or more presynaptic pyramidal neurons. These results are consistent with 20-80 Hz activity being generated by an interaction of both pyramidal and fast spiking interneurons - a model that is also supported by results obtained from dual recordings from these two cell types. The network implications of tight, millisecond-precision synchrony in fast spiking interneurons is currently under investigation.

Disclosures: **D.B. Salkoff:** None. **D. McCormick:** None.

Poster

040. Oscillations and Synchrony

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 40.02/D14

Topic: B.09. Network Interactions

Title: Loss of network functionality due to a cumulative one-cell-at-a-time deletion of constituent neurons: A simulation and modeling study of pre-Bötzinger laser ablation experiments

Authors: *H. SONG[1], D. LAMAR[2], C. DEL NEGRO[3];
[1]The Col. of William and Mary, Williamsburg, VA; [2]Dept. of Biol., [3]Dept. of Applied Sci., The Col. of William & Mary, Williamsburg, VA

Abstract: The mammalian breathing rhythm originates from the pre-Bötzinger complex (preBötC) of the ventral medulla. The preBötC core consists of interneurons derived from progenitors that express transcription factor Dbx1. Experiments showed that cumulative deletion of ~15% of Dbx1 preBötC neurons stopped respiratory rhythm. Here we simulated these experiments to help explain rhythm cessation due to Dbx1 neuron ablations. We generated random directed graphs that model the preBötC. Each neuron was populated by the Rubin-Hayes (2009) preBötC neuron model. Synaptic connections were modeled as ionotropic glutamatergic synapses. Rhythmic model networks were subjected to cumulative ablations, which lead to irreversible rhythm cessation, like the experiments. By computing several metrics for graph connectivity, we found that the network remained intact even after the rhythm stopped, which led us to hypothesize that the network function depends on a subset of neurons whose specific properties are key rhythmogenic elements. We sought to identify that putative critical subset of neurons and the characteristic properties that distinguished them. In numerical simulations, we discovered a sub-network of neurons with a strong inward current (CAN current). Deleting this subset of neurons terminated the respiratory rhythm at lower cell-ablation tallies. One key feature for neurons in this CAN-subnetwork is high in-degree (i.e., a large number of incoming excitatory synapses). Furthermore, by monitoring the time for the first burst of each individual neuron in its pre-inspiratory phase (strongly correlated with leakage-K current) we also discovered that constituent deletions of random neurons would impair the recurrent excitation, eventually terminating the network functionality to generate a network-wide burst when the network fails to achieve some threshold rate of recurrent excitation. These simulation and modeling results suggest that neurons in the rhythm-generating network have heightened importance based on topological properties (i.e., high in-degrees) as well as high levels of CAN current and low levels of leakage-K current. The loss of respiratory rhythm would be largely attributed to the impairment of network excitability or the disturbance of the process of recurrent excitation.

Disclosures: **H. Song:** None. **D. LaMar:** None. **C. Del Negro:** None.

Poster

040. Oscillations and Synchrony

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 40.03/D15

Topic: B.09. Network Interactions

Title: Spatiotemporal analysis of bicuculline-induced synchronous neuronal activities in rat hippocampal slices

Authors: Y. HONGO[1], *K. OGAWA[1], Y. TAKAHARA[1], K. TAKASU[1], M. HASEGAWA[1], G. SAKAGUCHI[1], Y. IKEGAYA[2];
[1]SHIONOGI & CO., LTD., Toyonaka-Shi, Osaka, Japan; [2]Lab. of Chem. Pharmacology, Grad. Sch. of Pharmaceut. Sci., The Univ. of Tokyo, Bunkyo-ku, Japan

Abstract: Synchronous neuronal activity in the hippocampus plays an important role in both physiological and pathological states. A number of studies have demonstrated that GABAergic inhibitory neurons regulate synchronous neuronal activities; however, less is known about the behavior of excitatory neurons in synchronous events. In this study, we monitored the spatiotemporal patterns of synchronous neuronal activities in the CA1 or CA3 pyramidal cell layer of acute hippocampus slices prepared from P6-8 rats using functional multineuron Ca^{2+} imaging. When GABAergic transmission was inhibited by bicuculline, synchronous Ca^{2+} elevations of CA1 spontaneously occurred in pyramidal cells. These activities were abolished by surgical incision of CA3-CA1 axonal pathways. We analyzed the behavior of individual neurons in synchronous Ca^{2+} activities and clarified that the onset times of individual neurons depended on the cell location relative to the stratum oriens (SO). Synchronous Ca^{2+} activities could be also induced by field stimulation of Schaffer collaterals, but they did not exhibit the location dependence of the onset time. In CA3, the onset times of Ca^{2+} activities did not strongly correlate with the distances from the SO. On the other hand, cells that showed earlier onset times were located close to each other, forming hot spot-like foci of activity initiation. Finally, we investigated the temporal fluctuations in synchronous Ca^{2+} activities. Synchronous events were classified into three groups depending on the propagation speed of their sequential activities, 1) compression, 2) normal, and 3) expansion. Synchronous Ca^{2+} activities fluctuated across these three states during our observation period of 24 min, and the state of an event could be predicted by the inter-event interval after the immediately preceding event. Thus, even without GABAergic transmission, the spatiotemporal patterns of synchronous neuronal activities are non-randomly organized, and the temporal states drift dynamically. Therefore, excitatory neurons may also serve as a regulator of synchronous neuronal activities.

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Poster

040. Oscillations and Synchrony

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Topic: B.09. Network Interactions

Support: TÉT_0389

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KMR_0214

Title: Dendritic integration in fast-spiking, parvalbumin-expressing interneurons during sharp wave-ripple activity

Authors: *B. ROZSA[1], C. BALÁZS[1,2], D. PÁLFI[1,2], G. F. TÚRI[1,3], A. KASZÁS[1,2], P. MAÁK[4], G. SZABÓ[1], G. SZALAY[1], Z. SZADAI[1], S. KÁLI[1], M. MADARÁSZ[2], G. KATONA[1]; [1]Inst.of Exptl. Med., Budapest, Hungary; [2]The Fac. of Information Technol., Pázmány Péter Catholic Univ., Budapest, Hungary; [3]Dept. of Neurosci., Columbia Univ., New York, NY; [4]Budapest Univ. of Technol. and Econ., Department of Atomic Physics, Hungary

Abstract: Sharp wave-ripples are transient oscillatory events in the hippocampus which are associated with the reactivation of neuronal ensembles within specific circuits during memory formation. Fast-spiking, parvalbumin-expressing interneurons (FS-PV-INs) are thought to provide fast integration in these oscillatory circuits by suppressing regenerative activity in their dendrites. Here, using fast 3D two-photon recording from multiple dendritic segments we challenge this classical view by demonstrating that passive, well-dampened FS-PV IN dendrites can be transiently activated by a high number of spatially and temporally coincident excitatory inputs. In this active state, several physiological properties of FS-PV INs are changed. For example, in contrast to the silent slice conditions, FS-PV INs now can generate propagating Ca2+ multiple dendritic hot-spots, propagate toward neighboring dendritic segments, and are mediated dominantly by L-type Ca2+ with membrane potential oscillations, that we call ‘interneuronal ripple oscillations’. These interneuronal ripple oscillations had the same frequency as the field potential oscillations associated with sharp wave-ripples, and controlled the phase of action potentials in FS-PV INs. Our results suggest that FS-PV IN dendrites could play a key role in mechanism of network oscillations.

Disclosures: **B. Rozsa:** None. **C. Balázs:** None. **D. Pálfi:** None. **G.F. Túri:** None. **A. Kaszás:** None. **P. Maák:** None. **G. Szabó:** None. **G. Szalay:** None. **Z. Szadai:** None. **S. Káli:** None. **M. Madarász:** None. **G. Katona:** None.

Poster

040. Oscillations and Synchrony

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Topic: B.09. Network Interactions

Support: FNRS (Belgium) grants 9.4560.03 and T.0015.13

IAP (Belgium) grant P7/10

Title: Low dose quinpirole affects cocaine-induced locomotion and slows down theta rhythm frequency in the rat ventral tegmental area

Authors: A. MONTEFORTE[1], S. KOULCHITSKY[1], T. BEEKEN[1], J. DETHIER[1], E. QUERTEMONT[1], E. BULLINGER[2], *V. M. SEUTIN[1];
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Abstract: In any brain area, the simultaneous activity of large number of cells generates rhythms which rapidly fluctuate during wakefulness. This is also the case of the ventral tegmental area (VTA) where dopaminergic and GABAergic neurons are organized in “subnetworks” with specific inputs and outputs. How the brain coordinates the activity of various networks, such as the VTA network, to produce behavioral output is not clear. The VTA is involved in motor activity, motivation, reward and salience. Dopaminergic drugs such as cocaine induce locomotor activation and, in high and repeated doses, stereotypies. We previously showed that co-administration of a low dose of quinpirole (0.1 mg/kg, i.p.) and a moderate dose of cocaine (10 mg/kg, i.p.) alters the pattern of locomotor activation (as compared to the same dose of cocaine alone), making it look like a so-called locomotor stereotypy. In quantitative terms, co-administration of quinpirole modifies the relative amount spent at various speeds by the animal, with a clear and significant decrease in the relative amount of time spent at high speed. Using a telemetric recording system and 8-microelectrode-arrays spanning most of the extent of the VTA of freely behaving Wistar rats, we recorded the local field potentials (LFPs) within this area in order to test the hypothesis that this altered behavior could be due to changes in the activity of the VTA network. Indeed, the values of the LFP theta peak were significantly different

depending on the experimental conditions (AND when only considering very similar locomotor bouts). In control conditions, power spectrum analysis of the LPFs revealed a prominent peak in the theta frequency range during these periods (7.29±0.06 Hz). Injection of cocaine induced a shift of the peak of LFP theta power toward a slightly higher frequency (7.94±0.08 Hz). In contrast, when cocaine was co-administrated with a low-dose of quinpirole, the peak was shifted toward a lower frequency (~15% decrease, 6.65±0.10 Hz, $p<0.001$ vs cocaine alone). Our data show that an autoreceptor-selective dose of quinpirole markedly alters the quality of cocaine-induced locomotion and at the same time significantly decreases the macroscopic rhythm of the VTA network. To our knowledge, this is the first suggestion that a moderate shift in frequency of LFPs in the VTA is associated with a strong behavioral alteration. This reduced frequency may alter the ability of the VTA to communicate with its output structures.

Disclosures: **A. Monteforte:** None. **S. Koulchitsky:** None. **V.M. Seutin:** None. **T. Beeken:** None. **J. Dethier:** None. **E. Quertemont:** None. **E. Bullinger:** None.

Poster

040. Oscillations and Synchrony

Location: Halls A-C

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Program#/Poster#: 40.06/D18

Topic: B.09. Network Interactions

Support: JST, CREST, JAPAN

Title: Cortical membrane oscillatory activities induced by light stimulations

Authors: ***T. OTSUKA**, Y. KAWAGUCHI;
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Abstract: The cortex outputs the information to several brain areas through different sets of pyramidal cells. It has been shown that cortical pyramidal cells form intra- and inter-laminar subnetworks, depending on pyramidal projection subtypes. To understand cortical information processing related to the high order brain functions, it is important to know how activities of individual pyramidal cell subnetworks are regulated. In the present study, we investigated optogenetically induced network activities in the rat frontal cortical slices. Channel rhodopsin was selectively expressed in L2/3 pyramidal cells by *in utero* electroporation. Whole-cell recordings were obtained from L5 pyramidal cells and Fast-Spiking (FS) interneurons, while light stimulation was applied to layer 2/3 to induce network activities. Membrane oscillatory

activities at the frequency around 30Hz were observed in both pyramidal and FS cells during light stimulations. Moreover, we compared activities induced by light stimulations in L5 pyramidal cells projecting to the ipsilateral pontine nuclei (CPn) and the contralateral cortex (COM), identified by retrograde fluorescent tracers. Oscillatory activities were frequently found in CPn cells. Cross-correlation analysis suggests that pyramidal and FS cells highly interact with each other during oscillations. Our results suggest that oscillatory activities, induced by L2/3 pyramidal cell stimulation, in the cortical circuits depend on the interaction between specific pyramidal cell subtypes and FS interneurons.

Disclosures: **T. Otsuka:** None. **Y. Kawaguchi:** None.

Poster

040. Oscillations and Synchrony

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 40.07/D19

Topic: B.09. Network Interactions

Title: Optogenetic activation of cholinergic neurons in the medial septum causes a scopolamine-sensitive reduction in theta power in hippocampus

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Abstract: Cholinergic neurons in the medial septum-vertical limb of the diagonal band of Broca (MSvDB) are known to have an important role in memory formation and have been suggested to be an important player for theta rhythm generation. Although previous studies have focused on understanding how cholinergic neurons pace hippocampal theta oscillations, all cholinergic neurons are slow-firing and thus may not have the capacity to pace theta oscillations. Therefore, the function of these neurons, in modulating hippocampal activity remains unknown. We combined optogenetics with our recently developed *in vitro* septo-hippocampal preparation to address this question. For this study, we used ChAT-mhChR2-YFP BAC transgenic mice expressing channelrhodopsin-2/EYFP fusion protein (mhChR2:YFP) targeted selectively to cholinergic neurons. Characterization of septo-hippocampal neurons showed photocurrents of 190 +/- 50 pA (steady state: 160 +/- 50 pA) and depolarizations of 25 +/- 3 mV (n=9). Cholinergic neurons followed light stimulation from 1 to 20 ms pulse duration. Firing efficiency

increased with pulse size and stimulation response ratio reached peak value at 5, 10 and 20 ms, suggesting that cholinergic neurons could be finely activated with light. Surprisingly, optogenetic activation of cholinergic neurons caused a scopolamine sensitive reduction in theta power in the CA1 area in the intermediate hippocampus. In addition we found that MSvDB cholinergic neurons can directly modulate firing frequency hippocampal CA1 interneurons. Together, these results suggest that the role of slow firing septo-hippocampal neurons contribute negatively to theta oscillation power in the hippocampus.

Disclosures: **S. Mondragon:** None. **S. Glasgow:** None. **S. Williams:** None.

Poster

040. Oscillations and Synchrony

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Title: Transient competitive amplification in cortical circuits

Authors: *N. A. VASCONCELOS[1], J. BOURG[1], K. WIMMER[2], A. COMPTE[2], J. DE LA ROCHA[2], A. RENART[1];
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Abstract: Recurrent connections in cortical circuits might play a role in the selective amplification of different patterns of population activity. We explored this issue in population recordings of spontaneous activity from the auditory and somatosensory cortices of Urethane anesthetized rats during periods of cortical activation, which are also characteristic of attentive

wakefulness. PCA reveals that the correlation structure of the population is low-dimensional, with a gap in the amount of explained correlation between the first and subsequent principal components. The distribution of loadings onto the high-variance mode (PC1) across the population is wide and roughly centered at zero. Thus, the dynamics of the population is 'competitive': neurons with high positive or high negative loadings are negatively correlated with each other. Because PC1 is approximately orthogonal to the mean population activity, the population-averaged correlation is close to zero, as we showed previously (Renart et al., Science, 2010). What mechanisms could underlie the amplification of competitive fluctuations? We first show that randomly connected balanced networks do not generate competitive amplification. Competition in recurrent circuits has typically been modeled in a 3-population network with two excitatory populations interacting directly and through mutual inhibition (Wang, Neuron, 2002). Competition arises when the network operates close to a pitchfork bifurcation, so we refer to this mechanism as Normal Competitive Amplification (NCA). We developed a different mechanism using non-normal amplification (Murphy & Miller, Neuron, 2009) in the same 3-population network. When two eigenvectors of the effective connection matrix are approximately collinear with the competitive mode, strong amplification of competitive fluctuations arises in the absence of near-zero eigenvalues. Because the resulting strong fluctuations are short-lived, we refer to this phenomenon as Transient Competitive Amplification (TCA). We show that the TCA model requires asymmetric connectivity between the two competing populations. Then, using linear systems theory we examine the temporal structure of correlations in both models and compare them with those in our recordings. The TCA model naturally generates idiosyncratic but robust features of the real data, such as positive correlations within the two populations of clearly different magnitudes and a time delay in the negative correlations between them. Our work suggests that non-normal amplification indeed drives competitive spontaneous temporal fluctuations during states of cortical activation.

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Poster

040. Oscillations and Synchrony

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 40.09/D21

Topic: B.09. Network Interactions

Title: Early brain network alterations are correlated with β-CTF in Alzheimer's transgenic mouse model

Authors: S. MONDRAGÓN-RODRÍGUEZ[1], *F. MANSEAU[2,1], N. GU[1], R. BOYCE[1], S. WILLIAMS[1];
[1]Dept. of Psychiatry, McGill Univ., Douglas Mental Hlth. Univ. Inst., Montreal, QC, Canada; [2]EBRI, Rome, Italy

Abstract: Alzheimer's disease (AD) is defined by the presence of amyloid-β (Aβ) and tau protein aggregates but increasing data suggests that early brain network alterations may be at the root for the pathogenesis of the disease. In the present study we performed extracellular field recording at the CA1/subiculum area in an *in vitro* complete hippocampal preparation from p30 J20-AD mice. We found that theta oscillation strength (a measure of rhythmicity) in J20 was significantly lower than that from the control group (tg: 16.3±1.3; ctrl: 32.8±3.6, t-test: $p<0.01$, n=10). In addition, J20 displayed significantly reduced theta-gamma cross-frequency coupling (CFC) at low (25-45 Hz) and high (150-250 Hz) gamma range, as the peak modulation index for low/high gamma were significantly reduced (low gamma: tg: 2.8±0.6; wt:14±2.9; high gamma: tg: 5.0±1.3; wt: 15.0±1.4, t-test: $p<0.01$, n=10). We then used optogenetics to explore the possible alterations in pyramidal cell and interneuronal network in CFC. Optogenetics was performed in offspring from CamKII-cre mice crossed with J20, injected with AAV2-Cheta-eYFP virus. Light driven pyramidal cell networks elicited theta oscillations (4-12 Hz) as well as nested low (25-50 Hz) and high gamma (150-250 Hz). However, we found that when driven with fixed frequency light stimulations, the J20 group showed significantly less CFC at all frequencies tested compared to controls. When applying sinusoidal injections of light of increasing frequencies (0-12 Hz) to drive the hippocampal network, it was found that the optimal CFC found between 4-6 Hz in controls was nearly absent in the J20 (n=8, $t<0.05$). Interestingly, the changes in CFC in the J20 mice were significantly associated with the Aβ precursor β-CTF (t-test: $p<0.005$, n=8). Despite the high β-CTF expression and the changes in network activity, no significant changes in learning and memory were noted at this age in the J20. In summary, our data suggests that brain network alterations precede the canonical Aβ protein deposition.

Disclosures: **S. Mondragón-Rodríguez:** None. **F. Manseau:** None. **N. Gu:** None. **R. Boyce:** None. **S. Williams:** None.

Poster

040. Oscillations and Synchrony

Location: Halls A-C

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Topic: B.09. Network Interactions

Support: NIH Grant NS54281

Title: Estimation of optimal neuron parameters to obtain minimal variability of periods in an oscillatory hybrid network

Authors: *R. M. HOOPER[1], R. A. TIKIDJI-HAMBURYAN[2], C. C. CANAVIER[2,3], A. A. PRINZ[4];
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Abstract: Rhythmic neuronal activity is a hallmark of many functions of the nervous system−including expression of motor patterns, cognition, memory, arousal, and sleep. Networks that give rise to this rhythmicity often consist of pacemaker neurons coupled to non-pacemaking neurons, and produce activity that is stereotyped and reliable, yet with some level of variability that appears to be specific to the requirements of each network; e.g. some circuits exhibit high levels of activity variability in order to accomplish some behavioral goal [1,2]. While it is understood that existence of synaptic feedback from non-pacemaking neurons to pacemaker neurons is one strategy by which networks can scale the level of activity variability [3,4], it is not understood how specific neuronal dynamics of networks influence variability scaling, or what the neuronal dynamics must be to induce minimal network variability. Here we show how network activity variability changes with altered phase response dynamics of non-pacemaking neurons, and using previously developed analyses [5] demonstrate a theoretical approach's predictive efficacy in hybrid networks established in the stomatogastric nervous system (STNS) of *Cancer borealis.* These hybrid networks consist of one biological pacemaker neuron (Anterior Burster/Pyloric Dilator complex [AB/PD]) and one artificial non-pacemaker neuron (designed to mimic the burst timing of the Lateral Pyloric [LP] neuron) established with dynamic clamp, which allows us to vary the phase response dynamics of the latter element. Qualitatively similar simulations are used in the theoretical approach. Our experimental results show that a network with a non-pacemaker neuron using a positive slope in the response interval to stimulus interval relationship (t_S-t_R curve) results in different network variability levels compared to both an isolated oscillator neuron or a hybrid network with a zero slope t_S-t_R curve. Initial theoretical predictions indicate that a negative slope in the non-pacemaker neuron t_S-t_R curve would result in opposite effects on network variability compared to the initially examined positive slope and zero slope t_S-t_R network configurations. This prediction was confirmed in further experiments. Finally, we use realtime t_S-t_R measurements of the pacemaker neuron and theoretical methods to estimate and implement a network with minimal activity variability. 1. Hooper S. *J Neurophysiol* 2004, **92**:40-41. 2. Horn C, et al**.** *J Neurophysiol* 2004, **92**:157-180. 3. Mamiya A, et al. *J*

Neurosci 2003, **23**: 9557-9564. 4. Nadim F, et al. *J Neural Eng* 2011, **8**:065001 5 Tikidji-Hamburyan R, et al. *Network: Computation in Neural Systems* 2014*,* **25**: 38-62

Disclosures: R.M. Hooper: None. **R.A. Tikidji-Hamburyan:** None. **C.C. Canavier:** None. **A.A. Prinz:** None.

Poster

040. Oscillations and Synchrony

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Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

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Topic: B.09. Network Interactions

Support: DFG support through GK "Emotions"

Title: Analysis of single unit and neuronal activity in the human subthalamic nucleus during reach-to-grasp movements

Authors: *U. E. RAMIREZ PASOS[1], M. PÖTTER-NERGER[2], R. REESE[1], F. STEIGERWALD[1], *. VOLKMANN[1];
[1]Universitätsklinikum Würzburg, Würzburg, Germany; [2]Neurologische Klinik, Universitätsklinikum Schleswig-Holstein, Campus Kiel, Kiel, Germany

Abstract: The basal ganglia (BG) are involved in processing of goal-oriented behavior, motor preparation, and action selection. Electrophysiological studies of the human BG point to a decrease in subthalamic nucleus (STN) oscillatory LFP activity upon movement onset, chiefly in the beta range (13-30Hz). However, the simplicity of these movements (e.g. arm flexion, hand suppination) may not represent more complex human motor processing. We have employed the reach-to-grasp (RtG) paradigm as a more functionally relevant task to observe whether the STN processes movement differently according to the kinematic aspects of a complex motor task involving coordinated distal or proximal muscle activation. 12 parkinsonian patients reached toward a panel and pressed a grip device in response to a warning cue while single-unit activity was intraoperatively being recorded from their STN within the 300-10,000 Hz band at ~25kHz. On average the patient performed the task 10 times per depth of insertion. Ultrasound-emitting devices were attached to thumb, index finger, wrist, and elbow to reconstruct the position and velocity curves of the wrist markers as well as the distance between fingers. Neuronal background unit activity (BUA) has been interpreted as a marker for synchorinized activity of the local population in the neuron vicinity. Following Moran's (2008) method, background

activity was extracted from neuronal spike trains by removing spikes identified with a spike sorter and inserting random segments from the rest of the signal into the empty slots. The resulting BUA signals were then analyzed relative to each aspect of the movement (beginning of transport phase (TP); maximum velocity; end of transport phase; maximum grip opening; and grip closing) to identify spectral properties using SPM. We observed a significant activation of the alpha band shortly before the warning cue followed by suppression, which we interpret as a marker for motor preparation. In half of the subjects a broadband activation is present at the beginning of the TP ans likewise during maximum grip opening for 5/12 patients. For the end of the RtG movement broadband desynchronization is observed in 5/12 patients. The last results run counter to LFP studies which support the idea that movement initiation specifically suppresses beta activity in the subthalamic nucleus. This suggests that changes in the beta band in STN activity relative to movement may be only specific to synchronized post-synaptic potentials but not to BUA. Reference: Moran A, Bergman H, Israel Z, Bar-Gad I. Subthalamic nucleus functional organization revealed by parkinsonian neuronal oscillations and synchrony. Brain. 2008; 131: 3395-3409.

Disclosures: **U.E. Ramirez Pasos:** None. **M. Pötter-Nerger:** None. **R. Reese:** None. **F. Steigerwald:** None. ***. Volkmann:** None.

Poster

040. Oscillations and Synchrony

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Topic: B.09. Network Interactions

Support: FINEP

INCT Incemaq

CNPq

FAPERN

AASDAP

Title: Phase Locking Value Analysis in active tactile discrimination task

Authors: *C. SARDETO DEOLINDO[1], A. C. B. KUNICKI[2], F. L. BRASIL[2], R. C. MOIOLI[2], M. A. L. NICOLELIS[2,3,4,5,6];
[1]Edmond and Lily Safra Intl. Inst. of N, Natal, Brazil; [2]Neuroengineering Grad Program, Edmond and Lily Safra Intl. Inst. of Neurosci. of Natal, Natal, Brazil; [3]Biomed. Engin., [4]Neurobio., [5]Psychology and Neurosci., [6]Ctr. for Neuroengineering, Duke Univ., Durham, NC

Abstract: Neuronal oscillations have a close, nontrivial relationship with brain signal processing and behavior, and are regarded as a fundamental mechanism for temporal coordination and information integration. The Local Field Potential (LFP) is one of the electrophysiological signals that captures this phenomenon, since it corresponds to the sustained currents in the tissue, and its variations over time represent how a neuronal population respond as a whole to a given stimulus. It is, therefore, a preeminent signal when we aim to analyze the changes in connectivity between different cortical regions. More specifically, the LFP synchronization within and between different brain regions is taken as an indicative of a decision-making process, and its dynamics may reflect the integration of different neuronal ensembles towards accomplishing a specific goal. To date, it is not clear how different brain regions interact and encode information. In this work, the relation between motor and somatosensory cortices (M1 and S1, respectively) was studied during an active whisker discrimination task. Long-Evans rats were trained to discriminate between a wide or narrow aperture employing only their mystacial vibrissae to receive a water reward. Over the task execution, the LFPs of sensorimotor cortex were analyzed around the instant the rat broke the photo beam at the discrimination bars. We present temporal analysis through the variation of the Phase Locking Value (PLV) to unveil information dynamics and interactions between M1 and S1 neurons.

Disclosures: C. Sardeto Deolindo: None. **A.C.B. Kunicki:** None. **F.L. Brasil:** None. **R.C. Moioli:** None. **M.A.L. Nicolelis:** None.

Poster

040. Oscillations and Synchrony

Location: Halls A-C

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Program#/Poster#: 40.13/D25

Topic: B.09. Network Interactions

Support: ERC SERRACO

OTKA K109790

Astellas Young Investigator's Grant 2012/2013

Title: Effect of median raphe manipulation on the hippocampal network

Authors: *A. DOMONKOS, L. NIKITIDOU, A. SZőNYI, C. CSERÉP, D. ZELENA, G. NYIRI, T. F. FREUND, V. VARGA;
Inst. of Exptl. Medicine, Hungarian Acad. of Sci., Budapest, Hungary

Abstract: The median raphe (MR) is a major source of the ascending serotonergic system involved in neuromodulation related to e.g. mood, fear and stress. Previous studies revealed the existence of a fast and vigorous glutamatergic component of the MR-hippocampal pathway. The aim of this study was to decipher the role of MR-manipulation in the regulation of hippocampal activity in acute and freely behaving (chronic) animals. Adeno-associated viral vectors containing channelrhodopsin 2 tagged with mCherry or YFP under the synapsin promoter were injected into the MR of C57Bl/6 mice. About 3-4 weeks after injection, a 32-channel 4-shank (Buzsáki) probe was implanted into the dorsal hippocampus for sampling hippocampal unit and field response to the optogenetic manipulation of the MR. In some cases, multiple single wires were implanted into different brain regions (prefrontal cortex, amygdala, dorsal and ventral hippocampus), instead of silicon probe. Different stimulation frequencies were tested in acute (urethane-anesthetized) and chronic (freely behaving) experiments. Preliminary data show layer-dependent response in the hippocampus during MR stimulation. However, the MR stimulation failed to induce state transitions in the hippocampus. In chronic recordings, pulses delivered in the MR at 20 Hz dramatically decreased the power of low-range theta oscillations in the hippocampus. Additionally, weak entrainment of the hippocampal network was detected during stimulation by 8 Hz. Importantly, the response amplitude was dependent on the testing environment (home cage vs. linear track). The correlation between evoked effect and precise localization of optic fiber and opsin-expressing cells will be analyzed. Additionally, selective optogenetic manipulation of serotonergic MR neurons will be also carried out to test the role of serotonergic component in the modulation of hippocampal activity.

Disclosures: **A. Domonkos:** None. **L. Nikitidou:** None. **A. Szőnyi:** None. **C. Cserép:** None. **D. Zelena:** None. **G. Nyiri:** None. **T.F. Freund:** None. **V. Varga:** None.

Poster

041. Alzheimer's Disease: Tau Animal Models

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 41.01/D26

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Rainwater Foundation

Title: Accumulation of A152T-variant tau causes synaptic changes and increases network excitability before leading to cognitive decline

Authors: *S. MAEDA[1], B. DJUKIC[2], R. PONNUSAMY[2], P. TANEJA[2], M. M. FINUCANE[2], I. LO[2], A. DAVIS[2], R. CRAFT[2], W. GUO[2], X. WANG[2], D. KIM[2], G.-Q. YU[2], E. MASLIAH[3], L. MUCKE[4];
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Abstract: Tau aggregation is a pathological hallmark of various neurodegenerative disorders, including frontotemporal dementia spectrum disorders (FTD-s) and Alzheimer's disease (AD). Recently, an amino acid substitution in tau (A152T) was reported to increase the risk of both FTD-s and AD, but the underlying mechanisms are unclear. To investigate the effects of A152T-variant human tau (hTau-A152T) on the integrity, function and survival of neurons *in vivo*, we generated transgenic mice with doxycycline (DOX)-regulatable expression of hTau-A152T in excitatory forebrain neurons. By 2–4 months of age, brains of these mice showed an abnormal accumulation of misfolded and phosphorylated tau and reactive astrocytosis. Suppressing hTau-A152T expression with DOX reversed these abnormalities. hTau-A152T expressing mice had no behavioral deficits until they were 17 months old, when they showed impairments in the Morris water maze test. Electrophysiological analysis of acute hippocampal slices from 4–8 month-old hTau-A152T mice revealed decreased intrinsic excitability of granule cells in the dentate gyrus and increased synaptic transmission strength at the granule cell to CA3 mossy fiber synapse. Electroencephalographic recordings of brain oscillations in awake behaving mice revealed network hyperexcitability in hTau-A152T mice, including enhanced epileptiform activity in response to pentylenetetrazol challenge. In addition, we found evidence for co-pathogenic interactions between hTau-A152T and human amyloid precursor protein (hAPP) or amyloid- (A) peptides. Breedings between hTau-A152T mice and hAPP transgenic mice from line J20 yielded 128 offspring, but only two of these mice co-expressed hTau-A152T and hAPP/A, and both of them died shortly after weaning. To confirm that the above effects are specifically caused by the A152T substitution and not by overexpression of hTau, we generated transgenic mice expressing comparable levels of wildtype hTau. Side-by-side analyses of hTau-A152T and hTau-Wildtype mice are in progress. The findings we have obtained so far suggest that accumulation of hTau-A152T leads to age-related cognitive decline, possibly by changing synaptic functions and enhancing neural network excitability. These effects may also contribute to the ability of this tau variant to increase the brain's susceptibility to pathologically elevated levels of A and, possibly, to other factors involved in the pathogenesis of AD and FTD-s.

Disclosures: **S. Maeda:** None. **B. Djukic:** None. **R. Ponnusamy:** None. **P. Taneja:** None. **M.M. Finucane:** None. **I. Lo:** None. **A. Davis:** None. **R. Craft:** None. **W. Guo:** None. **X. Wang:** None. **D. Kim:** None. **G. Yu:** None. **E. Masliah:** None. **L. Mucke:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Bristol-Myers Squibb.

Poster

041. Alzheimer's Disease: Tau Animal Models

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 41.02/D27

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Longitudinal assessment of behavior and tau phosphorylation in the brainstem of P301L tau-transgenic pR5 mice

Authors: M. PATERNO[1], K. MORCINEK[1], S. ARNDT[2], C. KÖHLER[1], H. SCHRÖDER[1], J. GÖTZ[3], *N. MOSER[1];
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Abstract: Alzheimer’s disease (AD), frontotemporal dementia and related tauopathies are characterized by aggregation of hyperphosphorylated tau. Several animal models have been established to reproduce the pathology at the level of the hippocampus and amygdala, but age-dependent changes in tau phosphorylation and aggregation in the brainstem and their possible behavioral sequels have not been studied intensively. As the occurrence of disturbed eating behavior suggests an involvement of brainstem nuclei in the pathogenesis of AD, we analyzed the behavior and patterns of tau phosphorylation in the trigeminal motor nucleus of senescent human P301L tau-transgenic pR5 (pR5) mice. Male pR5 mice on a C57BL/6OlaHsd (Harlan) background and gender-matched non-transgenic littermates (non-tg) at the age of 6, 15 and 18 months were tested for behavioral performance (T-maze, Morris water maze, Open field, Object recognition test, RotaRod, Fitness Center). At the age of 8, 16 and 19 months, the animals were sacrificed and coronal brainstem sections were cut for immunohistochemical staining. The number of phospho-tau (AT8- and AT180-)immunoreactive neurons in the trigeminal motor

nucleus was quantified and normalized to the number of choline acetyltransferase or Nissl-stained cells. Behavioral testing revealed no significant age- or genotype-dependent differences. Likewise, body weight of pR5 mice was unchanged compared to non-tg mice. Therefore eating-related behavior seemed to be unaffected. However, histological analysis revealed a significantly higher proportion of both, AT8- and AT180-immunoreactive neurons in the trigeminal motor nucleus of older compared to younger pR5 mice (8 versus 16 months: $p<0.01$; 8 versus 19 months: $p<0.05$; performed by means of Student's t-test). No significant differences in the number of AT8- and AT180- immunoreactive neurons of the trigeminal motor nucleus were observed between 16- and 19-months-old pR5 mice. Our findings in the pR5 mouse model show for the trigeminal motor nucleus an age-related increase of the number of neurons that display tau hyperphosphorylation at the AT8- and AT180-epitopes between 8 and 16 months of age. Since there were no detectable behavioral alterations, this pretangle tau hyperphosphorylation state does not appear to affect the functionality of neurons.

Disclosures: **M. Paterno:** None. **N. Moser:** None. **K. Morcinek:** None. **S. Arndt:** None. **C. Köhler:** None. **H. Schröder:** None. **J. Götz:** None.

Poster

041. Alzheimer's Disease: Tau Animal Models

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 41.03/D28

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Imhoff-Stiftung of the Faculty of Medicine, University of Cologne

Title: Preliminary electrophysiological characterization of hippocampal CA1 neurons in a mouse model of tauopathy (P301L tau transgenic pR5 mice)

Authors: *K. MORCINEK[1], B. DENGLER[1], F. NEUMAIER[2], T. SCHNEIDER[2], W. WALKOWIAK[3], H. SCHRÖDER[1], J. GÖTZ[4], S. HUGGENBERGER[1];
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Abstract: There is mounting evidence that soluble hyperphosphorylated tau species drive neuronal dysfunction and degeneration in tauopathy. Since the effect of progressive tau

pathology on intrinsic neuronal functions and synaptic plasticity still remains unclear, we investigated electrophysiological properties of hippocampal CA1 neurons in elderly tau transgenic mice. Electrophysiological recordings were performed in acute brain slices from P301L tau transgenic pR5 mice (tg) at the age of 13 to 17 months and age-matched non-transgenic littermate (nt) controls. Using the whole-cell configuration of the patch-clamp technique, intrinsic membrane properties and response to stimulation of the Schaffer collaterals were analyzed in a total of 25 neurons from tg and 12 neurons from nt animals. We found significant differences in the action potential firing threshold ($p<0.01$) and in the macroscopic activation kinetics of the hyperpolarization-activated inward current (IH; $p<0.05$). The higher firing threshold in tg (-47.2mV±11.8mV) in comparison to nt (-55.7mV±6.3mV) neurons may have substantial implications for the hippocampal network. The difference could represent a conceivable explanation for the pronounced loss of long-term potentiation detected in CA1 neurons of several tau transgenic models (Sydow et al. [2011] J Neurosci 31:2511-2525; Oddo et al. [2003] Neuron 39:409-421). The slowed macroscopic activation of IH in tg (84.7ms±92ms) versus nt (27.8ms±28ms) neurons may tend to facilitate temporal summation, possibly antagonizing the increased firing threshold. These results are in apparent contrast to former studies (Rocher et al. [2010] Exp Neurol 223:385-393; Crimins et al. [2012] Acta Neuropathol 124:777-795) where changes in the intrinsic properties of frontal cortical pyramidal neurons indicated an enhanced hyperexcitability in the rTg4510 mouse model of tauopathy. This may point to region-specific pathological changes in tau transgenic mice.

Disclosures: **K. Morcinek:** None. **B. Dengler:** None. **F. Neumaier:** None. **T. Schneider:** None. **W. Walkowiak:** None. **H. Schröder:** None. **J. Götz:** None. **S. Huggenberger:** None.

Poster

041. Alzheimer's Disease: Tau Animal Models

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 41.04/D29

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: C2N Diagnostics

Title: Passive immunization with an anti-tau antibody in P301S mice reduces tau accumulation and improves motor deficits

Authors: ***K. YANAMANDRA**[1,3,4], H. JIANG[1,3,4], T. E. MAHAN[1,3,4], S. E. MALONEY[2], D. F. WOZNIAK[2], M. I. DIAMOND[1,3,4], D. M. HOLTZMAN[1,3,4];

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Abstract: Abstract: Aggregation of tau is a hallmark in many neurodegenerative diseases including Alzheimer's disease, certain forms of frontotemporal dementia, and progressive supranuclear palsy. Tau is a cytosolic, microtubule binding protein. In disease conditions, tau protein dissociates from microtubules and aggregates to forms neurofibrillary tangles in neurons and dystrophic neurites. We recently showed by continuous intracerebroventricular infusion of three anti-tau antibodies into the lateral ventricle of P301S transgenic mice that tau pathology was markedly reduced and there was improvement of cognitive deficits (Yanamandra et al. 2013). Of all anti-tau antibodies we administered, HJ8.5 was the most potent in reducing tau pathology in all methods we analyzed. In the current study, we administered two doses (10 mg/kg and 50 mg/kg) of the HJ8.5 antibody intraperitoneally (I.P.) to 6 month old P301S tau transgenic mice. Control mice received PBS injection. Mice received one I.P. injection weekly for a period of 12 weeks. After treatment, we performed immunostaining, biochemical analysis and behavioral tests. Quantitative immunostaining showed that 50mg/kg treatment of HJ8.5 reduced hippocampal CA1 cellular layer staining as assessed AT8-phospho tau antibody compared to PBS/vehicle treated controls. We also observed that both 10 mg/kg and 50 mg/kg treatment with HJ8.5 antibody significantly reduced the loss of cortical and hippocampal tissue volumes compared to control mice. By biochemical analysis, we found that mice treated with HJ8.5 at 50 mg/kg showed an increase in soluble and a marked decrease in insoluble total tau levels. HJ8.5 is a human specific tau antibody and it resulted in a substantial decrease in insoluble human tau by ~75% in the 70% formic acid soluble fraction. We also observed a strong correlation between the amount of brain atrophy and insoluble tau levels measured by ELISA. HJ8.5 50 mg/kg treated mice showed a decrease in motor deficits compared to the control group in both the inverted screen and ledge tests. Using *in vitro* BV2-microglial cell lines, we observed significantly higher uptake of P301S tau aggregates in the presence of HJ8.5 antibody. Overall, our results indicate that systemically administered anti-tau antibody HJ8.5 potently decreases insoluble tau, decreases brain atrophy, and improves motor function in a mouse model of tauopathy.

Disclosures: K. Yanamandra: None. **H. Jiang:** None. **T.E. Mahan:** None. **S.E. Maloney:** None. **D.F. Wozniak:** None. **M.I. Diamond:** None. **D.M. Holtzman:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Grant to Washington University from C2N Diagnostics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); DMH is a co-founder of C2N Diagnostics and on the scientific advisory board.

Poster

041. Alzheimer's Disease: Tau Animal Models

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 41.05/D30

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Deutsche Forschungsgemeinschaft HO2402/6-1, HO2402/8-1

BMBF GEF 10-54

DAAD

FP7/2007-2013

Title: Memory deficits correlate with tau and spine pathology in P301S MAPT transgenic mice

Authors: *H. XU[1,2], T. W. RÖSLER[1], T. CARLSSON[3], W. H. OERTEL[3], G. U. HÖGLINGER[1,2,3];
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Abstract: Back ground As a model of frontotemporal dementia and parkinsonism linked to chromosome 17 with tau pathology (FTDP-17-Tau), P301S *MAPT* transgenic mice (P301S mice) have been identified with onset of locomotor deficits since 5 months of age in previous studies. However, no cognitive deficit correlated with sub-cellular level of tau pathology and dendritic spine loss was reported on the model at early age. **Aim of the study** Our study investigated memory deficit of P301S mice in relation to pathological tau species and dendritic spine pathology throughout adulthood. **Methods and results** Behavior tests One group of P301S mice was investigated monthly with the rotarod test from 2 to 6 months of age. Another group of P301S mice was subjected monthly to the novel open field from 2 to 6 months of age and the Morris water maze test at 2.5 and 5.5 months. Same numbers of the wild type mice were taken as control for all the testing sessions and time points. Immunoblot and immunohistochemistry P301S and wild type mice ($n \geq 3$ per group and time-point) were sacrificed by an intraperitoneal (i.p.) injection of pentobarbital at (30 g/kg) at 2, 3, 4, 5, or 6 months of age. Soluble and sarkosyl-insobuble tau from the brain homogenates were analyzed using the following tau antibodies: AT180 (Thermo scientific, Waltham, MA) for PHF-tau; CP13 and MC1 (kindly provided by the Department of Pathology, Albert Einstein College of Medicine, NY, NY) for S202-pTau and conformationally changed tau, respectively. Post-fixed free floating sections

were immunostained with the same antibodies for tau and the anti-NeuN antibody (Millipore, Darmstadt, Germany) for neurons. Golgi staining and dendritic spine quantification P301S and wild type mice were sacrificed at 2.5 and 5.5 months of age by cervical dislocation. Brains were quickly removed and briefly cleaned with Millipore water. The GolgiStain^{TM} kit (FD NeuroTechnologies, Columbia, MD) was used following the manufacturer's guidelines. Dendrites with more than 10 μm length were reconstructed into 3D images and then quantified spine density, length, cross-sectional area, and volume using the Imaris software (Bitplane, Zurich, Switzerland). **Conclusion** Tauopathy-induced impaired synaptic connectivity in the hippocampus leads to memory deficit in P301S mice at an early age (< 2.5 month of age). This newly recognized behavioral deficit and suggested patho-anatomical substrate may be helpful as a read-out for the development of new therapeutic tau-targeting interventions.

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Poster

041. Alzheimer's Disease: Tau Animal Models

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 41.06/D31

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIGMS Grant U54GM104942

Alzheimer's Association Grant 12-242187

Title: Riluzole attenuates spatial memory deficits in a TauP301L mouse model of Alzheimer's disease

Authors: *D. WEITZNER, M. REED, H. HUNSBERGER, C. RUDY;
Psychology, West Virginia Univ., Morgantown, WV

Abstract: Individuals at risk for developing Alzheimer's disease (AD) often exhibit neuronal hyperexcitability throughout the memory networks in the years preceding AD diagnosis. This hyperexcitability may be due to an increase in glutamatergic signaling, a hypothesis supported by our recent findings of increased glutamate release in the hippocampus of rTg(TauP301L)4510 mice, a commonly used tau mouse model of AD. Though TauP301L mice exhibit age-dependent memory deficits, neuron loss, and neurofibrillary tangle formation, the alterations in glutamate

signaling observed in these mice occurs early in the disease process, at a time prior to neuron loss or tangle formation, but coinciding with the onset of subtle memory deficits. The objective of this study was to examine whether riluzole, an FDA-approved disease-modifying drug for amyotrophic lateral sclerosis that effectively regulates glutamate levels, would attenuate the spatial memory deficits observed in TauP301L mice. To avoid alterations resulting from P301L tau expression during development, tau expression was suppressed until adulthood (approximately 2.5 months of age). Spatial reference memory was compared prior to the onset of P301L tau expression and at multiple time points after the onset of P301L tau expression. As anticipated, prior to the onset of P301L tau expression, there were no differences in memory between TauP301L mice and transgene negative littermates. In contrast, after the onset of P301L tau expression, our initial findings indicate TauP301L mice exhibit age-dependent memory deficits, an effect attenuated by riluzole. Thus, our findings could establish a potential therapeutic intervention for memory deficits and cell death caused by the accumulation of excess glutamate in the extracellular space.

Disclosures: **D. Weitzner:** None. **M. Reed:** None. **H. Hunsberger:** None. **C. Rudy:** None.

Poster

041. Alzheimer's Disease: Tau Animal Models

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 41.07/D32

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Beneficial effects of anatabine in a mouse model of tauopathy

Authors: *D. PARIS[1], D. BEAULIEU-ABDELAHAD[1], G. AIT-GHEZALA[1], V. MATHURA[1], M. VERMA[1], A. E. ROHER[2], F. CRAWFORD[1], M. MULLAN[1,3];
[1]Roskamp Inst., SARASOTA, FL; [2]The Longtine Ctr. for Neurodegenerative Biochem., Banner Sun Hlth. Res. Inst., Sun City, AZ; [3]Rock Creek Pharmaceuticals, Sarasota, FL

Abstract: We have previously shown that the natural alkaloid anatabine displays some anti-inflammatory and Alzheimer ß-amyloid lowering properties in the central nervous system associated with reduced STAT3, NFκB and microglial activation. Microglial activation and astrogliosis are preeminent in tauopathies and mitigation of neuroinflammation has been shown to delay the progression of tauopathies in animal models. We therefore investigated here the impact of a chronic oral treatment with anatabine in a mouse model of tauopathy (Tg P301S). We found that the incidence of paralysis and abnormal hind limb extension reflex was reduced in

Tg Tau P301S treated with anatabine suggesting that anatabine delays the disease progression in this model of tauopathy. Additionally, we show that anatabine suppresses tau phosphorylation (at multiple Alzheimer's disease pertinent epitopes) as well as tau oligomerization in the brain and spinal cord of Tg P301S further confirming this assumption. The reduction in pathological tau species observed in anatabine treated mice was accompanied by a reduction in microgliosis and STAT3 activation in the brain and spinal cord of Tg P301S revealing an anti-inflammatory activity of anatabine in Tg P301S mice. Additional data regarding the mechanism(s) of action of anatabine responsible for its beneficial activity against the development of tauopathy will be presented. Overall our data support further exploration of anatabine as a disease modifying agent for the treatment of tauopathies including Alzheimer's disease. Possible competing interests: MM is the CEO of Rock Creek Pharmaceuticals which sells anatabine as a nutraceutical supplement. The anatabine used in this study was provided by Rock Creek Pharmaceuticals. None of the other authors receive remuneration from Rock Creek Pharmaceuticals. This study was funded by the Roskamp foundation.

Disclosures: **D. Paris:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Rock Creek Pharmaceuticals. **D. Beaulieu-Abdelahad:** None. **G. Ait-Ghezala:** None. **V. Mathura:** None. **M. Verma:** None. **A.E. Roher:** None. **F. Crawford:** None. **M. Mullan:** A. Employment/Salary (full or part-time):; Rock Creek Pharmaceuticals.

Poster

041. Alzheimer's Disease: Tau Animal Models

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 41.08/D33

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH/NCATS Colorado CTSI Grant Number UL1 TR000154

NIH HL089049

Title: Aggrecan changes seasonally in hibernating ground squirrels: Insight into understanding Alzheimer’s disease

Authors: *K. B. BJUGSTAD[1], A. HINDLE[2], S. L. MARTIN[2];
[1]Medicine, Neuroscience, Bioengineering, [2]Cell and Developmental Biol., Univ. Colorado Denver, AURORA, CO

Abstract: The ability for hyperphosphorylated tau and amyloid-beta to aggregate into neurofibrillary tangles (NFTs) and amyloid plaques, the two hallmarks of Alzheimer's disease (AD) neuropathology, is dependent on the presence of sulfated proteoglycans in the brain's extracellular matrix (ECM). Phosphorylated tau is abnormally elevated in the brains of hibernating mammals, specifically during torpor, without evidence of tangle formation. Remarkably, tau phosphorylation returns to normal during each interbout arousal; this cycle between torpor and arousal repeats ~every two weeks throughout the 6-7 months of winter hibernation. Because of the prominent role that the ECM has in NFT formation, we hypothesized that the relative composition of the sulfated proteoglycans in the ECM of hibernating animals may differ from that of other animals, display a seasonal change, and/or change within torpor-arousal cycles. Septal brain ECM was assessed in summer active (SA) and two winter hibernating groups of 13-lined ground squirrels, Late-Torpor (LT) and Interbout Arousal (IBA); non-hibernating rats served as controls) Using immunohistochemistry, the relative abundance of 5 ECM proteoglycans was measured: brevican, agrin, aggrecan, glypican, and hyaluronic acid. There were no significant species differences between rats and ground squirrels, suggesting potential seasonal or euthermic changes in ground squirrel brain ECM would be small and within the natural variation of ECM expression. Analysis among ground squirrel groups revealed no significant differences in agrin, brevican, HA, or glypican. Aggrecan, however, was elevated in hibernating ground squirrels, especially in IBA animals, with a significant elevation compared to SA animals. It is possible that aggrecan cycles seasonally, allowing the brain to prepare for long-term hibernation. Human brain areas with aggrecan deficient ECM, such as the septum, typically contain greater tau pathology during AD. Identifying the proteoglycan changes that prevent tau pathology during hibernation may reveal a novel approach to understanding AD.

Disclosures: **K.B. Bjugstad:** None. **S.L. Martin:** None. **A. Hindle:** None.

Poster

041. Alzheimer's Disease: Tau Animal Models

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 41.09/D34

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: PAPIIT-DGAPA IN215114

UNAM-PAPIIT IA202214-2

Title: Cellular and morphological changes in hippocampus (CA1) in rats exposed to Vanadium Pentoxide

Authors: *E. MONTIEL-FLORES, SR[1,2], V. ANAYA-MARTÍNEZ[2], A. GUTIÉRREZ-VALDEZ[2], J. SÁNCHEZ-BETANCOURT[2], J. ESPINOSA-VILLANUEVA[2], M. AVILA-COSTA[2];
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Abstract: Vanadium (V) is a petroleum product widely distributed in nature, it is employed in the steel industry, car parts and as a pigment in paint manufacture. Its toxicity depends on its oxidation state, it seems that the vanadium pentoxide (V2O5) is the most toxic to the living cells. It has been reported that oral administration induces changes in motor activity and learning, in rats i.p. administration increases lipid peroxidation levels in cerebellum and the concentration of free radicals in hippocampus and cerebellum. Mice which inhale V2O5 presented reduced number of tubulin+ in Leydig and Sertoli cells; it has also been reported that inhaled V2O5 induces loss of dendritic spines, necrosis, and hippocampus neuropil alterations, and that these changes correlate with alterations in spatial memory. The purpose of this work is to characterize the morphological alterations and the number of cells with hyperphosphorylated tau-p-Ser262 that appears in the hippocampus (CA1) in rats exposed to V2O5. Male wistar rats inhaled 0.02 M of V2O5, 1 h two times a week for 6 months. The results show that tau protein is phosphorylated by V2O2, we observed significant increase in the number of immuno+ tau-pSer262 at 4, 16 and 24 weeks comparing to control group, also we observed morphological changes in the shape and size of nucleus, soma and dendrites of hippocampal cells. We consider that CA1 neurons hyperphosphorylation observed induced morphological alterations in cytoskeleton and these changes are similar to those reported in tauopathies such as Alzheimer's disease.

Disclosures: **E. Montiel-Flores:** None. **V. Anaya-Martínez:** None. **A. Gutiérrez-Valdez:** None. **J. Sánchez-Betancourt:** None. **J. Espinosa-Villanueva:** None. **M. Avila-Costa:** None.

Poster

041. Alzheimer's Disease: Tau Animal Models

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 41.10/D35

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: National Institutes of Aging Grant 1R15AG039008

Title: Preventative effects of chronic treadmill exercise on cognitive and non-cognitive behaviors in P301S tau transgenic mice

Authors: *O. OHIA, S. MONTAZARI, C. VOLLERT, J. ERIKSEN;
Pharmacol. and Pharmaceut. Sci., Univ. of Houston, HOUSTON, TX

Abstract: Alzheimer's disease (AD), as well as other tau-related dementias, are neurodegenerative diseases that are characterized in part by the pathological development of neurofibrillary tangles. Based on recent clinical evidence in patients with neurodegenerative tauopathy, it is possible that introducing physical exercise can be instrumental in delaying the onset of behavioral impairments. In this study, 4-month old P301S tau transgenic mice were subjected to 6 months of forced treadmill exercise and evaluated for effects on cognitive and non-cognitive behaviors. Exercise significantly impacted the motor coordination and muscular strength of exercised P301S mice compared to their non-exercised counterparts. Additionally, mice were assessed on their performance in the light/dark avoidance test, elevated-plus maze, pre-pulse inhibition, contextual and cued fear conditioning and Morris water maze. We observed that exercise altered the cognitive and non-cognitive impairments displayed in P301S mice. We believe this research will lead to a more comprehensive understanding of how treadmill exercise may help to prevent or delay the onset of neurodegenerative disease.

Disclosures: **O. Ohia:** None. **S. Montazari:** None. **C. Vollert:** None. **J. Eriksen:** None.

Poster

041. Alzheimer's Disease: Tau Animal Models

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 41.11/D36

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH: R01NS075487

the BrightFocus Foundation

Title: Tau reduction in adulthood in a new line of conditional knockout mice is safe and reduces seizure susceptibility

Authors: *Z. LI, S. BUCKINGHAM, A. HALL, S. WILSON, E. D. ROBERSON;
Neurobio., Univ. of Alabama At Birmingham, Birmingham, AL

Abstract: Microtubule-associated protein tau is emerging as target for treatment of Alzheimer's disease and potentially for forms of epilepsy. Tau reduction from conception has been well demonstrated to confer resistance to seizures in various seizure models. This seizure protective effect could possibly arise from compensatory mechanisms during development triggered by tau knockout from conception. Therefore, targeting tau in adulthood to treat seizures and Alzheimer's disease may or may not be effective. To address this question, we developed an inducible tau knockout system. We maintained normal levels of tau during development to avoid potential developmental effects of tau reduction. We then induced tau reduction in adult mice and tested seizure susceptibility in two different models. Tau reduction in adulthood decreased the maximum seizure severity and prolonged the latency of seizures induced by pentylenetetrazol, an antagonist of $GABA_A$ receptors. Tau knockout in adulthood also decreased the severity of seizures induced by kainic acid, a glutamate receptor agonist. The severity of seizures correlated with residual level of tau; lower levels of tau were associated with lower seizure severity. Tau reduction in adulthood did not cause overt abnormalities, suggesting overall safety of tau knockout in adulthood. In summary, we conclude that tau reduction in adulthood effectively protects mice from seizures without causing major abnormalities. These data support the idea that tau could be effectively and safely targeted for treatment of seizures and Alzheimer's disease.

Disclosures: Z. Li: None. **S. Buckingham:** None. **A. Hall:** None. **S. Wilson:** None. **E.D. Roberson:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Intellectual property.

Poster

041. Alzheimer's Disease: Tau Animal Models

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 41.12/D37

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH

Title: A new reporter system of tau spreading *in vivo*: AAV-delivered human tau crosses synapses in mice

Authors: *S. WEGMANN, S. NICHOLLS, S. TAKEDA, Z. FAN, B. T. HYMAN;
Neurol., Mass Gen. Hosp. / Harvard Med. Sch., Charlestown, MA

Abstract: In Alzheimer's disease, the progressive deposition of tau protein in neurofibrillary tangels (NFTs) appears to follow a certain pattern. Starting in the entorhinal cortex, pathological tau aggregation next occurs in the limbic system, then cortical areas, and finally throughout the brain of AD patients. In recent years, mouse models that mimic such tau pathology spreading pattern have been evolved. In these mice, transgenic human tau expression is locally restricted to the entorhinal cortex. In old mice (>18 month), human tau is also found in neurons that do not express the transgene and are directly downstream of the EC, in the dentate gyrus, the hippocampus, and more frontal cortical areas suggesting that tau can cross synapses along the perforant pathway. However, drawbacks of this model remain: 1) late onset of tau spreading, 2) restriction to perforant pathway, and 3) technical difficulties in discriminating between tau expressing and tau recipient neurons. Here, to study tau spreading *in vivo* and *in vitro* more efficiently, we created a tau expression reporter system, eGFP-2a-huTau, in which eGFP and wild-type human full-length tau (441aa) are expressed as separate proteins in the the same cell. This construct allows direct indentification and *in situ* visualization of tau expressing cells without creating a bulky GFP-tau fusion protein that may show cellular functions and distribution distinct from "naked" tau. We expressed eGFP-2a-huTau in neurons to examine the cellular consequences of tau overexpression. Furthermore, using immunofluorescence labeling of human tau (N-terminal epitope), we were able to identify neurons that recieved human tau from neighboring neurons (huTau+/eGFP-) *in vitro* after >7 days in culture. Next, we packaged eGFP-2a-huTau under teh CBA promotor into an adeno associated virus (AAV) and delivered it stereotactically into the entorhinal cortex of wild-type mice. Postmortem analysis of eGFP and human tau in these brains showed strong expression of eGFP and tau in the injected brain areas, as well as tau spreading to neighboring neurons and neurons in more distal areas of the brain as early as 4-6 weeks after AAV delivery. Phosphorylated tau (PHF1, CP13) was found in tau expressing but not tau rececieving neurons. However, more detailed analysis of the tau found in downstream neurons is in progress. Our early data suggest that i) wild-type tau is able to travel from neuron-to-neuron and ii) the mechanism of tau traveling maybe independent of phoshprylation and aggregation. Our eGFP-2a-huTau construct resembles a great tool to study diverse questions in tau biology and disease both *in vitro* and *in vitro* in parallel.

Disclosures: **S. Wegmann:** None. **S. Nicholls:** None. **S. Takeda:** None. **Z. Fan:** None. **B.T. Hyman:** None.

Poster

041. Alzheimer's Disease: Tau Animal Models

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 41.13/D38

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NEOMED COP Competitive Funding Award

Title: Phosphorylated tau levels in the brains of naked mole-rats (Heterocephalus glaber) are differentially elevated based on reproductive status

Authors: *C. M. DENGLER-CRISH[1], G. N. WILSON[2], M. A. SMITH[1], S. D. CRISH[1]; [1]Pharmaceut. Sci., Northeast Ohio Med. Univ., Rootstown, OH; [2]Biomed. Sci. Grad. Program, Kent State Univ., Kent, OH

Abstract: Previous studies have shown that naked mole-rats exhibit unusual accumulations of amyloid beta at young ages comparable those shown in advanced Alzheimer's disease (AD) transgenic mice *(Edrey et al. 2013; Neurobiol Aging 34: 2352)*. Our laboratory investigated whether hyperphosphorylated tau, a pathological marker of AD, was elevated in reproductively suppressed and recently reproductively-activated naked mole-rats (12-weeks after removal from reproductive suppression) using ELISA. Our results indicated that newly activated mole-rats exhibited higher total brain levels of tau isoform s396-404 than suppressed animals. These differences were also apparent when comparing cerebellum and cortex but were not shown to be different at the subcortical level. Conversely, total tau was higher in the reproductively suppressed animals in all three brain regions. Additionally, total tau levels as well as isoform s396-404 were elevated comparably to hTau (B6.Cg-*Mapt*$^{tm1(EGFP)Klt}$ Tg(MAPT)8cPdav/J) mutant mice that express elevated levels of hyperphosphorylated tau. These results suggest that, like amyloid beta, potentially damaging high levels of hyperphosphorylated tau are naturally expressed in naked mole-rat brains and this expression may vary as a function of reproductive experience.

Disclosures: C.M. Dengler-Crish: None. **G.N. Wilson:** None. **M.A. Smith:** None. **S.D. Crish:** None.

Poster

041. Alzheimer's Disease: Tau Animal Models

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 41.14/D39

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NS 76308

Alzheimer's association

Title: Metabolic phenotyping of Tg4510 tau transgenic mice indicates hyperactivity and circadian disruption

Authors: *A. JOLY AMADO, M. N. GORDON, D. MORGAN;
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Abstract: Tau is a microtubule-associated protein which can aggregates into neurofibrillary tangles (NFTs) in age-related neurodegenerative diseases. Unexplained weight loss and cachexia are frequent clinical findings in patients with Alzheimer's disease (AD) and eating disorders have been reported in other tauopathies like Frontotemporal Dementia (FTD). However, the impact of tau pathology on energy homeostasis remains unclear. Transgenic Tg4510 mice overexpressing P301L tau exhibit pretangles, cognitive impairments and neuronal loss, which increase with aging. Mice also exhibit a significant decrease in body weight compared to their nontransgenic littermates (NTG). In this study, 2 and then 7 months old Tg4510 and nontransgenic mice were placed in metabolic cages which allow the continuous evaluation of food intake, locomotor activity and measurements of CO2 production and O2 consumption. At 2 months of age, Tg4510 exhibit no differences in food intake or body weight compared to NTG, but do display a significant increase in nocturnal total activity ($5.2x10^4$ +/-0.30 counts/12h vs $3.7x10^4$ +/-0.31 counts/12h in NTG) along with decreased O2 consumption and CO2 production compared to NTG. At 7 months of age, Tg4510 mice show a massive increase in total activity during both night ($12.7x10^4$ +/-2.4 counts/12h vs $2.4x10^4$ +/-0.2 counts/12h in NTG) and day ($10.9x10^4$ +/-2.6 counts/12h vs $1.9x10^4$ +/-0.2 counts/12h in NTG). Interestingly, both O2 consumption and CO2 production were now increased at 7 mo in tau mice compared to their nontransgenic littermates. In a different cohort, 7 months old Tg4510 revealed tau deposits, phosphorylated tau and NFTs in the hypothalamus, particularly in the ventromedial nucleus, which could be related to the metabolic disturbances observed. Our data suggest that tau protein accumulation in the hypothalamus may be involved in energy homeostasis deregulation observed in Tg4510 mice and tauopathies such as AD and FTD. Understanding the origin of energy balance disorders in tauopathies may suggest new drug targets to modify some of the behavioral characteristics of the disorder, and possibly have impact on the disease process as well, providing benefit to both patients and caregivers. Supported by NS 76308 and Alzheimer's Association.

Disclosures: **A. Joly Amado:** None. **M.N. Gordon:** None. **D. Morgan:** None.

Poster

041. Alzheimer's Disease: Tau Animal Models

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Health Research Council of the Academy of Finland

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Sigrid Juselius Foundation

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Doctoral Programme of Molecular Medicine, University of Eastern Finland

Title: High-fat diet increases the expression and alternative splicing of tau in the brain of female mice independently from peripheral metabolic changes

Authors: M. TAKALO[1], *A. HAAPASALO[1], S. KEMPPAINEN[1], P. MÄKINEN[1], J. PIHLAJAMÄKI[1], H. SOININEN[1,2], M. LAAKSO[1], H. TANILA[1], M. HILTUNEN[1]; [1]Univ. of Eastern Finland, Kuopio, Finland; [2]Kuopio Univ. Hosp., Kuopio, Finland

Abstract: A number of epidemiological studies suggests that type 2 diabetes mellitus (T2DM) increases the risk of Alzheimer's disease (AD). However, molecular mechanisms underlying this co-morbidity are not well understood. Intraneuronal deposits of microtubule-associated protein tau are characteristic features of several neurodegenerative diseases, including AD. Moreover, abnormal splicing of tau exon 10 leads to the altered ratio of 4-repeat (4R) vs. 3-repeat (3R) tau isoforms in neurodegenerative diseases and T2DM.To investigate the effects of both genetically and high-fat diet (HFD)-induced diabetic phenotype on tau expression and exon 10 splicing, we used the well characterized AD mouse model (APdE9) with hyperglycemia induced by the overexpression of pancreatic insulin-like growth factor 2 (IGF2). Four genotype groups were included in the study; wild-type (AwIw), IGF2 overexpressing (AwI+), APdE9 overexpressing (A+Iw), and APdE9 x IGF2 co-overexpressing (A+I+) mice. Mice were fed either with standard chow or with HFD for four months. Before sacrificing, mice went through a behavioral test battery. Tau expression at mRNA level was assessed with quantitative PCR and capillary electrophoresis by using primers flanking exon 10. Tau phosphorylation and protein expression were assessed with Western blot. HFD significantly induced 4R-tau mRNA and protein

expression and tau phosphorylation in the temporal cortex of all female mice regardless of the genotype. The mRNA levels of 3R-tau were also significantly increased by the HFD, although the expression of 3R-tau was considerably lower as compared to that of 4R-tau. Moreover, HFD significantly increased the mRNA ratio of 4R-tau vs. 3R-tau in the temporal cortex of these mice. Increased 4R-tau and 3R-tau mRNA expression correlated with poor spatial memory and reduced exploratory activity. These effects were associated with diet-induced weight gain, but were independent of peripheral hyperglycemia, hyperinsulinemia, and insulin resistance. HFD did not affect neuroinflammation, activation of Akt/glycogen synthase kinase 3β signaling pathway, or the expression of tau exon 10 splicing enhancers in the temporal cortex of the mice. We are currently analyzing the brain metabolic profile of the HFD-fed mice in order to identify the specific lipid(s) responsible for the observed alterations in tau expression and splicing. Our findings provide experimental *in vivo* evidence that HFD, independently from diabetic or AD background, can modulate the expression and alternative splicing of tau. Thus, active dietary choices may modify tau-related pathological mechanisms.

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Poster

041. Alzheimer's Disease: Tau Animal Models

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 41.16/D41

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: DZNE

MPG

Tau Consortium

Title: Neuroinflammation and cognitive impairments in a novel transgenic mouse model expressing a rare Tau mutation A152T in progressive supranuclear palsy

Authors: *A. SYDOW[1], K. HOCHGRÄFE[2], S. KÖNEN[1], D. MATENIA[2], O. PETROVA[3], E.-M. MANDELKOW[1,2,3];
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Neurolog. Res. Cologne, Hamburg outstation, Hamburg, Germany; [3]CAESAR Res. Ctr., Bonn, Germany

Abstract: To mimic pathological hallmarks of genetically provoked tauopathies (e.g. progressive supranuclear palsy), we have generated a novel transgenic mouse model expressing human full-length Tau with a rare point mutation A152T (hTau40/A152T). The transgene is located inside the ROSA26 locus and the expression is controlled by the neuron-specific Thy1.2 promoter resulting in physiological expression levels in the brain and spinal cord of hTau40/A152T mice. The molecular ratios of Tau species (exogenous mutant Tau vs endogenous mouse Tau) are 1-2:1 (brain) and 3:1 (spinal cord), respectively. At young age immunohistological analysis of hTau40/A152T mice demonstrated the presence of pathological Tau conformation (MC1) and Tau hyperphosphorylation (pThr231/pSer235, pSer202/pThr205, pSer396/pSer404) combined with Tau missorting into the somatodendritic compartment of cortical and hippocampal neurons. The detection of Tau-aggregates by Gallyas silver staining and sarcosyl-extraction indicates a progressive co-aggregation of endogenous mouse Tau and exogenous human Tau with increasing age, leading to neuronal loss. Furthermore old hTau40/A152T mice show a prominent neuroinflammatory response as judged by activation of microglia and astrocytes and a rise of inflammatory markers such as inducible nitric oxide synthase (iNOS). In clear contrast to other Tau-transgenic models and Alzheimer disease patients with rather reduced protein clearance, hTau40/A152T mice show a strong induction of autophagy with increased levels of lysosomal (beclin, hsc70, LC3) and proteasomal (proteasome 26S subunit) markers in cortex and hippocampus. Although hTau40/A152T-expression induces Tau hyperphosphorylation and Tau aggregation in spinal cord and motor cortex, transgenic hTau40/A152T mice exhibit unaffected neuromotor performance similar to wild-type littermates as tested by a rotarod test. Importantly, deficits in spatial reference memory (Morris water maze) manifest in hTau40/A152T mice at the age of ~16 months and are accompanied by neurodegeneration as visualized by Fluoro Jade C staining. The pathophysiological characterization shows an excitotoxic phenotype including increased extracellular glutamate and intracellular calcium (see abstract by J. Decker et al.). In conclusion, the hTau40/A152T mouse model mimics pathological hallmarks of tauopathies (Tau hyperphosphorylation, Tau aggregation, neuroinflammation, cognitive decline) and offers potential to evaluate new therapeutic strategies and drug candidates against Tau-induced neurodegeneration.

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Poster

041. Alzheimer's Disease: Tau Animal Models

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

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Support: Fondation Plan Alzheimer (Primatau project)

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Lille Métropole Communauté Urbaine

Thanks to Peter Davis for providing MC1 antibody

Title: Characterization of a novel non-human primate model of tauopathy

Authors: *R. ARON BADIN[1], R. CAILLIEREZ[2], M. COLIN[2], A. BEMELMANS[1], N. DUFOUR[1], S. DUJARDIN[3], L. PONTOIZEAU[1], C. LACHAUD[2], S. LECOURTOIS[1], M. GUILLERMIER[1], L. EYMIN[1], Y. BRAMOULLÉ[1], P. GIPCHTEIN[1], C. JAN[1], N. VAN CAMP[1], N. DEGLON[4], L. BUÉE[2], P. HANTRAYE[1];
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Abstract: Aims: Generate and characterize a robust non-human primate model of progressive tauopathy by targeted overexpression of mutant or wild type human Tau protein in the hippocampus using adeno-associated viral (AAV) vectors. Background: Recently published results demonstrate the efficacy of lentiviruses to generate a genetic model of tauopathy in rodents. Longitudinal histopathological characterization revealed the presence of aggregates as early as 2 months and different stages of tau protein phosphorylation, conformational change and aggregation were clearly shown. This data supported the development of a non-human primate model of tauopathy where functional deficits could be explored longitudinally *in vivo*. Methods: Intracerebral stereotaxic injections of AAV vectors were performed under preoperative MRI guidance in 12 adult male macaques (M.fascicularis). Primates were injected into the hippocampus bilaterally with an AAV2/9 viral vector bearing a CBA promoter and overexpressing either the WT hTau46 (n=4), the hTau46 P301L mutated form of Tau (n=4) or a null GFP (n=4) where the reporter gene is transcribed but not translated. MRI was used to screen for potential inflammatory reactions and atrophy as well as individual determination of the targeted stereotactic coordinates. All primates were trained to perform a cognitive battery on tactile screens before and at 4, 8, and 12 months post-injection looking at working memory, spatial memory and executive function. Post-mortem histological analysis included MC1, AT8,

AT100, NeuN, GFAP, CD68, and Iba1. The control group is still ongoing. Results: Our preliminary results suggest that overexpressing either wild type or mutated forms of tau leads to a cognitive deficit that is detectable as early as 4 months after viral delivery and stabilizes at 8 months post-infection. The same animals showed no deficit in a visual discrimination task suggesting the specificity of the impairment in spatial, executive and recognition memory. MRI imaging did not evidenced inflammatory reactions or atrophy over time. Ongoing histological analysis confirms the presence of widespread AT8-positive inclusions in the hippocampus and connected regions like entorhinal cortex and the cingulate cortex. These results remain preliminary until the GFP group full data is available. Conclusion: This model of tauopathy based on the use of AAV9-CBA vector has good construct, neuropathological and functional validity and holds great promise for further investigation of therapeutic strategies and tau specific PET ligands in non-human primates.

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Poster

041. Alzheimer's Disease: Tau Animal Models

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 41.18/D43

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH/NINDS R21NS07708901A1

Alzheimer’s Association Grant NIRG11204995

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Title: Cell-restricted deletion of MyD88 affects tau pathology in an hTau mouse model of tauopathy

Authors: N. MAPHIS[1], X. LI[3], B. LAMB[3], *K. BHASKAR[2];
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Abstract: Tangle pathology (including hyperphosphorylated and aggregated tau) is a major neuropathological hallmark of Alzheimer's disease (AD) and related tauopathies. Increasing evidence suggests that inflammatory processes in the brain precede tau pathology. A previous study from our group has demonstrated that enhancing neuroinflammation either via a Toll-Like Receptor-4 (TLR-4) ligand lipopolysaccharide (LPS) or genetic ablation of the *Cx3cr1* (fractalkine receptor) led to increased tau phosphorylation, aggregation and working memory impairment in a manner dependent upon the activation of interleukin-1 receptor (IL-1R)-p38 mitogen activated protein kinase (p38 MAPK) pathway. To block IL-1R and TLR-4 receptor signaling, we targeted and performed cell-specific deletion of MyD88 - a common adapter protein of IL-1 and TLR-4 receptors using Cre-LoxP technology. First, microglia-restricted deletion of MyD88 (Cd11bCreMyD88$^{f/f}$) was confirmed by isolation of microglia followed by qRT-PCR for MyD88 expression. Second, increased infiltration of CD45+ cells (peripheral monocytes) within the brains of Cd11bCreMyD88$^{f/f}$ mice was observed compared to MyD88$^{f/f}$ mice via flow cytometry. Third, in contrast to what was expected, microglia-restricted deletion of MyD88 led to enhanced AT8- and AT180-site tau phosphorylation. Fourth, gene expression and multiplex protein analysis comparing the CD11bCreMyD88$^{f/f}$ to MyD88$^{f/f}$ suggested alterations in key inflammatory cytokines and chemokines. Finally, we studied the effect of neuronal-restricted deletion of MyD88 in an hTau mouse model of tauopathy (hTau-*Mapt*$^{-/-}$/CamKII[[Unsupported Character - Symbol Font ]]CreMyD88$^{f/f}$) on tau pathology. Notably, we observed a drastic reduction in the levels of truncated neurotoxic tau in the cortex of hTau*Mapt*$^{-/-}$/CamKII[[Unsupported Character - Symbol Font ]]CreMyD88$^{f/f}$ mice compared to our hTau*Mapt*$^{-/-}$ mice, however there was no difference in the phosphorylated-specific tau at the AT8-, AT180- or PHF1-sites. Further studies on the characterization of truncated tau species and the effect of microglia-restricted (CD11b) deletion of MyD88 on tangle pathology and cognitive function are ongoing.

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Poster

041. Alzheimer's Disease: Tau Animal Models

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: DOH101-TD-B-111-0002

Title: Hypertension increases tau hyperphosphorylation and Aβ production in 3XTg-AD mice

Authors: *Y.-H. SHIH[1], C.-W. LEE[1], T.-W. LIN[1], S.-F. TSAI[1], Y.-M. KUO[1,2];
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Abstract: Objective: Epidemiological studies suggest chronic hypertension (HTN) is associated with late-onset AD. However, whether HTN is a cause for AD or merely a comorbid remains unclear. This study is to investigate the role of HTN in the pathogenesis of AD. Methods: We use the modified two-kidney one-clip (2K1C) to induce HTN in 3XTg-AD mice. The blood pressure and AD-related pathology in the hippocampus were measured 1 month after the surgery. Sham operation served as controls. Results: 2K1C resulted to blood pressure elevated and consistent after surgery 1 month compared to sham groups. The performance of learning and memory was impaired in object recognition test but not in radial arm water maze in HTN group. The level of hippocampal Aβ40 and Aβ42 was increased and positively correlated to mean blood pressure changed ratio. The p412-tau-postive stains were enhanced in the ventral parts of hippocampus. The level of GSK3β, a tau protein phosphorylation kinase, was unaffected by 2K1C. Conclusion: These results suggest that HTN may not cause AD, yet it may contribute and aggravate the pathogenesis of AD.

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Poster

041. Alzheimer's Disease: Tau Animal Models

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Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Reduction/delay of phenotype in Tg4510 tauopathy model following gestational doxycycline treatment and removal of helicobacter infection

Authors: *D. M. BARTEN, A. EASTON, B. SNYDER, L. B. DECARR, C. BOURIN, G. HIRSCHFELD, G. W. CADELINA, S. KEENAN, D. BRYCE, A. CACACE, C. M. CONWAY,

M. K. AHLIJANIAN, N. DEVIDZE;
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Abstract: The phenotype of transgenic mouse lines often varies between colonies of animals at different sites, or can drift over time within a colony. The Tg4510 bi-genic tauopathy mouse model overexpresses the tetracycline-controlled transcriptional activator (tTA) driven by the Cam kinase II (CamKII) promoter, which initiates transcription of the human tau-P301L transgene through the tetracycline operon-responsive element (TRE). Doxycycline (Dox) treatment of the mice reduces transgene expression by 75 percent. Unlike literature reports, the Tg4510 colony at our facility showed a significant loss of CA1 neurons as early as 2 months of age. Based on this neuropathology, it is not surprising that 2 month Tg4510 mice were cognitively impaired, although previous reports found no deficits at this age. Doxycycline treatment of the dams from inception to P21 (weaning) or P0 (birth) prevented this developmental loss of CA1 neurons. Tg4510 mice treated with Dox during development were studied longitudinally to determine the onset of behavioral deficits, tau pathology and neurodegeneration. A significant delay in the tauopathy phenotype was observed when transgene expression was reduced during gestation. In a separate observation, we noted that the founder animals and our colony harbor helicobacter. Helicobacter species infect the gastrointestinal tracts of many of species, including over 50% of humans. Most subjects/animals show no clinical signs, and it is widespread in many otherwise specific-pathogen free colonies. Breeders at the BMS colony treated with a combination of amoxicillin, clarithromycin, metronidazole, and omeprazole (BioServ) for several generations were found to be helicobacter-free using PCR analysis of feces. Helicobacter-free CamKII tTA female and TRE tau-P301L male mice were bred to generate Tg4510 bi-transgenic mice. Importantly, the Tg4510 mice themselves had no antibiotic treatment during gestation or later. Helicobacter-free Tg4510 mice showed reduced tau pathology compared to helicobacter positive mice, but they still retained developmental loss of CA1 neurons measured at 2 months of age. These data demonstrate the impact of transgene expression during gestation, as well as the effects of a peripheral bacterial infection on a central tauopathy phenotye in transgenic mice. These observations may help to explain the phenotypic variability observed between colonies of a single line of transgenic mice.

Disclosures: **D.M. Barten:** A. Employment/Salary (full or part-time):; Bristol-Myers Squibb. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Bristol-Myers Squibb. **A. Easton:** A. Employment/Salary (full or part-time):; Bristol-Myers Squibb. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Bristol-Myers Squibb. **B. Snyder:** A. Employment/Salary (full or part-time):; Bradley.Snyder@bms.com. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Bradley.Snyder@bms.com. **L.B. DeCarr:** A. Employment/Salary (full or part-time):; Bristol-Myers Squibb. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual

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Poster

041. Alzheimer's Disease: Tau Animal Models

Location: Halls A-C

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Topic: C.02. Alzheimer's Disease and Other Dementias

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Title: Propagation of human tau in a mouse tau knockout model *in vivo*

Authors: *E. A. MAURY[1,2], S. WEGMANN[2], A. M. POOLER[3], B. T. HYMAN[2];
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Abstract: Alzheimer's disease (AD) is a neurodegenerative disorder that is characterized by the progressive deposition of amyloid plaques and neurofibrillary tangles (NFTs), composed of tau. Accumulation of these lesions is associated with synapse loss, neuronal death, and subsequent cognitive decline. In early stages of AD, NFT pathology occurs in the entorhinal cortex and medial temporal lobe from where it spreads along synaptic connections throughout limbic and association cortices. Understanding how tau pathology spreads in AD may reveal new therapeutic targets for AD. However, the mechanism underlying inter-neuronal transfer of pathological tau is unknown: pathological aggregates may be directly transported between synaptically-connected neurons, pathological tau may provide a template for the recruitment and subsequent misfolding of non-pathological tau, or both. In order to test these possibilities, we generated a novel transgenic mouse model of tauopathy. rTgTauEC mice overexpress human tau harboring the P301L mutation in layer II of the entorhinal cortex (EC). In these mice, NFTs form first in the transgene-expressing neurons in the EC and then progress to neurons without detectable transgene expression, including neurons in the dentate gyrus (DG), hippocampus, and frontal cortex. We crossed these mice with mice lacking murine tau (TauKO) to generate a new line that expresses human P301L tau within the EC in the absence of endogenous mouse tau. We examined human tau localization in 18 month-old brain sections from three mice strains: rTgTauEC, TauKO, and TauKO x rTgTauEC. Brain tissue was sectioned by cryostat into 10 micron horizontal sections, and human tau was visualized by immunohistochemistry using TauY9 antibody. As expected, brains from both rTgTauEC and TauKO x rTgTauEC mice showed localized human tau expression in the EC. In addition, we identified human tau-positive neurons in hippocampus and somatosensory cortex, indicating propagation of tau in both mouse lines. Interestingly, the numbers of human tau-positive neurons in the DG was not significantly different between rTgTauEC and TauKO x rTgTauEC mice. Levels of phosphorylated human tau, as identified by phospho-specific tau antibodies were similar in both mouse lines. However, only rTgTauEC brains showed tau aggregates, as visualized by both immunoreactivity to Alz50 antibody and staining with thiazine red. We conclude that inter-neuronal propagation of pathological human tau does not require the presence of endogenous mouse tau, suggesting that recruitment of non-pathological forms of tau is not necessary for spreading of NFT pathology.

Disclosures: **E.A. Maury:** None. **S. Wegmann:** None. **A.M. Pooler:** None. **B.T. Hyman:** None.

Poster

042. Beta Amyloid Toxicity

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 42.01/D47

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIRG-12-242598

Title: Sustained oligomeric Aβ exposure decreases GLT-1 steady state levels in astrocytes without altering its transcription

Authors: *J. M. ZUMKEHR, C. HAWKINS, M. KITAZAWA;
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Abstract: The disruption of the glutamatergic neuronal network has been suggested to contribute to synaptic loss and cognitive decline in Alzheimer's disease (AD). Astrocytes play an important role in regulating this network through the expression of the glutamate transporter 1 (GLT-1). Impairment of glutamate uptake by astrocytes has been reported in AD and the knockdown of GLT-1 in AD mice exacerbates cognitive decline. We previously demonstrated that GLT-1 levels decreased in an age-dependent manner in 3xTg-AD mice and that the pharmacological up regulation of GLT-1 in these mice rescued cognition, restored synaptic density and ameliorated tau pathology without affecting Aβ pathology. Our *in vivo* evidence suggests that a functional loss of GLT-1 is mediated by Aβ toxicity. Therefore, we hypothesize that certain toxic Aβ assembly states downregulate GLT-1 in astrocytes and promote other AD neuropathology including tau and synaptic loss. To test our hypothesis, we examine the mechanistic link between GLT-1 and Aβ *in vitro*. We find that pathologically-relevant, naturally secreted Aβ oligomers from the conditioned media of 7PA2 cells significantly decrease the steady state levels of GLT-1 in the primary neuron and astrocyte cell co-culture system. Aβ oligomers, however, did not change mRNA expression levels of GLT-1 which suggest that Aβ oligomers functionally inactivate GLT-1 by facilitating its degradation. Our results also suggest that sustained Aβ oligomers exposure significantly decrease GLT-1 steady state levels in astrocytes which may lead to the development of tau pathology, synaptic loss and neuronal loss in AD.

Disclosures: **J.M. Zumkehr:** None. **C. Hawkins:** None. **M. Kitazawa:** None.

Poster

042. Beta Amyloid Toxicity

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 42.02/D48

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Toxic events of beta-amyloid oligomers on cortical neurons and protective effect of beta-Estradiol: A mechanistic study

Authors: *N. CALLIZOT, M. COMBES, P. POINDRON;
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Abstract: Alzheimer disease (AD) affects mainly people over the age of 65 years, suffering from different clinical symptoms such as progressive decline in memory, thinking, language, and learning capacity. The toxic role of beta amyloid peptide (Ab) has now shifted from insoluble Ab fibrils to smaller, soluble oligomeric Ab aggregates (AbO). Many evidences suggest that the neurodegenerative process would be due to the interaction of AbO with binding targets, activation of stress kinases, hyperphosphorylation of tau protein, caspase activation, loss of synapse, neuronal death, loss of cholinergic function, generation of reactive intermediates of oxygen (oxidative stress), or glutamate excitotoxicity. Urgent need for efficient new therapies is high, but could only be successful with an extensively comprehension of AbO degeneration process. In the present work, based on an *in vitro* primary cell culture treated with AbO preparation, we have carefully studied the cytopathological effects of AbO on neuronal death and then we have investigated the effect of 17-beta Estradiol (B-estr) on the degeneration process induced by AbO. Briefly we used rat cortical neurons (from E15). The cells were seeded in 96-well plates and intoxicated with AβO solution after 11 days of culture for 24 hours. B-estr was used at 100 nM (final concentration) and was added as pretreatment (1h before injuries). A co-incubation with selective inhibitors was performed for the mechanistic study. In parallel, western blotting (WB) analysis was done to quantify protein levels and their activation. We showed that B-estr was able to significantly protect neurons as well as glial cells from degeneration decreasing the caspase 3 activation and the massive mitochondrial stress (induced by AbO). Preservation of neurite network and synapsis integrity was also observed. Moreover, the large hyperphosphorylation of tau protein induced by AbO was significantly reduced with B-Estr. A mechanistic study was also performed co-incubating inhibitors of main survival pathways to try to better understand the mode of action of B-estr and the pathway involved in the AbO toxicity. We showed that the effects of b-Estr were fully abolished blocking the MEK pathway as well as the DNA repair pathway (PARP-g) or the mitochondrial anti-apoptotic pathway (Bcl2). Interestingly the effect was inexisting coincubating B-estr with TrK receptor or Ras/Raf inhibitors showing the predominant role of growth factors paythway in its neuroprotective effect. Finally, we showed a large inactivation of AKT protein in presence of AbO that was reversed even over activated in presence of B-Estr.

Disclosures: **N. Callizot:** None. **M. Combes:** None. **P. Poindron:** None.

Poster

042. Beta Amyloid Toxicity

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 42.03/D49

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Aβ1-42 reduces P-glycoprotein in the blood-brain barrier through RAGE-NF-κB signaling

Authors: *J. PARK, R. PARK, S.-Y. KOOK, S.-M. SON, S.-H. HAN, I. MOOK-JUNG; Dept. of biomedical science, Seoul Natl. Univ., Seoul, Korea, Republic of

Abstract: The reduced clearance of amyloid-β peptide (Aβ) from the brain partly accounts for the neurotoxic accumulation of Aβ in Alzheimer's disease (AD). Recently, it has been suggested that P-glycoprotein (P-gp), which is an efflux transporter expressed on the luminal membrane of the brain capillary endothelium, is capable of transporting Aβ out of the brain. Although evidence has shown that restoring P-gp reduces brain Aβ in a mouse model of AD, the molecular mechanisms underlying the decrease in P-gp expression in AD is largely unknown. We found that Aβ1-42 reduced P-gp expression in the murine brain endothelial cell line bEnd.3, which was consistent with our *in vivo* data that P-gp expression was significantly reduced especially near amyloid plaques in the brains of five familial AD mutations (5XFAD) mice, which are used as an animal model for AD. A neutralizing antibody against the receptor for advanced glycation end products (RAGE) and an inhibitor of nuclear factor-kappa B (NF-κB) signaling prevented the decrease in Aβ1-42-induced P-gp expression, suggesting that Aβ reduced P-gp expression through NF-κB signaling by interacting with RAGE. In addition, we observed that the P-gp reduction by Aβ was rescued in bEnd.3 cells receiving inductive signals or factors from astrocytes making contacts with endothelial cells (ECs). These results support that alterations of astrocyte-EC contacts were closely associated with P-gp expression. This suggestion was further supported by the observation of a loss of astrocyte polarity in the brains of 5XFAD mice. Taken together, we found that P-gp down regulation by Aβ was mediated through RAGE-NF-κB signaling pathway in ECs and that the contact between astrocytes and ECs was an important factor in the regulation of P-gp expression.

Disclosures: **J. Park:** None. **R. Park:** None. **S. Kook:** None. **S. Son:** None. **S. Han:** None. **I. Mook-Jung:** None.

Poster

042. Beta Amyloid Toxicity

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 42.04/D50

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Identification of 5-methoxyflavone as a novel DNA polymerase-beta inhibitor and neuroprotective agent against beta-amyloid toxicity

Authors: S. MERLO[1], L. BASILE[2], M. SORTINO[1], F. NICOLETTI[3], S. GUCCIONE[2], *A. G. COPANI[2];
[1]Dept. of Clin. and Mol. Biomedicine, [2]Dept. of Drug Sci., Univ. of Catania, Catania, Italy;
[3]Dept. of Human Physiol. and Pharmacol., Univ. of Rome, Rome, Italy

Abstract: DNA replication is an obligatory step in the apoptotic pathway triggered by beta-amyloid in neurons. Neuronal DNA replication is unusual, since it is carried-out by DNA polymerase-beta (pol-beta), and likely lasts months before the occurrence of neuronal Death (reviewed in Copani et al., Curr Med Chem 2008). Pol-beta might therefore represent a relevant target for neuroprotection in Alzheimer's disease (AD). Known pol-beta inhibitors are not selective for the enzyme; dideoxycytidine, which we have shown to prevent beta-amyloid-induced DNA replication and apoptosis (Copani et al., Faseb J. 2002), is a preferential inhibitor of pol-beta over DNA polymerase-alpha and delta. We searched for selective pol-beta inhibitors by virtual screening of a database containing more than 4,000 natural and over 20,000 drug-like compounds. Twenty compounds were selected for their best-fit pose on the 8-kD domain of the enzyme. So far, we have tested nine of the selected compounds on both wild type and pol-beta-null mouse fibroblasts, which are hypersensitive to the DNA-methylating agent methylmethanesulfonate (MMS). Among the tested compounds, only 5-methoxyflavone was able to enhance cellular sensitivity to MMS in wild type but not in pol-beta null cultures, and the resulting sensitivity to MMS in wild type cells mimicked that observed in pol-beta null cells. Thus, 5-methoxyflavone was able to inhibit the base-excision repair activity of pol-beta required for MMS resistance. Similarly, the compound directly inhibited human pol-beta activity on a gapped DNA substrate. In pure cultures of rat cortical neurons, 5-methoxyflavone was devoid of intrinsic toxicity when applied for 48 hrs and up to a concentration of 10 microM. These cultures are a useful model to investigate potential inhibitors of beta-amyloid-induced DNA replication and apoptosis (Copani et al., Faseb J. 2002; Copani et al., J. Neurosci. 2006). Consistent with an inhibition of pol-beta, 5-methoxyflavone (5-10 microM) was able to reduce both the number of S-phase neurons and apoptosis triggered by soluble oligomers of beta-amyloid.

Disclosures: **S. Merlo:** None. **L. Basile:** None. **M. Sortino:** None. **F. Nicoletti:** None. **S. Guccione:** None. **A.G. Copani:** None.

Poster

042. Beta Amyloid Toxicity

Location: Halls A-C

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Program#/Poster#: 42.05/D51

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Grant 13-04-00326 from Russian Foundation for Basic Research

Grant 517.2014.4 from the Foundation of Russian Scientific Schools

Title: Agonists of sigma1-receptor, donepezil and PRE-084, rescue hippocampal long-term potentiation impaired by beta-amyloid peptide

Authors: *E. I. SOLNTSEVA, N. A. KAPAI, O. V. POPOVA;
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Abstract: Donepezil is a potent acetylcholinesterase inhibitor used for the treatment of Alzheimer's disease (AD). Additional therapeutically relevant target for donepezil is sigma1 receptor (Sig1-R). Beta-amyloid peptide (Aβ) is believed to contribute to the pathogenesis of AD. In our previous work (Kapai et al., 2012) we have shown that donepezil antagonizes the suppressive action of Aβ(1-42) on long-term potentiation (LTP) in rat hippocampal slices. The purpose of the present study was to determine whether Sig1-R is involved into the mechanisms of donepezil action. For this purpose, we have tested whether agonist of Sig1-R PRE-084 mimics, and antagonist of Sig1-R haloperidol abolishes the effect of donepezil. Population spikes (PS) were recorded from the pyramidal layer of the CA1 region of rat hippocampal slices. LTP of PS was induced using a train of high frequency stimulation (100 Hz, 1 s). Drugs were applied by addition to the perfusate starting 15 min before and ending 5 min after the tetanus. In the control group, the amplitude of PS 30 min post-tetanus reached 153±10%. Aβ1-42 (200 nM) markedly suppressed the LTP magnitude or even caused the suppression of baseline PS (82±8%, $P<0.001$). This suppression of LTP could be markedly prevented when 1 μM donepezil was co-administered with Aβ (136±11%, $P<0.05$). Further, we co-administered three substances: Aβ, donepezil and 0.5 μM haloperidol, and have found that haloperidol antagonized the stimulating effect of donepezil on LTP (92±6%, $P<0.05$). Neither donepezil, nor haloperidol changed control LTP. Agonist of Sig1-R PRE-084 at concentration of 1 μM did not influence PS evoked by

single stimuli but significantly augmented the LTP (209.3 ± 15.5%, P<0.05). Then PRE-084 was co-administered with Aβ in the following concentrations: 0.1 μM, 0.5 μM, 1 μM and 10 μM. The drug was found to reverse LTP impairment in a dose-dependent manner. The concentration of 0.1 μM was not effective (79.8 ± 12.0%), while addition of higher concentrations of the drug to Aβ solution caused significant improvement of LTP. The LTP amplitude 30 min post-tetanus restored to 139.0 ± 11.5% for 0.5 μM (P < 0.05), to 172.4 ± 5.5% for 1 μM (P < 0.01) and to 183.0 ± 7.3% for 10 μM PRE-084 (P < 0.01). In conclusion, our observations show for the first time that selective agonist of Sig1-R PRE-084 can facilitate both control and Aβ impaired LTP, and this effect mimics the effect of donepezil. In addition to that, antagonist of Sig1-R haloperidol was found to abolish the stimulating effect of donepezil on LTP impaired by Aβ, which confirms the involvement of Sig1-R in the observed effect of donepezil.

Disclosures: **E.I. Solntseva:** None. **N.A. Kapai:** None. **O.V. Popova:** None.

Poster

042. Beta Amyloid Toxicity

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 42.06/D52

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Mechanism of toxic effect of amyloid beta peptide on Kv1.1 channel activity

Authors: *K. DEBOEUF[1,2], M. ISLAM[2], M. PACE[2], B. HALLAHAN[3], J. FARLEY[1,2]; [1]Psychology, [2]Neurosci., Indiana Univ., Bloomington, IN; [3]Pharmaceut. Sci., Univ. of Kentucky, Lexington, KY

Abstract: Amyloid beta-peptides [e.g., Aβ (1-42)] have frequently been implicated in the pathogenesis of Alzheimer's disease. Previous studies have found that neurotoxic Aβ peptides lead to the eventual disruption of calcium (Ca2+) homeostasis and can even disrupt synaptic transmission long before cell death occurs. However, the precise mechanism of these effects remains unclear. Our work suggests that Aβ- suppression of voltage-dependent K+ channel activity is among the earliest key steps in these processes. Using Xenopus oocytes, we have elucidated a pathway in which Ca2+-dependent activation of protein phosphatase 2B (PP2B/calcineurin), protein kinase C (PKC), an as yet unidentified protein tyrosine kinase (PTK), and the small G-protein RhoA all participate to produce profound suppression of Kv1.1 activity. This pathway is recruited by a variety of stimuli that increase [Ca2+]i in oocytes, including exogenous and endogenous GPCRs that couple to Gq/11 -PLC, calcium ionophore

(A23187), and ligand-gated ion channels that flux Ca2+. Because Kv1.1 and related K+ channels are activated during an action potential, are potent regulators of Ca2+ influx during depolarization, and pharmacological inhibition of Kv channels is often neurotoxic, we speculate that Aβ could disrupt Kv channels and thus lead to hyperexcitability, altered synaptic transmission, disruption of Ca2+ homeostasis, and neurotoxicity. We assessed the effects of Aβ (1-42) peptide (monomers and low-n oligomers) on heterologously expressed Kv1.1 channels in oocytes. Low (1uM-10 nM) concentrations produced a rapid, dose-dependent, suppression of macroscopic Kv1.1 current: ~50% reductions within 30 m. Reverse sequence (40-1) control peptide, as well as solvent-alone, failed to suppress Kv1.1. Additional studies showed Aβ suppression was partially Ca2+-dependent, and was reduced by ~50% when oocytes were loaded with BAPTA-AM. Aβ suppression of Kv1.1 also involves PP2B, since it was partially blocked (~50%) by the PP2B-inhibitor, cyclosporine A. The Aβ-produced Ca2+ increase did not occur via Ca2+ influx (e.g., through Aβ-mega pores), since complete removal of extracellular Ca2+ failed to attenuate Aβ-suppression of Kv1.1. Instead, it appears that Aβ is releasing Ca2+ from internal stores. Current lab work suggests aging (1 hr vs. 12 hrs.) may affect Aβ aggregate forms (soluble monomers vs. low-n oligomers), thus altering the extent of Kv1.1 channel activity suppression. Preliminary single channel work suggests the possibility that Aβ-suppression of Kv1.1 may also involve direct protein-protein interaction of Aβ with Kv1.1 channel subunits.

Disclosures: **K. Deboeuf:** None. **M. Islam:** None. **M. Pace:** None. **B. Hallahan:** None. **J. Farley:** None.

Poster

042. Beta Amyloid Toxicity

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 42.07/D53

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: CIHR MOP 74465

NSERC

Title: Role of somatostatin in β-amyloid induced cytotoxicity in human brain endothelial cells

Authors: S. PAIK, R. K. SOMVANSHI, C. SINGH, S. ZOU, *U. KUMAR;
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Abstract: Introduction: We recently demonstrated that somatostatin (SST) improves inflammation induced impaired distribution of ZO-1 in human brain endothelial cells (hCMEC/D3). In addition, we have also described changes in SST release and accumulation in cultured cortical neurons in response to β-amyloid. In several neuropathological conditions including Alzheimer's disease (AD), impaired blood brain barrier (BBB) play crucial role in brain damage. However, nothing is currently known whether receptor proteins exert any role in the protection of BBB. Accordingly in the present study, we used human brain endothelial cells and β-amyloid as a model of AD and determined concentration and time-dependent effect of SST in response to β-amyloid induced toxicity. In addition, we also determined the changes in expression and distributional pattern of SSTR and NMDAR subtypes at the level of mRNA and protein, respectively. Methods: hCMEC/D3 cell line was used for the experiments. β-amyloid induced toxicity was determined by using MTT assay in presence or absence of SST in concentration dependent manner. To determine whether the cells were displaying apoptosis or necrosis, Hoechst and PI staining was used. Subcellular distribution of SSTRs and NMDARs was determined by using immunofluorescence staining. Quantitative expression of SSTRs and NMDARs was determined by qRT-PCR and western blot analysis. Results: MTT assay revealed that β-amyloid consistently elicited dose-dependent toxicity. Interestingly, SST showed biphasic effect against β-amyloid by displaying protection at low concentration (1nM), however, the protective effect was gradually decreased with increasing concentration of SST. In addition, we have also characterized that SSTR and NMDAR subtypes are expressed in hCMEC/D3 cell in a receptor specific manner. Conclusion: SST elicit protective role against β-amyloid induced toxicity in hCMEC/D3 cells via alteration of SSTR and NMDAR subtype specific interaction.

Disclosures: **S. Paik:** None. **R.K. Somvanshi:** None. **C. Singh:** None. **S. Zou:** None. **U. Kumar:** None.

Poster

042. Beta Amyloid Toxicity

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH grant 1R01AG044404

Title: H2o2-upregulated rage on ab-induced oxidative pathway and membrane phase changes in bend3 cells

Authors: *C. EST, H. WANG, J. LEE;
Univ. of Missouri - Columbia, Columbia, MO

Abstract: Although the implication of amyloid-β (Aβ) in the pathogenesis of Alzheimer's Disease is still a matter of debate, increasing evidence has shown that Aβ is a pivotal component which directly influences cellular membrane alterations and triggers cellular signaling cascades leading to responses such as neuro-inflammation. The overall goal of this study is to investigate the role of hydrogen peroxide (H2O2) upregulated RAGE (receptor for advanced glycation end products) on the Aβ induced oxidative pathway and membrane phase changes in mouse cerebral endothelial cells (CECs). b.End3 cells were treated with H2O2, 5 μM Aβ, or the two in succession. First, the levels of RAGE normalized by β-actin were determined by the immune-blotting of RAGE rabbit/anti-rabbit primary and secondary antibodies. Following a positive MTT analysis, the expression of Aβ-induced superoxide after H2O2 treatment was elucidated by fluorescent microscopy of dihydroethidium (DHE), a flourescent DNA probe. The intensity of fluorescence directly correlates to activity of reactive oxygen species (ROS). Next, cytosolic phospholipase2 (c-PLA2) expression was measured by Western blotting, as c-PLA2 is known to play a role in the mediation of the neurotoxic effects of Aβ. Lastly, fluorescent microscopy of Laurdan was utilized to determine cell membrane molecular order; two fluorescent images at different wavelengths are overlaid to determine the generalized polarization (GP), where a high GP indicates high molecular order, and a low GP indicates low molecular order. Analysis of RAGE levels shows a maximal expression at H2O2 concentrations of 25 μM, and this concentration was used in subsequent experiments. DHE experiments show that H2O2 increases ROS expression after 24 hours, but at a lower extent than a two hour treatment of Aβ. Interestingly, Aβ treatment immediately following the 24 hour H2O2 treatment increases ROS expression at levels significantly lower than either treatment alone. Further supporting these findings, the Laurdan data shows a decrease in GP initially following H2O2 treatment, then a shift towards high GP, maximizing at the 24 hour observation. However, the H2O2/Aβ successive treatment shifts back towards a slightly high GP, but with a significantly less ordered membrane than the 24 hour treatment H2O2. Controlling oxidative stress is considered to be an important therapeutic method for AD, and the data of this experiment shows that a mechanism must exist that counteracts the combined damage of H2O2 and Aβ in succession, so further research is necessary to elucidate the structure of this response, which may lead to strong candidates for therapeutic treatment.

Disclosures: **C. Est:** None. **H. Wang:** None. **J. Lee:** None.

Poster

042. Beta Amyloid Toxicity

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 42.09/D55

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: E0745201

Title: Changes to tight junctions in the blood-brain barrier mediated by $A\beta^{25-35}$-RAGE interaction

Authors: *S. M. LANTZ-MCPEAK[1], E. CUEVAS[1], H. R. HERNANDEZ[2], M. G. PAULE[1], S. F. ALI[1], S. Z. IMAM[1];
[1]Div. of Neurotoxicology, NCTR/FDA, JEFFERSON, AR; [2]Lab. de Fisiología Celular, Facultad de Ciencias Químicas, Univ. Autónoma de San Luis Potosí, San Luis Potosí, Mexico

Abstract: Due to its high-energy demands, sensitivity to reactive oxygen species (ROS) and apoptosis, the neuron is one of the cell types most vulnerable to mitochondrial damage. There have been numerous reports of associations between mitochondrial damage and diverse neurodegenerative diseases including Parkinson's disease (PD) and Alzheimer's disease (AD). Such associations are thought to be mediated by oxidative stress. Overproduction and accumulation of amyloid-β peptide (Aβ) is a hallmark pathologic feature of AD. Although $A\beta^{1-42}$ accumulates in the neuritic plaques in AD patients, it has been suggested that $A\beta^{25-35}$ is the toxic domain of this peptide. Recently, we demonstrated that microinjection of $A\beta^{25-35}$ into the rat hippocampus produced neurotoxicity mediated by the receptor for advanced glycation end products (RAGE). It has been demonstrated that RAGE interacts with Aβ and mediates Aβ transport across the blood-brain barrier (BBB), contributing to the deposition of Aβ in the brain. However, the molecular mechanisms behind the Aβ-RAGE interacts that underlie such transport is poorly understood. In order to determine whether the toxicity associated with $A\beta^{25-35}$-RAGE interactions involves tight junction disruptions in the BBB, a primary culture of rat brain microvessel endothelial cells (rBMECs) was prepared by enzymatic digestion and differential centrifugation as an *in vitro* model of the BBB. Confluent rBMEC monolayers (10-14 days) were treated with $A\beta^{25-35}$ or $A\beta^{35-25}$ (as control) at 20 μM for 24 hours. Changes in RAGE, occludin (OC), and claudin-5 (C-5) expression were determined utilizing Western blot techniques. Cytotoxicity was evaluated using an XTT assay and Mitotracker for assessment of mitochondrial function, detection of DCFH-DA to measure ROS production (oxidative stress), and an LDH assay for membrane integrity. Trans epithelial electric resistance (TEER) and fluorescein flux were used as indicators of rBMEC permeability. $A\beta^{25-35}$ treatment resulted in an increase in RAGE, OC, and C-5 expression, indicating disruption of tight junctions. Significantly decreased mitochondrial function and TEER, as well as increased oxidative stress and fluorescein flux were also observed. These data suggest that toxicity associated with the $A\beta^{25-35}$-RAGE interaction is mediated by oxidative stress, which ultimately leads to cell death and disruption of BBB integrity, thereby increasing the permeability of the BBB and decreasing its protective function.

This compromised functionality of the BBB warrants further studies using markers of $A\beta^{25-35}$ and RAGE to further elucidate their interaction and involvement in neurodegenerative diseases.

Disclosures: **S.M. Lantz-McPeak:** None. **E. Cuevas:** None. **H.R. Hernandez:** None. **M.G. Paule:** None. **S.F. Ali:** None. **S.Z. Imam:** None.

Poster

042. Beta Amyloid Toxicity

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 42.10/D56

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Role of Adiponectin in the pathogenesis of Alzheimer's disease

Authors: *U. YUN, J. JEONG, U. YUN, Y. CHOI;
PHARMACY, SUNGKYUNKWAN UNIVERSITY, SUWON, Korea, Republic of

Abstract: Extensive epidemiological studies have shown that unexplained weigh loss is associated with the Alzheimer's disease but the mechanism linking AD and weight loss remains obscure. Currently, possibilities that hormones secreted from adipocytes may have roles in the development of Alzheimer's disease have suggested in several studies. The adipocyte-derived bioactive protein, adiponectin, is specifically expressed in adipose tissue. Adiponectin has attracted attention in recent years as therapeutic target for the metabolic syndrome. A recent finding revealing the correlation of elevated adiponectin levels in plasma and CSF to AD patients provides evidence suggesting adiponectin as a clue for linking AD. Adiponectin receptor 1 (ADR1) and adiponectin receptor 2 (ADR2) are the major receptors for adiponectin and can be activated by all forms of adiponectin found in the circulation. In the brain, ADR1 and 2 are expressed in the arcuate and the paraventricular nuclei of the hypothalamus, where they regulate feeding behaviors and cerebral cortical neurons. However, functions of adiponectin and ADRs in Alzheimer's disease are still poorly understood. In this study, we showed that adiponectin expression was consistently and significantly increased in the brains of Alzheimer's disease mouse model. It suggests that further studies of the functions of adiponectin signaling in Aβ metabolism and toxicity are warranted

Disclosures: **U. Yun:** None. **J. Jeong:** None. **U. Yun:** None. **Y. Choi:** None.

Poster

042. Beta Amyloid Toxicity

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Program#/Poster#: 42.11/D57

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Fondecyt 1100502

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Conicyt

Title: Differential membrane toxicity of Aβ fragments by pore forming mechanisms

Authors: *C. M. PETERS[1], E. FERNÁNDEZ-PÉREZ[1], D. BASCUÑÁN[1], M. ESPINOZA[1], C. OPAZO[2], L. G. AGUAYO[1];
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Abstract: Introduction. Alzheimer´s disease (AD) is a progressive neurodegenerative pathology that affects the human brain and causes cognitive and behavioral disorders. A major characteristic of AD is the presence of β-amyloid peptide (Aβ) oligomers in the brain. We have previously shown that Aβ oligomers (Aβo) associate with the neuronal membrane and induce the formation of perforations, causing an influx of calcium ions and increasing the release of synaptic vesicles that leads to a delayed synaptic failure produced by vesicle depletion. Several studies suggest that Aβ fragments, produced in the brain by enzymatic processing, can produce toxic effects similar to those of Aβ42. Here, we evaluated three Aβ fragments from different regions of Aβ; Aβ1-28 from the N-terminal region, Aβ25-35 from the central region and Aβ17-42 from the C-terminal region. Materials and methods. With the aim of determining the role of the different regions of Aβ on neurotoxicity, we performed a series of experiments to evaluate the toxicity of Aβ fragments. We used patch clamp, immunofluorescence, western blot, calcium imaging and viability assays. Results. Our results show that Aβ readily associated to the plasma membrane of several cell types such as CA1 hippocampal neurons, primary hippocampal cultures and HEK cells after 1 h of incubation. Moreover, Aβ changed the normal membrane conductance inducing the formation of pore-like structures. The latter produce a delayed synaptic failure. Although all the tested fragments of Aβ were found to produce toxicity when we used MTT viability assays, only Aβ25-35 was able to induce membrane perforation. Moreover, Aβ25-35 increased the intracellular calcium levels in acute applications, observing similar results with Aβ42 and Aβ1-28, but not with Aβ17-42. Conclusion. We conclude that Aβ fragments produced

toxic effects similar to those observed with Aβ42. The results suggest that the center region of Aβ (25-35) is important for membrane perforation and calcium increase; the N-terminal region (1-28) contributes to the increase in intracellular calcium, but not in the membrane perforation, likely by interacting with calcium receptors; and the C-terminal region, represented with the fragment produced by the α-secretase (17-42), induced mitochondrial toxicity, but not membrane perforation and calcium increase, supporting the idea of less toxicity in the non-amyloidogenic pathway.

Disclosures: **C.M. Peters:** None. **E. Fernández-Pérez:** None. **D. Bascuñán:** None. **M. Espinoza:** None. **L.G. Aguayo:** None. **C. Opazo:** None.

Poster

042. Beta Amyloid Toxicity

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 42.12/D58

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: The Ministry of Health, Labor and Welfare (Research on Nanotechnical Medical: No.H21-007)

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New Energy and Industrial Technology Development Organization (Translational Research Promotion Project)

Title: Calcium-mediated neurotoxicity of high-mass amyloid-beta assembly, amylospheroids (ASPD) to mature hippocampal neurons through activation of voltage-gated calcium channels

Authors: ***T. OHNISHI**[1,2], T. SASAHARA[1,2], H. KOMURA[1,2], Y. ARAI[1,2], T. NISHIYAMA[1,3], M. HOSHI[1,3];
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Abstract: Amyloid β protein (Aβ) plays a central role in pathogenesis of Alzheimer disease (AD). Recently nonfibrillar Aβ assemblies (termed oligomers), varying in size from dimers to multimers, are considered to play more proximal roles in AD. To elucidate the molecular identities of pathogenic Aβ oligomers and their neuronal targets leading to neurodegeneration in AD, we have previously isolated highly toxic spherical Aβ assemblies termed "amylospheroids" (ASPD) from AD patients' brains (Hoshi M et al. PNAS2003, Noguchi A et al. JBC2009). Patient-derived ASPD were toxic to human mature neurons and their amount in AD susceptible regions well correlated with pathological severity of AD. Furthermore, we found that ASPD bind to mature neurons specifically and cause severe neurodegeneration. In this study, as a first step to dissect the molecular mechanism of ASPD neurotoxicity, we examined whether ASPD exposure to mature neurons affected their intracellular free calcium level ($[Ca^{2+}]i$). Measurement of $[Ca^{2+}]i$ responses to ASPD showed that $[Ca^{2+}]i$ responses were enhanced in mature neurons treated with ASPD. The addition of EGTA, calcium chelator, abolished the progressive elevation of $[Ca^{2+}]i$ caused by the ASPD treatment and BAPTA-AM, $[Ca^{2+}]i$ chelator, blocked ASPD neurotoxicity. Taken together, ASPD neurotoxicity requires Ca^{2+} influx. Further studies using inhibitors against Ca^{2+} related channels, we found that N-type voltage gated calcium channels (VGCC) are involved in ASPD neurotoxicity. Interestingly, the abnormal Ca^{2+} overload induced by ASPD activated two tau protein kinase, CDK5 and GSK3β, and increased the phosphorylation of tau. ASPD binding to mature neurons is likely to cause such VGCC activation and eventual tau phosphorylation. However, whether or not ASPD directly bind to VGCC has remained to be elucidated. Therefore, we are currently searching for ASPD target protein(s) present on the mature neuronal surface, which are responsible for the above Ca^{2+}-mediated neurotoxicity of ASPD.

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Poster

042. Beta Amyloid Toxicity

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant R37-DK27083

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Swedish Research Council (VR) Internationell Postdok Grant DRN 637-2013-503

Title: Alzheimer's amyloid degradation by secreted lysosomal enzymes

Authors: *S. SOLÉ-DOMÈNECH[1], D. L. WAKEFIELD[2], E. CAPETILLO-ZARATE[1], D. CRUZ[1], B. A. BAIRD[2], F. R. MAXFIELD[1];
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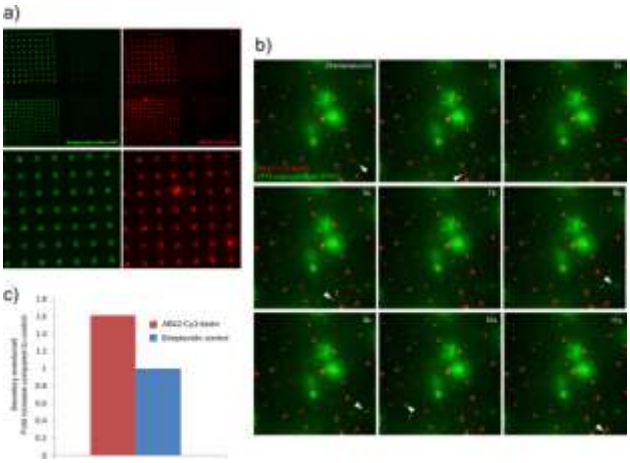
Abstract: In Alzheimer's disease (AD), some brain regions associated with neurodegeneration present abundant amyloid-beta (Aβ) fibrillogenesis. It is unclear how microglia or macrophages in the brain can proteolytically degrade Aβ fibrils (fAβ) and plaques that are significantly larger than these cells. A new possible mechanism is suggested by an earlier study published by our lab in which it was found that macrophages could degrade very large aggregates of LDL by creating an extracellular, acidic compartment that we called the "lysosomal synapse" into which lysosomal contents are secreted (Haka et al. 2009). To study the interaction of microglia and macrophages with fAβ, and in collaboration with the Cornell NanoScale Science & Technology Facility (CNF), we fabricate surfaces to present spatially defined fluorescently labeled streptavidin on glass cover slips which are thereafter incubated with Aβ-Cy3-biotin fibrils of 100-200nm in length. Biotinylated fAβ is captured by the multivalent streptavidin, thus immobilizing fAβ onto the glass surface (Fig. A). Macrophages and microglia, for which their lysosomes were previously labeled with FITC-dextran, are subsequently incubated on the fAβ-coated micro-pattern and imaged using Total Internal Reflection Fluorescence Microscopy (TIRFM). TIRFM reveals rapid FITC flashes (indicated by white arrowheads in Fig. B) at the pattern surface which are indicative of lysosomal content release. As FITC exits the lysosomes, it undergoes fluorescence increase associated with the transition from the acidic lysosomal environment to the more alkaline pH of the extracellular environment. Measurements from a reduced number of samples indicate that macrophages incubated on fAβ-coated surfaces present higher secretory activity when compared to those exposed to streptavidin-coated surfaces (Fig. C). The proposed studies are aimed at exploring new ground for treatments based on the use of therapeutic agents to increase lysosomal activity. These could potentially lead to an enhancement in microglia fAβ lysosomal degradation and clearance.



Disclosures: **S. Solé-Domènech:** None. **D.L. Wakefield:** None. **E. Capetillo-Zarate:** None. **D. Cruz:** None. **B.A. Baird:** None. **F.R. Maxfield:** None.

Poster

042. Beta Amyloid Toxicity

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 42.14/D60

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: CIHR ROP 108435

RDC contract 5404.1139.102

Title: Treatment of neurons with coconut oil and constituent fatty acids attenuates the effects of amyloid beta *in vitro*

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[1]Div. Biomed. Sci., Mem. Univ. Newfoundland, St John's, NL, Canada; [2]Div. of BioMedical Sci., Mem. Univ. of Newfoundland, St. John's, NL, Canada

Abstract: Dietary supplementation has been studied as an approach to ameliorating deficits associated with aging and neurodegeneration. In particular, recent anecdotal evidence has touted the use of coconut oil as having major benefits in lessening the cognitive deficits associated with Alzheimer's disease. Coconut oil has a high percentage of medium chain triglycerides (MCTs, approx. 70% of C:6-12), with caprylic and capric acid comprising ~ 21% of these MCTs in

virgin coconut oil. While there is rather limited scientific evidence that such treatment would have a significant effect on disease, there is scientific basis behind the use of MCTs, such as those found in coconut oils. MCTs can be rapidly metabolized to induce metabolic ketosis and ketone bodies can then be used as an alternative to glucose for energy requirements. We recently reported that treatment of cultured cortical neurons with coconut oil could rescue the cells from the deleterious effects of Aβ exposure. In the current study we extend our investigation to include coconut oil and its major constitutent fatty acids caprylic/octanoic (C8) acid and lauric (C12) acid, as well as an examination of treatment timing and duration in cultures of neonatal cortical and hippocampal neurons. Cultures of cortical or hippocampal neurons were exposed to a variety of treatment paradigms. In initial experiments, cultures were concomitantly treated with Aβ and oil/FAs; subsequently we investigated treatment with Aβ for 1, 6 or 24 hrs followed by addition of oil/FAs for a further 24 hrs. Neuronal survival was assessed using standard survival assays, while confocal microscopy was employed to analyze mitochondrial parameters, cleaved caspase3 labelling, and axonal and dendritic morphology. Our preliminary results suggest that the complete oil is more effective than the individual FAs in ameliorating the toxic effects of Aβ on the neurons, based on survival assay and cleaved caspase3 labelling. In terms of the treatment timing, preliminary data suggest that the oil can rescue cells pre-exposed to Aβ for 1 or 6 hrs, but is much less effective when the pre-exposure has been 24 hrs. Continuing work is focused on mechanisms that may be involved in this protective influence.

Disclosures: **K.M. Mearow:** None. **F. Nafar:** None.

Poster

042. Beta Amyloid Toxicity

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 42.15/D61

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant AG042804

Title: Novel microRNAs regulate specific neuronal genes important for Alzheimer's Disease

Authors: ***N. CHOPRA**[1,2], J. M. LONG[3], P. T. NELSON[4], P. REDDY[5], R. VASSAR[6], N. H. GREIG[7], D. K. LAHIRI[2];
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Cell and Mol. biology, The Feinberg Sch. of Med., Chicago, IL; [7]Translational gerontology branch, Natl. institute of aging, Baltimore, MD

Abstract: Alzheimer's disease (AD) is characterized by the presence of amyloid-β (Aβ) peptide plaques and believed to result from the misregulation of the production or clearance of Aβ. The rate-limiting step in the generation of Aβ is the processing of Aβ precursor protein (APP) by β-site APP-cleaving enzyme (BACE1). Elucidating how expression of these proteins is regulated will ultimately reveal new drug targets. Our aim is to utilize novel approaches to define the regulation of these gene products by microRNAs (miRNAs). MiRNAs are an abundant class of small RNAs that mediate potent inhibitory effects on global gene expression. Using multiple bioinformatic tools and a series of functional studies in neuronal and glial cultures, we previously reported specific microRNA species that regulate levels of APP and BACE1. Herein we present data demonstrating miR20b and miR-298 mediated regulation of APP alone or with BACE1. First, we prepared chimeric APP 3'-UTR and BACE1 3'-UTR reporter constructs downstream of a reporter *Renilla* luciferase gene and then delivered the reporter construct along with several miRNAs predicted to target the APP and BACE1 3'-UTR into human astroglial U373 cells. Several "hits" (e.g. miR20b and miR-298) resulted in reduced reporter expression. We further validated the reporter expression data for miR-298 (and miR-339-5p) by Western analysis of native BACE1 levels, which were significantly reduced following their transient delivery, with a potential to lower toxic soluble Aβ levels. Moreover, miR-20b or miR-298 delivery significantly reduced native APP levels in these human cells. Thus, miRNA-298 targets both APP and BACE1 expression. Our results reveal a novel regulatory interaction between two important AD-related genes (APP and BACE1) and specific endogenously expressed miRNA species, represented by miR-298. Our ongoing studies evaluating the expression of miR-298 in brain tissue specimens from two patient cohorts may further unveil its role in AD. Additional experiments suggest that miR-20b has another target, specifically voltage-gated calcium channels, within AD pathology. Summarizing, we show that two miRNAs, namely miR-298 and miR-20b, are able to affect AD pathology by targeting more than one gene. These regulatory interactions likely serve as novel therapeutic targets and may enable the development of treatment strategies beneficial in the fight against AD. This work is supported by grants from the Alzheimer's Association and NIH.

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Poster

042. Beta Amyloid Toxicity

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 42.16/D62

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Aβ-induced synaptic loss requires glutamate binding to NMDA receptors but not ion-flux

Authors: *C. TACKENBERG, J. BIRNBAUM, R. M. NITSCH;
Univ. of Zurich, Schlieren, Switzerland

Abstract: Aggregates of beta-amyloid (Aβ) and tau represent the major histopathological hallmarks of Alzheimer's disease (AD). AD patients suffer from cognitive impairments which are caused by massive neuronal brain atrophy and synaptic loss. The reduction in synapse numbers is the best neuropathological correlate to the degree of dementia in AD. N-methyl-D-aspartate receptors (NMDARs) have been shown to mediate downstream effects of Aβ. NMDARs can trigger intracellular cascades via calcium entry, however also calcium-independent (metabotropic) functions of NMDARs have been described. We found that endogenous Aβ as well as exogenously added synthetic Aβ oligomers induced dendritic spine loss and reductions in pre- and postsynaptic protein levels in slice cultures. Synaptic alterations were mitigated by blocking glutamate-binding to NMDARs using NMDAR-antagonist APV but not by preventing ion-flux with calcium-chelator BAPTA or open channel blocker MK-801 or memantine. Synapse loss involved activation of p38 MAPK, which has been implicated in metabotropic NMDAR functions. Our data suggest that Aβ-induced synaptic loss depends on metabotropic-like mechanisms caused by glutamate binding to NMDARs but does not require calcium-influx.

Disclosures: **C. Tackenberg:** None. **J. Birnbaum:** None. **R.M. Nitsch:** None.

Poster

042. Beta Amyloid Toxicity

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Program#/Poster#: 42.17/D63

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Swedish Brain Fund (Hjärnfonden) postdoc grant

Title: beta-Amyloid acutely changes astrocytic energy metabolism and glucose utilisation

Authors: *M. Y. ZILBERTER[1], R. VALDEBENITO[3], A. FISAHN[2], F. BARROS[3]; [2]Alzheimer's Dis. Res. Ctr., [1]Karolinska Institutet, Stockholm, Sweden; [3]Ctr. de Estudios Científicos, Valdivia, Chile

Abstract: Alzheimer's disease (AD) disrupts brain glucose utilization as its earliest clinical manifestation, preceding any cognitive impairment. We have previously reported that AD's hallmark toxic peptide amyloid beta (Aβ(1-42)) induces an imbalance in neuronal energy metabolism with downstream disruption of basic neuronal electrogenic properties, leading to network hyperactivity. Here we report that Aβ also perturbs astrocytic cytosolic glucolysis as well as mitochondrial respiration, evident as modified astrocytic NAD(P)H and FAD autofluorescence transients during hippocampal network activation. This disruption is underlain by Aβ's acute effect onto astrocytic glucose metabolism, seen as changes in both glucose uptake and glucolysis in cultured astrocytes. As astrocytes are crucial for ensuring proper energy supply to neurons, such a fundamental disruption is potentially a key part of AD effect on overall energy homeostasis and would explain our earlier finding that the AD model mice display up to 50% reduction in brain glycogen levels. Our results highlight the early, multi-faceted effect of AD pathology on brain energy metabolism across cell types.

Disclosures: **M.Y. Zilberter:** None. **R. Valdebenito:** None. **F. Barros:** None. **A. Fisahn:** None.

Poster

042. Beta Amyloid Toxicity

Location: Halls A-C

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: CNPq

INNT

FAPERJ

CAPES

Title: Loss of choline acetyltransferase activity caused by oxidative damage: Mechanisms and possible implications for Alzheimer's disease

Authors: *L. E. SANTOS[1], C. FIGUEIREDO-FREITAS[2], N. NUNES-TAVARES[1], S. T. FERREIRA[1], F. G. DE MELLO[1];
[1]Inst. de Biofísica Carlos Chagas Filho, [2]Inst. de Bioquímica Médica Leopoldo De Meis, UFRJ, Rio De Janeiro, Brazil

Abstract: Acetylcholine is a major neurotransmitter in both central and peripheral nervous systems, normally synthesized near pre-synaptic terminals in a reaction involving the transfer of an acetyl group from acetyl-coenzyme A to choline. This reaction is carried out by an enzyme known as choline acetyltransferase (ChAT, EC 2.3.1.6) [J. Neurophysiol. 1943; 6, 7], which is also regarded as a marker of cholinergic neurons. Disturbances of cholinergic neurotransmission have long been implicated in the development of several central nervous system (CNS) pathologies. Among them, Alzheimer's disease (AD) - an alarmingly prevalent form of dementia - stands out for having one of the most well established cholinergic hypothesis, first presented formally in the early 1980s [Science 1982; 217:408-414]. In 2001, using chick retina as a CNS model, our group showed that ChAT activity in cultured or *ex vivo* neurons can be markedly and quite specifically down-regulated by excitotoxic stimuli, such as excitatory amino-acid (EAA) treatments, long before any significant changes in cell viability or enzyme levels occur. This effect was shown to be dependent on calcium influx and at least partially on nitric oxide (NO) production [J Neurochem. 2001; 77:1136-1144]. More recently, we extended these results to a more specific context, and observed a similar loss of ChAT activity after treating cultured cholinergic neurons with oligomeric forms of the amyloid-β peptide (AβOs). These small oligomers are diffusible toxins found in AD brains and are currently regarded as possible culprits of the disease [JBC 2012; 287:19377-19385]. In the current work, we address whether nitrative or nitrosative stress-induced modifications are involved in this loss of ChAT activity. Several free-radical donors and thiol reagents are shown to directly inactivate the enzyme with efficiency dependent on the type of reaction or reactive species produced. Moreover, using S-nitrosothiol resin-assisted capture (SNO-RAC), 4-acetamido-4'-maleimidylstilbene-2,2'- disulfonic acid (AMS) labeling of reduced thiols and immunodetection with nitrotyrosine antibodies we find that treatments with EAAs, AβOs or NO donors change the oxidation pattern of ChAT molecules in cysteine and tyrosine residues. We also observe that such modifications correlate well with the loss of enzyme activity. These results suggest a novel mechanism for cholinergic deficit that precedes neuronal death and may be relevant in early-stage AD pathology.

Disclosures: L.E. Santos: None. **C. Figueiredo-Freitas:** None. **N. Nunes-Tavares:** None. **S.T. Ferreira:** None. **F.G. de Mello:** None.

Poster

042. Beta Amyloid Toxicity

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH (OADC) 5 P30 AG008017 24

NIH NCCAM T32 AT002688

NIH 3P30- AG008017 24 S1

Department of Veteran's Affairs Merit Review Grant

Title: Centella asiatica protects against oxidative damage and mitochondrial dysfunction

Authors: *N. E. GRAY[1], C. J. HARRIS[2], A. SOUMYANATH[2], J. F. QUINN[2,3];
[1]Oregon Hlth. and Sci. Univ., Portland, OR; [2]Neurol., Oregon Hlth. & Sci. Univ., Portland, OR; [3]Dept. of Neurol. and Parkinson's Dis. Res. Educ. and Clin. Care Ctr. (PADRECC, Portland Veteran's Affairs Med. Ctr., Portland, OR

Abstract: Background: The plant *Centella asiatica* is used in traditional Chinese and Ayurvedic medicine to enhance cognition. We have previously shown that treatment with a water extract of *Centella asiatica* (CAW) attenuates learning and memory deficits in a mouse model of Aβ accumulation, and prevents cell death in *in vitro* models of Aβ toxicity. Yet the mechanism by which CAW exerts its neuroprotective effects has yet to be elucidated. In these studies we evaluated the effects of CAW on antioxidant response and mitochondrial function in response to Aβ *in vitro,* and the *in vivo* effects of CAW in healthy, aged animals. **Mechanism:** Using two neuroblastoma models of Aβ toxicity (MC65 and SH-SY5Y cells) we determined intracellular levels of reactive oxygen species (ROS) and the expression of the antioxidant response element (ARE) containing gene NRF2 and its target genes. We also measured expression of a several genes encoding protein components of the electron transport chain and intracellular ATP as well as mitochondrial respiratory flux, using the Seahorse XF Analyzer. Because mitochondrial dysfunction is also observed during normal aging, we investigated the effects of CAW on 20 month old wild type (WT) mice. Following two weeks of treatment with CAW, we analyzed learning and memory using the Morris Water Maze and measured the expression of ARE and mitochondrial genes in the brain. **Results:** CAW reduced intracellular ROS generated in response to Aβ and induced the expression of the ARE genes in both cell types. CAW also prevented the decrease in ATP in response to Aβ and induced the expression mitochondrial genes. Additionally CAW increased both basal and maximal mitochondrial respiration and attenuated the decrease in respiration observed in response to Aβ but had no effect on the spare respiratory capacity of mitochondria in those cells. CAW treatment significantly improved memory in aged WT mice an effect that persisted several days beyond the final trial. There was also a robust increase in the expression of mitochondrial and ARE genes in

both the hippocampus and cortex of CAW-treated animals. **Discussion:** Our data demonstrate that CAW can prevent Aβ-induced alterations ROS levels and ATP production. The data also show that CAW increases mitochondrial respiration but not the overall spare capacity of mitochondria *in vitro*. These data, together with the increase in mitochondrial gene expression in response to CAW, suggest that CAW may be inducing mitochondrial biogenesis. The experiments in WT mice support this hypothesis and show that the beneficial effects of CAW are not limited to Aβ toxicity but rather may be very broadly relevant in other contexts of mitochondrial dysfunction.

Disclosures: **N.E. Gray:** None. **C.J. Harris:** None. **A. Soumyanath:** None. **J.F. Quinn:** None.

Poster

042. Beta Amyloid Toxicity

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 42.20/D66

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Different segments of Amyloid-β drive distinct steps of toxicity

Authors: *A. K. DAS, R. PANDIT, B. CHANDRA, B. SARKAR, M. CHANDRAKESAN, S. MAITI;
Tata Inst. Fundamental Res., Mumbai, India

Abstract: Oligomers of the Amyloid-β (Aβ) peptide, but not the monomers or the mature fibrils, are thought to be the key toxic species in Alzheimer's disease. We have earlier shown that small Aβ oligomers (n-mers with n<10) display much higher membrane affinity than the monomers [1], and this is correlated with the emergence of a fibril-like tertiary fold in the structure [2]. Our recent structural study shows that the conformation of Aβ40 oligomers differs from the fibrils specifically at the turn (residues 22-29) and the N terminus (residues 1-10) regions [3]. Here we ask if these specific segments drive distinct steps leading to toxicity. We know that the core part of Aβ (Aβ18-35, which contains the turn region) folds similar to the full length peptide [4], so its properties can be studied independently. We find that the enhancement of membrane affinity is driven by this core region, but it lacks toxicity. Even a much longer fragment (Aβ10-40) shows similar properties. Interestingly, the N terminal fragment (Aβ1-9) by itself cannot initiate toxicity and neither can it bind to neuronal membranes. We create a minimal sandwich peptide comprising of just the core and the N-terminus (Aβ1-9/18-35) and find that while it has high

membrane affinity, it is still non-toxic. Thus interaction of Aβ1-9 with the rest of the peptide, or with another cellular component, enabled in turn by the right length of Aβ, is necessary for toxicity. Next, we find that a D enantiomer variant of Aβ1-9, attached to Aβ10-40, is also toxic. This suggests that N terminus is not involved in any stereospecific interactions (e.g. with receptors). It is likely that the N terminus non-stereo-specifically controls a critical post-attachment event, such as assembly formation on the membrane, internalization or vesicle dynamics. We are utilizing membrane diffusion measurements and fast quantitative multiphoton imaging of serotonergic vesicles [5] to probe these events. References [1] Sarkar, B. et al., Front. Physiol. 2013, 4(84), 1 [2] Nag, S. et al., Phys.Chem.Chem.Phys. 2013, 15, 19129 [3] Sarkar, B. et al., Angew.Chem.Int.Ed 2014, doi:10.1002/anie201 [4] Chandrakesan, M. et al., Chemical Physics. 2013, 422, 80 [5] Sarkar, B. et al., Front. Physiol. 2012, 3(414), 1

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Poster

042. Beta Amyloid Toxicity

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Alzheimer's Association Grant NIRG 10-173589

Mary E. Groff Surgical Medical Research Grant

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Title: Elevated Intracellular Adhesion Molecule (ICAM1) levels are associated with lower levels of amyloid-beta in white matter in Alzheimer's disease

Authors: E. Y. GRIFFITH[1], L. E. COLLINS[1,4], Y. FRANCIS[1], A. WIEGMAN[1], J. URBACH[1], A. LAWTON[1], L. S. HONIG[1,2], E. CORTES[3], J. VONSATTEL[3], P. CANOLL[3], J. E.

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Abstract: Alzheimer's disease (AD) is a neurodegenerative disease, characterized pathologically by extracellular amyloid-beta (Aβ) plaques and intracellular neurofibrillary tangles. The "amyloid cascade hypothesis" proposes that aggregation of Aβ42 peptides into oligomers and plaques triggers a cascade of pathological events, leading to widespread neurodegeneration. There is increased interest in the role of inflammation in this process. Chronic inflammation may affect mechanisms of amyloid deposition and/or clearance, but many findings are contradictory. Such investigations may be particularly relevant in white matter, where local effects of toxic Aβ peptides have been proposed as a potential mechanism of primary white matter damage. Previously, we showed that soluble forms of Aβ40 and Aβ42 are higher in hemispheric white matter tissue of patients with AD. The current study explored cytokine levels in white matter tissue of AD patients and non-AD controls, and examined their association with soluble Aβ40 and Aβ42 measured in the same regions. We also examined white matter microglial activation and its association with myelin integrity. Fresh frozen post-mortem tissue was sampled from white matter in the prefrontal center semi-ovale and the parieto-occipital lobe at the level of the atrium from brains of AD patients (n=12) and non-AD controls (n=10). ELISA was used to measure soluble Aβ40 and Aβ42 levels. To measure quantitatively the levels of 40 cytokines, tissue from the same regions was analyzed with a Quantibody® array (Ray Biotech). To investigate microglial activation, we took mirror image samples from the contralateral formalin fixed hemisphere of each subject and rated the tissue on a four-point scale on CD68 stained slides. Myelin pathology was quantified from slides stained with Luxol fast blue. Microglial activation did not differ between AD patients and controls, but microglial activation was associated with loss of myelin integrity. Cytokine levels were similar in AD patients and controls, except for Intercellular Adhesion Molecule 1 (ICAM-1), which was higher in the AD group. Stratified by diagnosis, ICAM-1 levels correlated negatively with Aβ42 levels in anterior and posterior regions among AD patients, but not among controls. Previous work suggests that ICAM-1 induces expression of the amyloid-degrading enzyme neprilysin in microglia, which could account for the observed relationship between ICAM-1 and Aβ42. Taken together, these exploratory analyses highlight the complexity of the inflammatory response in white matter tissue in AD, which could have critical importance for the development of novel treatments for the disease.

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Poster

042. Beta Amyloid Toxicity

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 42.22/D68

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Alzheimer's Association (NIRG 10-173589)

Mary E. Groff Surgical Medical Research and Education Charitable Trust

Taub Institute of Columbia University, and NIH (AG034189, AG008702, AG037212)

Title: Soluble amyloid beta levels are elevated in the white matter of Alzheimer patients, independent of cortical plaque burden

Authors: *L. E. COLLINS[1,2], Y. FRANCIS[2], A. WIEGMAN[2], E. Y. GRIFFITH[2], J. URBACH[2], A. LAWTON[2], L. S. HONIG[2,3], E. CORTES[4], J. P. VONSATTEL[4], P. CANOLL[4], J. E. GOLDMAN[2,4], A. M. BRICKMAN[2,3];
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Abstract: Alzheimer's disease (AD) is the most common neurodegenerative disease and the leading cause of dementia. In recent years, in addition to grey matter pathology, white matter changes have come to be recognized as an important pathological feature in the emergence of the disease. Multiple studies suggest that there is axonal damage and loss, demyelination, death of oligodendrocytes, gliosis and other pathological white matter changes in the brains of AD patients. Despite the growing recognition of the potential importance of white matter abnormalities in the pathogenesis of AD, the pathological basis of white matter degradation remains to be elucidated. While multiple studies have attributed this white matter degeneration to concomitant small vessel disease or Wallerian-like degeneration, others suggest that primary white matter pathology may be due, at least in part, to other mechanisms, including local effects of toxic Aβ peptides. The current study investigated levels of soluble amyloid-beta (Aβ) in white matter of AD patients. Fresh frozen tissue was sampled from anterior and posterior regions of cerebral white matter from post-mortem brains of AD patients (n= 12) and non-AD controls (n=10). ELISA was used to examine levels of both Aβ40 and Aβ42. Compared with controls, AD white matter samples had higher levels of both Aβ40 and Aβ42. While no regional white matter differences were found in Aβ40 levels, Aβ42 levels were higher in anterior regions than in posterior regions across both groups. After statistically controlling for total cortical plaque

load in the analysis, differences in soluble Aβ40 and Aβ42 between the groups remained, suggesting that white matter Aβ peptides accumulate independent of overall grey matter fibrillar amyloid pathology. Taken together, the results of the current study suggest potential pathological mechanisms that may contribute to white matter degeneration in AD. Given that white matter degeneration may be an early marker of disease, preceding grey matter atrophy, understanding the mechanisms and risk factors that may lead to white matter loss could help to identify those at high risk and to intervene earlier in the pathogenic process.

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Poster

042. Beta Amyloid Toxicity

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 42.23/D69

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: FIRB

Title: LRP1 activates ERK1/2 in lipid rafts in neuron-like cells

Authors: *E. MANTUANO[1,2], V. MATTEI[3], V. TASCIOTTI[3], V. MANGANELLI[2], S. MARTELLUCCI[3], F. SANTILLI[3], T. GAROFALO[2], M. SORICE[2], S. L. GONIAS[1], R. MISASI[2];
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Abstract: The cellular form of prion protein (PrPc) is a highly conserved cell surface GPI-anchored glycoprotein that was identified in cholesterol-enriched, detergent-resistant microdomains ('rafts') in neural and non-neural cells. Several physiological functions have been described for PrPC, including oxidative stress defense, metal ion homeostasis in the brain, and neuroprotection. Aβ oligomers, but not monomers or fibrils, bind tightly to PrPc (Kd ~ 0.4 nM) and, in hippocampal slices, PrPc PrPc is required for Aβ oligomer-mediated inhibition of long-term potentiation. Our results suggest that the activity of PrPc-Aβ requires lipid rafts and the transmembrane receptor, low-density lipoprotein receptor-related protein (LRP1). LRP1 functions as an endocytic receptor for a broad range of structurally and functionally diverse

ligands. LRP1 also functions in cell signaling, directly, in response to ligand-binding, and indirectly, by regulating levels of other signaling receptors. In neurons and neurite-generating cell lines, ligands control the signaling activity of LRP1 by directing the co-receptors that are recruited into a functional signaling complex with LRP1. LRP1 is also known to control surface and biosynthetic trafficking of PrPc in neurons. Our results show that PrPc is strictly associated with gangliosides in lipid rafts in neuroblastoma cells. Scanning confocal microscopy analysis revealed co-localization of PrPc with GM1, as well as TrkA with GM1, indicating the existence of a glycosphingolipid-enriched molecular complex. In order to analyze the mechanism by which recombinant PrPc initiates cell signaling in SK-N-BE2 and PC12 neuron-like cells, we examined ERK1/2 phosphorylation. ERK1/2 was robustly phosphorylated in cells that were treated with recombinant PrPc. ERK1/2 activation required LRP1 and was strictly dependent on the integrity of rafts. ERK1/2 activation was blocked by altering sphingolipid metabolism with fumonisin B1 or by disruption of lipid microdomains with methyl-β-cyclodextrin. These findings support a model in which LRP1 initiates signal transduction specifically in lipid rafts, where it forms multimolecular complexes that may include PrPc, TrkA and gangliosides. Although association of LRP1 with lipid rafts has been reported before, this is the first study to demonstrate a potentially significant LRP1 activity that depends on its distribution between rafts, clathrin-coated pits and other membrane domains.

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Poster

042. Beta Amyloid Toxicity

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 42.24/D70

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Internationale Stichting Alzheimer Onderzoek (ISAO)

Title: Aβ plaque formation and impaired protein quality control interact via γ secretase and result in a behavioural phenotype consistent with Alzheimer’s disease

Authors: ***F. VAN LEEUWEN**[1], R. J. G. GENTIER[1], M. L. M. VERHEIJEN[1], H. W. M. STEINBUSCH[1], M. O. GRIMM[2], V. J. HAUPENTHAL[2], T. HARTMANN[2];

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Abstract: Aβ plaque formation is a prominent cellular hallmark of Alzheimer's disease (AD). To date, immunization trials in AD patients have not been effective in terms of curing or ameliorating dementia. In studies with transgenic animals (line #85; APP Swe PSEN1Δexon 9) it was shown that there is limited clearance of pre-existing amyloid plaques. Therefore, more knowledge about the mechanism and relevance of Aβ plaque formation is required before reconsidering trials. We discovered that misframed ubiquitin (UBB+1) is unable to ubiquitinate degradable targets and cannot be deubiquitinated, thereby dose dependently inhibiting the ubiquitin proteasome system (UPS). Thus results in ERAD dysfunction, stress of mitochondria, their neuritic clogging and a phenotype of impaired contextual behaviour (Dennissen et al., Prog. Neurobiol., 96, 190-207, 2012). Recently, pooled GWAS studies, pathway analysis and proteomics identified protein ubiquitination as one of the key modulators of AD and support our hypothesis of a dysfunctional UPS as a causative factor in AD (Manavalan et al., Exp. Mol. Med., 45, E39, 2013). For more than a decade a relation between a dysfunctional UPS and Aβ plaque formation has been surmised but never proven in detail. It is now possible to address this issue by crossbreeding 2 transgenic lines in a pure C57Bl6 genetic background (e.g., lines #3413 with postnatal UBB+1 over expression, proteasomal inhibition and a proteome partly compatible with AD, line #85 with Aβ plaque formation starting at 4 months of age) and their crossbreed (e.g., lines #3413 x #85, van Tijn et al., Neurochemistry International, 61, 739-748, 2012). The results revealed that in line 85 with the Swedish double mutation, γ secretase activity was specifically decreased significantly at 3, 6 and 9 months of age, whereas α and β secretase activities were unaffected. However, in the crossbreed with a dysfunctional UPS the γ secretase activity (and not those of α and β) was enhanced at the age of 6 months; a critical period where Aβ plaque generation is attenuated. In the crossbreed, nest building and contextual memory, as determined in the Morris water maze, are impaired. Our new data implicate that there is strong interaction between a failing protein quality control by the UPS and Aβ plaque formation being mediated specifically via γ secretase. Currently the multimeric γ secretase complex (e.g., nicastrin, PSEN1) is analysed. These results show a striking inverse correlation between γ secretase activities and Aβ plaque load and will contribute to a better understanding of strategies to ameliorate or cure AD, via γ secretase modulation.

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Poster

043. Alzheimer's Disease: Proteinopathy, Non- Abeta, and Non-Tau

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 43.01/D71

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: PO1AG014449

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Brinson Foundation

Title: Preservation of hippocampal mTOR/p62 autophagy signaling pathways in AD

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Abstract: The mammalian target of rapamycin (mTOR) and p62 play a role in the cascade of cellular events associated with tau phosphorylation, and endosomal-lysosomal (E-L) autophagy processes early in the onset of Alzheimer's disease (AD). Previously, we have shown a down-regulation of the early endosome Rab5 and an up-regulation of the late lysosomal hydrolase cathepsin D (Cat D) in the hippocampus of people who died with a premortem clinical diagnosis of mild cognitive impairment (MCI) compared to no cognitive impairment (NCI). However, whether levels of mTOR and p62 are dysregulated and their relation with Aβ and tau pathology during the progression of AD is unknown. Here, we quantified changes in hippocampal mTOR phosphorylated at serine 2448 (pmTORs2448) and serine 2481 (pmTORs2481), total mTOR, p62 and the novel human Aβ receptor leukocyte immunoglobulin-like receptor B2 (LilrB2) in tissue harvested from subjects with a premortem clinical diagnosis of NCI, MCI and mild/moderate AD within 12 months prior to death. All groups were matched by age and postmortem interval and underwent detailed postmortem neuropathologic evaluations. Western blot analysis of tissue homogenates revealed stable levels of phosphorylated mTOR proteins, total mTOR and p62 as well as LilrB2 across the three clinical groups examined. pmTORs2448, pmTORs2481 and total mTOR were positively correlated with each other, but not with p62. pmTORs2448 levels showed a weak negative association with increased levels of Cat D, but not Rab5. Conversely, p62 showed a strong positive association with total (soluble and insoluble) $A\beta_{1-42}$ and $A\beta_{1-40}$ levels, whereas LilrB2 was weakly correlated with both, $A\beta_{1-42}$ and $A\beta_{1-40}$. Surprisingly, only pmTORs2481 and total mTOR values were weakly correlated with global cognitive z-score, but not with MMSE or episodic memory z-score, or with the neurofibrillary tangle Braak staging. Collectively, our findings demonstrate that the downstream levels of

mTOR/p62 autophagy signaling molecules and LilrB2 amyloid receptor remain relatively unchanged in these cases, suggesting that these E-L signaling factors as well as the Aβ receptor LilrB2 may not play a key role in the initiation of hippocampal neuronal degeneration during the onset of AD.

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Poster

043. Alzheimer's Disease: Proteinopathy, Non- Abeta, and Non-Tau

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 43.02/D72

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: N-acetylglucosamine polymers impede synaptic plasticity, are neurotoxic and accumulate in sporadic alzheimer's disease Brains

Authors: *G. BUSETTO, M. FAVERO, E. TURANO, B. BONETTI;
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Abstract: For familial Alzheimer's disease (AD), a large body of evidence supports a causative role of β-amyloid (Aβ). Unfortunately this hypothesis does not fully explain the pathogenesis of sporadic AD, which accounts for the majority of AD cases. In fact, recent pharmacological treatments aimed at reducing the Aβ burden failed to obtain clinical benefits. It is known that, besides Aβ, other molecules are present in amyloid plaques of sporadic AD brains, including insoluble polymers of N-acetylglucosamine (GlcNAc, possible result of a metabolic shift of glucose utilization from glycolysis to the hexosamine pathway) of whom the potential role in the pathogenesis of AD remains to be elucidated. We found that the acute application of precursors of GlcNAc polymers (5 mM N-acetylglucosamine and 150 μM UDP- N-acetylglucosamine) to murine hippocampal slices does not affect basal synaptic transmission (measured as excitatory post synaptic field potentials [fEPSPs] and paired-pulse ratio), but reduces the expression of long term potentiation (LTP) of CA1 glutamatergic synapses, similarly to Aβ 25-35 (0.5 μM) (fEPSPs slope relative to baseline: control 165 ± 6%, n=11; experimental 131 ± 10%, n=10, p=0.01; Aβ 135 ±3%, n=6). This effect is mainly postsynaptic (no modification of paired-pulse ratio after LTP induction). We also found that cultured neurons (SH-SY5Y cell line and primary hippocampal neurons) and microglia (N9 cell line and primary microglia) synthesize polymers of GlcNAc if treated with polymers' precursors. Moreover, polymers' precursors have a specific

neurotoxic effect, reducing to 40% the survival of hippocampal cultured neurons after 48hrs, while leaving fibroblasts unperturbed. We further tested the effect of polymers' precursors on hippocampal slice cultures (up to 7 days) and found evidence of synaptic damage (reduction of synaptophysin and syntaxin with immunoblotting). Finally, polymers' precursors activate microglia to a same extent as Aβ (assessed with MTT assay). These data support the hypothesis that, in case of sporadic AD, polymers of GlcNAc and high doses of their precursors may cause both neuronal damage and microglia activation, thus contributing to the Aβ-derived neurotoxicity.

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Poster

043. Alzheimer's Disease: Proteinopathy, Non- Abeta, and Non-Tau

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 43.03/E1

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Konkuk University research support program (2014)

Title: Time-dependent alterations of MMP9 and ANG-2 in hippocampus of rat with chronic bilateral common carotid artery occlusion

Authors: M.-S. KIM[1], *C. CHUNG[1], W. JEON[2], J.-S. HAN[1];
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Abstract: Chronic cerebral hypoperfusion causes neurological dysfunction including cognitive impairment. Chronic decreased cerebral blood flow is associated with neuropathological changes of vascular dementia and Alzheimer's disease (AD). An experimental animal model of chronic cerebral hypoperfusion is the bilateral common carotid artery occlusion (BCCAo) using the rat. In the BCCAo model, it has demonstrated that not only neural damage and memory deficits are occurred, but also angiogenesis is promoted. Angiogenesis may help in neuronal reorganization following initial occlusion, and also associate with reduced neurological deficits in rats with chronic BCCAo. The present experiment was conducted to examine the time-dependent alterations in angiogenesis-related proteins and factors. We examined an alteration of angiogenesis-related proteins in the hippocampus of rats with chronic BCCAo. Male Wistar rats (12 weeks-old) were randomly divided into sham-operated group, BCCAo-group with1 week,

BCCAo-group with 4 weeks, and BCCAo-group with 8 weeks by the time elapsed after the BCCAo. We found out time-dependent alterations of the expression levels of angiogenesis-related proteins using western blotting. The proteins associated with angiogenesis, such as angiopoietin-2, were altered as time progresses after the BCCAo. In addition, we confirmed the cellular location of the angiogenesis-related proteins, which are located in neurons and astrocytes, using double-immunofluorescence staining. Altered microvascular density depended on the time elapsed after the BCCAo. Overall, our findings would provide a better understanding of angiogenic factors on cerebral angiogenesis and could serve as effective therapeutic strategies for chronic cerebral hypoperfusion-associated neurological disorders such vascular dementia and AD.

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Poster

043. Alzheimer's Disease: Proteinopathy, Non- Abeta, and Non-Tau

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 43.04/E2

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Consortium for Frontotemporal Dementia

Title: Selective progranulin deficiency in neurons produces frontotemporal dementia-like deficits

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Abstract: Frontotemporal dementia (FTD) is a progressive, fatal neurodegenerative disorder that is the second most common cause of dementia after Alzheimer's disease. Loss-of–function mutations in progranulin (*GRN*) that result in progranulin deficiency are a major genetic cause of FTD, producing 5-10% of all cases. Progranulin-deficient mice ($Grn^{+/-}$ and $Grn^{-/-}$) have been generated to model progranulin deficiency, and develop an FTD-like phenotype including abnormal social and emotional behavior and amygdala dysfunction by 6 months of age. $Grn^{-/-}$, but not $Grn^{+/-}$, mice also develop inflammation, gliosis and lipofuscinosis that increases with age. A key question for FTD with *GRN* mutations is how progranulin deficiency causes disease. Progranulin is a secreted glycoprotein that is expressed by both neurons and microglia in the brain, so loss of progranulin from either or both cell types could cause of FTD. We investigated

whether loss of progranulin from neurons is sufficient to cause FTD-like behavior by generating neuron-specific progranulin knockout (N-KO) mice by crossing CaMKIIa:Cre mice with $Grn^{fl/fl}$ mice. N-KO mice had 32% lower progranulin protein levels in the cortex than Cre-negative mice, showing that a substantial fraction of brain progranulin is neuron-derived. We then tested 9-month-old N-KO mice and Cre-negative littermates for social and emotional behaviors. Similarly to our previous observations of global progranulin-deficient mice (Filiano et al, 2013 J. Neurosci.), N-KO mice exhibited increased social dominance and a trend for reduced sociability. N-KO mice also had reduced c-Fos expression in the amygdala after exposure to a novel, social environment. These data show that neuronal progranulin deficiency is sufficient to produce similar social behavioral and amygdala deficits as those seen in global progranulin-deficient mice. Markers of inflammation, gliosis, and lipofuscinosis were not significantly elevated in N-KO mice, showing that neuronal progranulin deficiency is not sufficient to reproduce the gliosis and lipofuscinosis of $Grn^{-/-}$ mice.

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Poster

043. Alzheimer's Disease: Proteinopathy, Non- Abeta, and Non-Tau

Location: Halls A-C

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: The Davee Neurobiology Research Initiative Fund

The Louis Family Foundation

NIA Center Grant AG13854

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Title: Concordance between inclusions, microglia activation and disease phenotype in primary progressive aphasia with progranulin mutations and TDP-43 pathology

Authors: M. PETERSON, S. S. AHMADIAN, S. WEINTRAUB, *E. J. ROGALSKI, E. BIGIO, M.-M. MESULAM, C. GEULA;

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Abstract: Aggregation of misfolded proteins in abnormal inclusions is a common feature of most neurodegenerative diseases. The exact relationship of these inclusions to the disease phenotype and patterns of atrophy is incompletely understood, particularly in frontotemporal lobar degeneration (FTLD) and its variants. While there have been dazzling advances in the genetics and cellular biology of FTLD-TDP, quantitative information on the regional distribution of TDP-43 inclusions, other pathology such as microglial activation and their relationship with disease phenotype are glaringly absent from the literature. The major goal of this study was to clarify the relationship between transactivation response element DNA binding protein-43 (TDP-43) inclusions, microglial activation and disease phenotype in primary progressive aphasia (PPA-TDP), a variant of FTLD-TDP in which aphasia is the salient clinical feature and is accompanied by atrophy in perisylvian language network in the dominant hemisphere (usually on the left). We used brains from three cases of PPA-TDP with progranulin mutations for this study. Inclusions were visualized immunohistochemically using an antibody to human phosphorylated TDP-43 and activated microglia were identified using an antibody to HLA-DR. Modified stereological techniques were used to quantify TDP-43 inclusions and activated microglia in inferior frontal gyrus (IFG), inferior parietal lobule (IPL), and superior temporal gyrus (STG), cortical areas involved in the processing of language, and in entorhinal cortex / hippocampus (ERC / Hip), areas involved in the processing of memory. When counts from all areas were combined across cases, significantly higher density of TDP-43 inclusions and activated microglia were observed in the left hemisphere when compared with the right ($p<0.02$). The lowest density of TDP-43 inclusions occurred in the memory related ERC / Hip. Two cases were classified as the logopenic subtype of PPA, in which greatest cortical atrophy has been observed in IPL; in these cases, substantially higher densities of TDP-43 inclusions were detected in the left IPL. A third case was classified at the agrammatic subtype and displayed the highest density of TDP-43 inclusions in IFG / prefrontal cortex, the area known to show the greatest atrophy in this subtype. The distribution of microglia displayed a similar pattern. These observations suggest concordance between TDP-43 inclusions, microglial activation, disease phenotype and putative sites of greatest atrophy in PPA-TDP with progranulin mutations. It remains to be seen if similar concordance exists in sporadic PPA-TDP cases.

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Poster

043. Alzheimer's Disease: Proteinopathy, Non- Abeta, and Non-Tau

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 43.06/E4

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Osteopathic heritage research foundation

UMDNJ foundation

Title: Role of S100B in defining pathological changes in Alzheimer's disease

Authors: *H. WU[1], R. NAGELE[2], V. VENKATARAMAN[1];
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Abstract: Alzheimer's disease (AD) is a devastating neurodegenerative disease for which no cure (other than palliative) exists. The search for a cure demands knowledge of the early pathological changes - critical necessity both for diagnostic as well as treatment purposes - which has remained elusive. Several biomarkers for AD are reported. S100B is one of them, although there is conflicting evidence. Our laboratory is investigating the role of S100B, if any, in AD. In order to explain the onset and progression of AD, this laboratory has been proposed a "multi-hit hypothesis", with a breach in the blood brain barrier, elevation of serum abeta42 peptide interaction with serum auto antibodies and change in cellular calcium as the critical determinants. An animal model system was designed based on these "hits" and the results were compared between wild-type and S100B KO mice. The results demonstrate that the pathological changes in the S100B KO mice are more advanced than that observed in the wild-type. Furthermore, we have identified at least one site of action for S100B: establishment and/or maintenance of an intact blood brain barrier. Further investigation is in progress.

Disclosures: H. Wu: None. **R. Nagele:** None. **V. Venkataraman:** None.

Poster

043. Alzheimer's Disease: Proteinopathy, Non- Abeta, and Non-Tau

Location: Halls A-C

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Program#/Poster#: 43.07/E5

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: The role of a splice variant of carboxypeptidase E in neurodegeneration with links to Alzheimer's disease

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Abstract: Recent work has demonstrated a functional role of carboxypeptidase E (CPE) in neuroprotection and cancer cell survival. A non-redundant nucleotide sequence database search with human CPE, against the GeneBank EST database, identified an EST sequence entry from Alzheimer cortex tissue representing a splice variant of CPE. The three adenosine insertion mutations results in a CPE protein with 9 new amino acids within the first beta-pleated sheet after the pro-domain. We have named this mutant, QQ CPE, due to the presence of two glutamine residues in the new sequence. Transfection and expression of QQ CPE in Neuro2a cells demonstrated that it was poorly made and failed to be secreted into the culture medium, in contrast to WT CPE that was efficiently made and secreted. Western blot analysis showed the presence of the pro-form of QQ CPE indicative of accumulation in the ER. QQ CPE levels were increased after treatment of the cells with MG132, a proteosomal inhibitor, indicating that the mutant CPE was degraded by the proteosome. Further inhibitor studies with E64d, a cell permeable thiol protease inhibitor, also increased the levels of QQ CPE, indicating that a portion of the mutant was targeted for degradation by the lysosome. Expression of the mutant in Neuro2a cells resulted in the initiation of ER stress induced cell death signaling as evidenced by the expression of CHOP, a transcription factor induced under conditions of ER stress. Overexpression of QQ CPE in rat hippocampal and cortical neurons also resulted in increased levels of CHOP in these neurons, in addition to increased cytotoxicity and neuronal cell death as measured by LDH and MTT assays. Co-expression of WT and QQ CPE resulted in the degradation of both forms of the protein and reduction in the secretion of WT CPE, indicating that the mutant was acting in a dominant recessive manner. Preliminary behavioral experiments on ~8 month old QQ CPE transgenic mice have demonstrated a reduced learning and memory capacity compared to their WT littermates as measured by the Morris water maze protocol. We speculate that aged neurons expressing WT and QQ CPE may result in 1) a reduction in overall levels of CPE due to degradation initiated by the mutant and 2) may become more susceptible to ER stress-induced cell death leading to neuronal loss. This would result in the loss of the neuroprotective functions of CPE and possibly lead to neurodegenerative diseases including Alzheimer’s disease.

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Poster

043. Alzheimer's Disease: Proteinopathy, Non- Abeta, and Non-Tau

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 43.08/E6

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant AG027297

PhRMA Foundation

Hazel Embry Research Fund

Title: Calcineurin proteolysis is associated with astrocyte and small vessel pathology

Authors: *M. PLEISS[1], H. MOHMMAD ABDUL[2], J. L. FURMAN[5], R. P. GUTTMANN[3], E. PATEL[2], D. M. WILCOCK[4], P. T. NELSON[2], C. M. NORRIS[2];
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Abstract: The Ca2+ dependent protein phosphatase calcineurin (CN) has been implicated as a causative factor in multiple neuropathological features of Alzheimer's disease (AD) including synapse dysfunction, neuroinflammation, and amyloidosis. Dysregulation of CN activity during AD appears to arise, in part, from the disruption or complete removal of the CN autoinhibitory domain located near the C terminus of the CN catalytic subunit. Commercially available antibodies that target the N terminus of the CN catalytic subunit reveal the presence of an approximately 48 kDa fragment in human brain tissue during early stages of cognitive decline and also in a variety of experimental models of neurodegeneration. While useful for determining the extent of CN proteolysis in Western blot applications, N terminus antibodies do not reveal the cellular location of the proteolysis. Knowing where CN is proteolyzed in nervous tissue seems critical to understanding the mechanistic basis of its many deleterious actions, particularly because CN is found at high levels in both neurons and glial cells where it is involved in different cellular functions. To address this gap in our understanding of CN regulation, we generated custom rabbit polyclonal antibodies to CN A based on previously identified calpain (CP)-dependent cleavage sites. One of these antibodies (referred to here as "ΔCN48") detects a 48 kDa fragment in Western blot assays, but does not detect full-length CN. The ΔCN48 antibody was then used for immunohistochemical labeling of human brain sections characterized by both AD and mixed AD/vascular pathologies. The anatomical features labeled by the ΔCN48 antibody included astrocyte clusters and vascular associated elements and/or processes. We also observed numerous ΔCN48-positive astrocytes associated with microinfarcts. Surprisingly, we

have seen very little neuronal labeling with this antibody. The results suggest that astrocytes and, perhaps astrocyte end-feet, are a primary locus for CP-dependent CN proteolysis in injured or diseased nervous tissue. This work may provide new mechanistic insights into the impact of Ca2+ dysregulation on neurodegenerative diseases.

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Poster

043. Alzheimer's Disease: Proteinopathy, Non- Abeta, and Non-Tau

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant AGR01AG041507

Bristol Myers Squibb Grant

Title: Identification of novel calcineurin/NFAT transcriptional targets mediating Alzheimer's disease synaptic dysfunction

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Abstract: Previous studies in Alzheimer's disease (AD) patients and in AD transgenic animals have suggested that the presence of soluble amyloid ß peptides and/or tau potentially leads to chronic activation of the neuronal calcium dependent phosphatase calcineurin (CaN, also termed protein phosphatase 2B). Upon activation, CaN is responsible for at least two broad types of effects biologically relevant to AD pathophysiology. First, CaN leads to a rapid post-translational modulation of post-synaptic proteins such as cofilin and AKAP79, a process associated with long-term depression. Second, CaN dephosphorylates the nuclear factor of activated T cells (NFAT), which leads to its translocation to the nucleus and to the expression of target genes encoding important proteins implicated in neuronal survival. Importantly, our previous work has shown that targeted inhibition of CaN/NFAT alleviates Aß-mediated neurotoxic events,

suggesting that the transcriptional impact of this particular cascade may be of relevance in the disease. In order to characterize the expression profile changes dependent upon CaN/NFAT-activation in neurons, intrahippocampal injections of adeno-associated vectors coding for wild-type CaN (wtCaN), constitutively activated CaN (CACaN) and GFP (control vector) were performed in wild-type mice. After laser capture microdissection of the transduced neuronal cells, a whole genome micro-array assay identified 6 genes differentially modulated by CACaN (as compared with wtCaN and GFP): Neuronatin (NNAT), Nurr1 (Nr4a2), VGF nerve growth factor (VGF), Hippocalcin (HPCA), Heat shock protein 5 (Hspa5) and corticotropin-releasing hormone (CRH). Further validation by qRT-PCR in human hippocampal tissue confirmed the significant increased expression of VGF and CRH in AD compared with aged-matched controls. By using *in vivo* gene transfer, we demonstrate that CaN activation impacts the expression level of a set of genes in neurons. In particular, our findings highlight for the first time a possible link between a chronic induction of calcineurin and CRH, a peptide hormone and neurotransmitter potentially involved in the stress response in Alzheimer's disease. Further studies will decipher if interfering with the CRH cascade, may alleviate amyloid induced, CaN-dependent, neurotoxic effects. Supported by NIH grant AGR01AG041507 and a grant from Bristol Myers Squibb

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Poster

043. Alzheimer's Disease: Proteinopathy, Non- Abeta, and Non-Tau

Location: Halls A-C

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Program#/Poster#: 43.10/E8

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: PO1AG14449

RO1AG043375

R01AG042475

Brinson Foundation

Title: Hippocampal Cathepsin D and APP/Aβ processing in Alzheimer's disease

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Abstract: Endosomal-lysosomal (E-L) dysfunction occurs prior to β-amyloid (Aβ) and tau-containing neurofibrillary (NFT) pathology and may underlie neuronal selective vulnerability during the progression of Alzheimer's disease (AD). Western-blot data have demonstrated an up-regulation of the lysosomal hydrolase Cathepsin D (Cat D) in the hippocampus of people who died with a clinical diagnosis of mild cognitive impairment (MCI) compared to no cognitive impairment (NCI). The hippocampus is involved early in the onset of AD as evidenced by the mediation of cognition and, NFT and Aβ pathology. Since E-L pathways are major routes of amyloid precursor protein (APP) processing and tau dysregulation, we examined whether hippocampal intraneuronal Cat D was associated with Aβ and/or NFT pathology early in AD. Hippocampal neuronal Cat D, NFT density and intraneuronal APP/Aβ levels were quantified using immunohistochemistry and densitometry in paraffin sections from subjects with a premortem clinical diagnosis of NCI, MCI or mild to moderate AD. Adjacent hippocampal sections were immunolabeled with antibodies against oligomeric tau TOC1 and TNT, phosphorylated AT8 and truncation Tau C3 as well as with 6E10 an APP/Aβ marker. Optical density (OD) analysis revealed a significant increase in Cat D-immunoreactive (-ir) levels within CA4 hippocampal neurons in AD compared to NCI, but not in CA2/3 or CA1 perikarya. The density of the CA1 hippocampal AT8 and TOC 1-ir neurons was significantly increased in AD compared to NCI, whereas intraneuronal APP/Aβ-ir OD levels were unchanged in the different hippocampal subfields examined across groups. Hippocampal neuronal Cat D OD values did not correlate with NFT density, Braak staging or mini-mental state examination (MMSE) during AD progression. Conversely, AT8 and oligomeric TOC1-ir neuronal density showed the strongest association within all hippocampal subfields across groups, but only AT8 and TOC1 NFT density within CA1 showed a strong association with Braak staging and MMSE scores. Remarkably, there was a positive association between intraneuronal OD measurements of APP/Aβ and Cat D-ir values in CA2/3 across groups and a trend in the CA1 pyramidal cell layer. These data suggest that Cat D alterations in hippocampal neurons occur late in AD and may be associated to APP/Aβ processing rather than tau pathology.

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Poster

043. Alzheimer's Disease: Proteinopathy, Non- Abeta, and Non-Tau

Location: Halls A-C

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Program#/Poster#: 43.11/E9

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NSFC Major Research Grant 91132718

Title: Abnormity of myelination in Alzheimer's Disease mouse models

Authors: *Y. WU[1], Y. ZHANG[2];
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Abstract: Abnormity of myelination in AD mouse models Yu Wu, Yan Zhang The abnormity of myelination has been reported in Alzheimer's disease (AD) patients. We now report a pathway involved in regulating myelination, which acts abnormally in APP/PS1 transgenic mouse (AD mouse models). Ankyrin G, DR6, caspase6 and neuregulin1 are four important factors in the pathway. We found that the expression of Ankyrin G is decreased in APP/PS1 transgenic mouse, which finally regulate the activation of neuregulin1 to affect myelination by DR6 and caspase6. Schwann cells and DRG neurons *in vitro* co-culture systems were used to observe the myelination. So far, our data suggested that the abnormity of myelination might play important role in AD pathology, which may affect the spread of action potential.

Disclosures: Y. Wu: None. **Y. Zhang:** None.

Poster

043. Alzheimer's Disease: Proteinopathy, Non- Abeta, and Non-Tau

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Center for Biomolecular Therapeutics

Institute of Bioscience and Biotechnology Research Seed Grant

Title: The Ca2+ sensor S100A1 modulates neuroinflammation, histopathology and Akt activity in the PSAPP Alzheimer's disease mouse model

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Abstract: The contribution of the Ca^{2+} sensor S100A1 to *in vivo* Alzheimer's disease (AD) pathobiology has not been elucidated although S100A1 regulates numerous cellular processes linked to AD. This study uses genetic ablation to ascertain the effects of S100A1 on neuroinflammation, beta-amyloid (Aβ), plaque deposition and Akt activity in the PSAPP AD mouse model. PSAPP/S100A1$^{-/-}$ mice exhibited decreases in astrocytosis (GFAP burden), microgliosis (Iba1 burden) and plaque load/number when compared to PSAPP/S100A1$^{+/+}$ mice at six and twelve months of age. The presence of detectable S100A1 staining in human AD specimens is consistent with a detrimental gain of S100A1 function in AD. Plaque associated PO_4-Akt and PO_4-GSK3β staining was also reduced while non-plaque associated staining was increased. S100A1·Akt complexes were undetectable in PC12 cells and AD brain tissue suggesting that S100A1 indirectly modulates Akt activity. In contrast, S100A1·RyR (ryanodine receptor) complexes were present in human/mouse AD brain and exhibited Ca^{2+}-dependent formation in neuronal cells. This is the first direct demonstration of an S100A1 target protein complex in tissue/cells and identifies the RyR as a primary S100A1 target protein in the brain. However, over 20 *in vitro* target proteins have been reported for S100A1 and it is unlikely that the RyR is the only intracellular S100A1 target protein in the AD brain. Studies are underway to determine if Hsp70/Hsp90 multichaperone complex, protein phosphatase 5, L-type Ca2+ channel, SERCA pump, tau, tubulin and synapsin are *in vivo* S100A1 target proteins.These data suggest that S100A1 inhibition may be a novel strategy for normalizing aberrant Ca^{2+} signaling in AD.

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Poster

043. Alzheimer's Disease: Proteinopathy, Non- Abeta, and Non-Tau

Location: Halls A-C

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Hong Kong University of Science and Technology

National Key Basic Research Program of China (2013CB530900)

The NIH - NS071022

Title: The role of ATM in Alzheimer’s disease pathogenesis

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Abstract: Neuronal cell loss is one of the hallmarks of Alzheimer's disease (AD). Although its mechanism is elusive, unscheduled cell cycle reentry has been proposed to be an important part of the process. ATM (ataxia telangiectasia [A-T], mutated) is involved in cell cycle checkpoint control and DNA damage repair. ATM also plays a role in epigenetic histone modification through its regulation of the subcellular localization of the histone deacetylase, HDAC4. We believe that this epigenetic function raises the possibility that although A-T is a disease of childhood, ATM may play a role in AD. We hypothesized that reductions in ATM activity may be one of factors leading to cell cycle related neuronal cell loss in AD. We performed immunohistochemistry on postmortem brain tissue from human AD patients and age-matched control individuals using several ATM-related antibodies and cell cycle markers. Using the translocation of HDAC4 to the nucleus as a marker for the loss of ATM activity, we found that a significant decrease in ATM function in hippocampus of individuals with AD compared with controls. Curiously, the CA2 sub-region was the region with the most levels of HDAC. Correspondingly, S1981-phosphosphorylated ATM (an activating phosphorylation) was found localized in cytoplasmic granules in hippocampal pyramidal cells. We hypothesize that the neurons under the stress of lower ATM activity initiate a protective response by activating ATM. We are currently analyzing brain stem, frontal cortex and cerebellum of these cases, and will correlate the levels of ATM activity with the presence of cell cycle events and other markers. Thus, the decrease in ATM activity was confirmed in AD, yet its mechanism and downstream effects are not fully revealed.

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Poster

043. Alzheimer's Disease: Proteinopathy, Non- Abeta, and Non-Tau

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant AG027224

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Title: VILIP-1 protein in Alzheimer disease and frontotemporal lobar dementia postmortem brain tissue

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Abstract: Visinin-like protein 1 (VILIP-1) is a neuronal calcium sensor in a subfamily of closely related EF-hand Ca2+-binding proteins that mediate receptor trafficking and are required for induction of long term depression. VILIP-1 has recently been reported to be increased in cerebrospinal fluid and plasma of Alzheimer disease (AD) subjects relative to normal controls, and to individuals with other dementias, suggesting a potential role as an AD biomarker. Higher cerebrospinal fluid VILIP-1 levels were also predictive of more rapid cognitive decline. However, how VILIP-1 expression levels are altered in the brain tissue of AD and other dementia subjects remains largely unknown. Using a targeted mass spectrometry approach, we measured VILIP-1 protein levels in superior frontal gyrus gray matter from a set of Braak Stage 3 - 5 AD (n=59) subjects, along with both frontemporal lobar dementia (FTLD) (n=11) and normal control (n=12) subjects. A trend towards reduced VILIP-1 levels in AD subjects compared to controls was observed. Within AD subjects, VILIP-1 levels were unaffected by a number of indicators of more rapid progression or disease severity: presence of psychosis, Braak stage, MMSE score, and the presence of Lewy bodies. Similarly, measures of soluble tau, phosphotau, and amyloid-β did not correlate with VILIP-1 levels. FTLD subjects had significantly lower levels of VILIP-1 compared to controls, which could indicate that loss of VILIP-1 is due to neuronal loss rather than being due to a neuropathologic process specific for AD. An expanded evaluation of VILIP-1 levels in mesial temporal gray matter, a region highly compromised in early stages of AD, and the parietal cortex, a region largely unaffected in FTLD, from a subset set of AD and FTLD subjects will also be presented to further test this hypothesis.

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Poster

043. Alzheimer's Disease: Proteinopathy, Non- Abeta, and Non-Tau

Location: Halls A-C

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: R01 AG15819

P30 AG10124

P30 AG10161

Title: In the hippocampal region of Alzheimer's disease, IRS-1 pS616, a candidate biomarker of brain insulin resistance, rises first in CA1 and later in the subiculum and perirhinal cortex

Authors: *A. SAMOYEDNY[1], M. P. BALDASSARI[2], H. KAZI[2], L.-Y. HAN[2], S. E. ARNOLD[2], J. Q. TROJANOWSKI[2], D. A. BENNETT[3], K. TALBOT[1];
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Abstract: BACKGROUND: *Ex vivo* tests of insulin responsiveness in AD brain tissue show that it is profoundly insulin resistant compared to that of normal, age- and sex-matched controls (Talbot et al., JCI 122: 1316-1338, 2012; Wang et al. J. Neurosci. 32: 9773-9784, 2012). Among the most consistent correlates of such brain insulin resistance is elevation in basal levels of insulin receptor substrate-1 phosphorylated at serine 616 (IRS-1 pS616). Viewed immunohistochemically, this phenomenon reflects accumulation of the phosphospecific antigen in neuronal cytoplasm and neuropil (Talbot et al., 2012). In normal cases, IRS-1 pS616 is restricted to neuronal nuclei. As we reported earlier, this candidate biomarker of brain insulin resistance is elevated in hippocampal field CA1 in AD dementia cases and to a lesser degree in mild cognitive impairment (MCI) cases. Here we studied the same phenomenon throughout the hippocampal region (HR = CA1-3 + dentate gyrus [DG] + subiculum + combined pre- and para-subiculum + entorhinal cortex [ERC]) + perirhinal cortex). METHODS: We studied the two cohorts analyzed in our earlier report (Talbot et al., 2012), namely 24 pairs of cognitively normal and AD dementia cases matched for age and sex in the University of Pennsylvania brain bank and a set of 30 cognitively normal, 29 MCI, and 31 AD dementia not significantly different in age or male/female ratio from the Religious Orders Study (ROS) at Rush University. Immunohistochemistry was performed on coronal sections of the HR using an Invitrogen anti-IRS-1 pS616 antibody (44-550) at 1:500 as specified in Talbot et al. (2012). The density of neurons with extra-nuclear IRS-1 pS616 in each anatomical area was quantified using ImagePro Plus. RESULTS: Elevated extra-nuclear IRS-1 pS616 in MCI or AD dementia cases was seen only in neurons, specifically glutamatergic (i.e., pyramidal) cells in the HR. This antigen was found in clustered microdomains around cell nuclei, as well as in primary dendrites, neuritic

processes, neuropil threads, and perhaps axons. In MCI cases, the density of HR neurons with extra-nuclear IRS-1 pS616 was significantly ($p < 0.05$) increased only in CA1, but in AD dementia cases, the density of such cells was significantly increased in CA1, the subiculum, and the perirhinal cortex. The density of these pathological neurons was not elevated in CA2, CA3, DG, pre- and para-subiculum, or the ERC of MCI or AD dementia cases. CONCLUSION: These findings suggest that a candidate biomarker of brain insulin resistance (IRS-1 pS616) in the HR is limited to glutamatergic neurons and appears first in CA1 as seen in MCI cases and only later in the subiculum and perirhinal cortex as seen in AD dementia cases.

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Poster

043. Alzheimer's Disease: Proteinopathy, Non- Abeta, and Non-Tau

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH P30 AG10124

Title: Cerebrocortical IRS-1 pS616, a candidate biomarker of brain insulin resistance, may be an AD biomarker

Authors: *K. TALBOT[1], B. M. SAGGU[2], S. A. RIZVI[3], M. A. KAHN[4], A. J. SAMOYEDNY[1], J. Q. TROJANOWSKI[5];
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Abstract: BACKGROUND: Among the most consistent correlates of brain insulin resistance in AD is an elevation in basal levels of insulin receptor substrate-1 phosphorylated at serine 616 (IRS-1 pS616) (Talbot et al., JCI 122: 1316-1338, 2012). Since this elevation can be produced by beta amyloid (Bomfim et al., JCI 122: 1339-1353), IRS-1 pS616 may be a biomarker of AD and other neurodegenerative disorders (NDD) with abnormally high beta amyloid levels (e.g., dementia with Lewy bodies, DLB). To test this, we quantified IRS-1 pS616 in cerebrocortical neuronal cell bodies and neuropil in AD, DLB and NDDs with typically low beta amyloid levels: corticobasal degeneration (CBD, a tauopathy) and a TDP-43 positive, tau-negative form of

frontotemporal lobar degeneration (FTLD/TDP-43). METHODS: Seventeen quintuplets of normal, AD, DLB, CBD, and FTLD/TDP-43 cases matched for age (within 5 y), sex, and postmortem interval (within 4 h) were selected in the brain bank of the Center for Neurodegenerative Disease Research at the University of Pennsylvania. In each case, sections were cut from fixed blocks of neocortical areas (motor, prefrontal, and temporal) and hippocampus. These were reacted immunohistochemically for IRS-1 pS616 using Invitrogen antibody 44-550 (1:500). In all brain areas, we calculated the density of neurons (cells per unit area) with extra-nuclear IRS-1 pS616 (usually restricted to cell nuclei in normal cases) and the optical density of IRS-1 pS616 immunoreactivity in neuropil. RESULTS: A one-way ANOVA showed highly significant ($p < 0.002$) differences among the case groups in the density of neurons with extra-nuclear IRS-1 pS616 in all brain areas studied and in IRS-1 pS616 neuropil in all those areas except motor cortex. Post-hoc tests showed that this was due to higher levels of both IRS-1 pS616 measures in AD cases compared to all the other groups with one exception, namely higher than normal neuropil IRS-1 pS616 in CA1 of DLB cases. CONCLUSIONS: These results support the hypothesis that IRS-1 pS616 in the neocortex and/or CA1 is associated with NDDs often characterized by elevated beta amyloid. The consistent increase in IRS-1 pS616 across cortical areas in AD and its consistent absence in a tauopathy and a TDP-43 proteinopathy suggests that elevated cerebrocortical IRS-1 pS616 may be a reliable and relatively selective biomarker of AD.

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Poster

043. Alzheimer's Disease: Proteinopathy, Non- Abeta, and Non-Tau

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: BR3706/3-1

Title: Mitochondria dictate the cytotoxicity of Alzheimer disease-associated aberrant ubiquitin

Authors: ***R. J. BRAUN**[1], C. SOMMER[2], R. J. G. GENTIER[3], V. I. DUMIT[4], C. LEIBIGER[5], K. PADUCH[5], T. EISENBERG[2], L. HABERNIG[2], G. TRAUSINGER[6], C. MAGNES[6], J. DENGJEL[4], F. W. VAN LEEUWEN[3], G. KROEMER[7], F. MADEO[2];
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Abstract: Neuronal accumulation of UBB+1, a frameshift variant of ubiquitin B, is a hallmark of Alzheimer disease (AD). How UBB+1 contributes to neuronal dysfunction remains poorly understood. Here we show that, in hippocampal brain regions of AD patients with neurofibrillary tau tangles, UBB+1 co-exists with VMS1, the mitochondrion-specific component of the ubiquitin-proteasome system (UPS). Expression of human UBB+1 in yeast disturbs the UPS, leading to mitochondrial stress and cytotoxicity. Inhibition of UPS activity exacerbates UBB+1-triggered cytotoxicity, while UPS stimulation by the transcription activator Rpn4 reduces UBB+1 cytotoxicity. High levels of the Rpn4 target protein Cdc48 and its conserved cofactor Vms1 are sufficient to relieve UBB+1-triggered cytotoxicity. The fact that AD-induced cellular dysfunctions can be avoided by UPS activity at mitochondria has far-reaching pathophysiological implications.

Disclosures: **R.J. Braun:** None. **C. Sommer:** None. **R.J.G. Gentier:** None. **V.I. Dumit:** None. **C. Leibiger:** None. **K. Paduch:** None. **T. Eisenberg:** None. **L. Habernig:** None. **G. Trausinger:** None. **C. Magnes:** None. **J. Dengjel:** None. **F.W. van Leeuwen:** None. **G. Kroemer:** None. **F. Madeo:** None.

Poster

043. Alzheimer's Disease: Proteinopathy, Non- Abeta, and Non-Tau

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 43.18/F1

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH GRANT P50AG025688

NIH GRANT P30NS055077

Title: Aggregation properties of the small nuclear ribonucleoprotein U1-70K in Alzheimer Disease

Authors: I. DINER[1], C. M. HALES[2], L. RABENOLD[1], I. BISHOF[1], D. DUONG[1], H. YI[1], O. LAUR[1], G. MARLA[3], L. J. JAMES[2], A. LEVEY[2], *N. T. SEYFRIED[4];
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Abstract: Recent evidence indicates that U1-70K and other U1-specific small nuclear ribonucleoproteins (snRNPs) are sarkosyl-insoluble and associate with tau neurofibrillary tangles (NFTs) selectively in Alzheimer disease (AD). Currently, the mechanisms underlying the conversion of highly soluble nuclear U1 snRNPs into insoluble cytoplasmic aggregates remain elusive. In this study, immunogold electron microscopy was used to demonstrate that U1-70K associates with filamentous structures in the cytoplasm that resembled twisted-ribbon NFTs. Following biochemical fractionation nearly all U1-70K was sarkosyl-insoluble in AD brain tissue, but not in control brain. Herein we demonstrate that total homogenate fractions from AD brains function as seeds for aggregation of soluble U1-70K from control brain homogenate or recombinant U1-70K to become sarkosyl-insoluble. This indicates that biomolecules harbored within AD brain sequester normal U1-70K into an insoluble form. This effect was not dependent on RNA, and did not correlate with tau levels as AD homogenates with reduced levels of these components were still capable of seeding U1-70K. By contrast, proteinase K-treated AD homogenates and sarkosyl-soluble fractions were unable to seed soluble U1-70K, suggesting that aggregate-prone proteins in AD brain are responsible for seeding U1-70K. Finally, by expressing recombinant N- and C-terminal truncations of U1-70K, we determined that the highly disordered low complexity C-terminus is necessary and sufficient for U1-70K aggregation. These results support that AD homogenate can induce the aggregation of soluble U1-70K in a protein dependent manner.

Disclosures: **I. Diner:** None. **C.M. Hales:** None. **L. Rabenold:** None. **I. Bishof:** None. **D. Duong:** None. **H. Yi:** None. **O. Laur:** None. **G. Marla:** None. **L.J. James:** None. **A. Levey:** None. **N.T. Seyfried:** None.

Poster

043. Alzheimer's Disease: Proteinopathy, Non- Abeta, and Non-Tau

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: ARM is a Postdoctoral Fellow of the Michael Smith Foundation for Health Research

CIHR Grant MT-14037

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Title: Differential regulation of cortical Munc18-1 splice variants in Alzheimer's disease: Correlations with the severity of cognitive decline and neuropathology in a community-based aging study

Authors: A. RAMOS-MIGUEL[1], C. HERCHER[1], C. L. BEASLEY[1], A. M. BARR[2], J. A. SCHNEIDER[3], D. A. BENNETT[3], *W. G. HONER[4,1];
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Abstract: Loss of synaptic connections stands among the earliest events in the pathophysiology of Alzheimer's disease (AD). The mechanisms involving synaptic damage are unclear. We previously showed that weak interactions of the soluble NSF attachment protein receptor (SNARE), the molecular engine of neurosecretion, were associated with cognitive decline and AD pathology, without a significant loss of syntaxin-1 and SNAP-25 monomers. Munc18-1 (M18) is a critical molecule for SNARE assembly. We hypothesized that the age-related SNARE decline could be caused by abnormal M18 expression. Cortical samples from the middle-frontal gyrus were collected from deceased participants enrolled in the Memory and Aging Project, a community-based aging study. Cognitive abilities of the participants were evaluated annually, and neuropathological assessments met Reagan criteria. Participants were grouped into non- ($n = 56$) or mild ($n = 50$) cognitive impairment (NCI/MCI), or AD ($n = 70$). Selective antibodies targeting M18a and M18b splice variants were used for quantitative Western blotting, immunohistochemistry, and co-immunoprecipitation. Cortical levels of M18a were dramatically downregulated in AD (64%, $p < 0.001$) compared with NCI or MCI. Only a marginal reduction of M18b (9%, $p = 0.033$) was found in the same samples. Other presynaptic proteins (synaptophysin, syntaxin-1, SNAP-25) were not significantly altered, indicating that the M18 deficit may precede synaptic loss. M18a density was strongly related to global cognitive score ($r = 0.323$, $p < 0.001$) and neuropathology ($r = -0.266$, $p < 0.001$), whereas these correlations were weaker for M1b ($r = 0.179$, $p = 0.018$; $r = -0.216$, $p = 0.004$, respectively). Comparative analyses of human cortical sections revealed substantial differences in the distribution of M18 variants. While M18b was ubiquitously expressed across all layers, M18a showed perinuclear localization, mainly in layer IV-VI neurons. M18b, but not M18a, co-localized with synaptophysin in presynaptic terminals. Tau-positive cells (AT8 and Alz-50) barely expressed M1a, whereas M1b was intact regardless the presence of these pathological tau forms. Moreover, the amount of syntaxin-1 co-immunoprecipited with M18b was 5-fold larger than observed with M18a. The results suggest that loss of M18a in cortical neurons is an important event in the pathogenesis of AD. M1a deficiency may be linked to early neuronal damage, since weak to null expression of this variant was observed in tau positive cells. The cytosolic localization and its low affinity for syntaxin-1 suggest that M1a activity is unrelated to neurosecretion.

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Poster

043. Alzheimer's Disease: Proteinopathy, Non- Abeta, and Non-Tau

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 43.20/F3

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Alzheimer´s Association IIRG-11-205127

Title: Amyloid peptides associated with human dementias, Aβ42, ABri and ADan cause differential neurotoxicity in *Drosophila* brain

Authors: *M. S. MARCORA[1], A. C. FERNANDEZ-GAMBA[2], R. VIDAL[3], L. MORELLI[2], M. F. CERIANI[2], E. M. CASTAÑO[2];
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Abstract: Several neurodegenerative diseases share common features such as their clinical progression with age, synaptic abnormalities followed by neuronal loss and the presence of amyloid deposit in the brain. Alzheimer's disease (AD), the most common cause of dementia, is characterized by the aged-associated dysfunction of cholinergic neurons and the progressive accumulation of amyloid β (Aβ) and hyperphosphorylated tau, major components of senile plaques and neurofibrillary tangles (NFT), respectively. Familial British and Danish dementias (FBD and FDD) are autosomal dominant neurodegenerative disorders associated to the accumulation of amyloids ABri and ADan, respectively. Internal proteolysis of the precursor protein BRI2 carrying different mutations leads to the release of ABri and ADan, which are highly amyloidogenic *in vitro* as compared to Aβ. *Drosophila* melanogaster is a suitable animal model to study the relationship between different amyloids and their specific neurodegenerative and behavioral phenotype. We generated transgenic *Drosophila* lines that over-express human amyloids Aβ42, ABri and ADan, respectively, or a control peptide Bri2-23 (the non-amyloidogenic product of the internal proteolysis of wild type BRI2) using the Gal4/UAS binary system. Pan-neuronal expression revealed an age-dependent toxicity of amyloids as determined by the ability of flies to climb in a geotaxis paradigm when compared to Bri2-23. ADan-

expressing flies displayed a significant impairment in climbing ability at 7 days post eclosion (p.e) ($p< 0.001$), while ABri and Aβ42 showed a significant toxicity at 15 and 21 days respectively ($p<0.01$). Histological analysis of paraffin brain sections showed thioflavine-S-negative accumulation of amyloids peptides along with mild vacuolization. In addition, we analyzed the levels of Bruchpilot (Brp), a protein involved in clustering and vesicle release at the presynaptic active zones. Western blots from fly heads showed an age-dependant reduction of Brp in amyloid-expressing as compared to controls. At 7 days p.e. no differences were found while at 21 days Brp levels in amyloid-expressing flies were reduced (approximately a 20%) as compared to Bri2-23 control flies. Our results indicate that different amyloids display differential neurotoxicity in the CNS. However, all of them appear to affect synaptic transmission in an aged-dependent manner. These *Drosophila* models will allow a systematic and unambiguous assessment of differences and similarities in the mechanisms of toxicity of diverse amyloid peptides associated with dementia.

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Poster

044. Alzheimer's Disease: Imaging and Biomarkers

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 44.01/F4

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH/NIA 1 R21 AG037843

Brigham Young University, College of Life Sciences, Start-Up Grant

Brigham Young University, School of Family Life, Gerontology Program

Brigham Young University, Graduate Research Fellowship

Title: Relaxation properties of iron-bound AD-associated proteins

Authors: *R. D. ADHIKARI[1], S. R. BURT[2], N. K. BANGERTER[3], R. K. WATT[2], H. V. VINTERS[4], J. J. WISCO*[1,5];
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Abstract: INTRODUCTION Alzheimer's disease (AD) is a progressive, neurodegenerative and incurable disorder. Many studies have attributed iron-related oxidative stress as a factor promoting neuronal damage. Free iron in the neurons leads to the formation of reactive oxygen species that are neurotoxic, hence neuronal damage. Immunohistochemistry studies have shown co-localization of iron with amyloid plaques (Aβ) and hyper-phosphorylated Tau proteins (HP-tau) which are the hallmark histopathological changes seen in AD. In fact, we hypothesize that Aβ and HP-tau sequester the excess iron that overwhelms the ferritin protein, the primary iron storage protein. Such co-localizations are also seen with the Magnetic Resonance Imaging (MRI). However, we do not know the pathophysiological mechanism or related stages of disease with regard to Aβ and HP-tau iron sequestration. Nuclear Magnetic Resonance (NMR) is based on the principal that a ferromagnetic substance would have different Longitudinal (T1) and Transverse (T2) relaxation based on their environment they are in. We titrated NMR relaxation values for iron-bound AD-associated protein complexes. METHODS Hydrogen atoms are ferromagnetic; hence, they align so that their axis is parallel to in the applied magnetic field of NMR. A radiofrequency pulse was delivered to tip these atoms from their position. The atoms again try to align according to magnetic field. These atoms, based on their environment, relax back to their original position giving the longitudinal relaxation (T1). Atoms also have their spin and when they are tipped off their position, they precess back giving the transverse relaxation (T2). Phosphate NMR is based on phosphorous atoms. RESULTS We conducted NMR (proton and phosphate) on different iron-bound AD-associated protein complexes at varying concentration levels. Phosphate saline buffer in deuterium was used as a control. The T1 relaxation ranges from 65.3 ± 0.2 ms to 10.96 ± 0.5 s. T2 values ranges from 20 ± 2 ms to 24.3 ± 13.5 s and T2* ranges from 13 ms to 774 ms. CONCLUSIONS We have been able to determine T1, T2 and T2* values for varying concentrations of iron-bound proteins and appropriate controls. These values were not only different for individual iron-protein complexes but also different for varying concentrations of same iron-protein complexes. We also conducted the phosphate based NMR: the result showed different relaxation properties for iron phosphate complexes at different concentrations. These relaxation properties can be used to inform MRI for generating contrast images. Our ultimate goal is to develop a diagnostic imaging technique for AD in murine and human brains.

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Poster

044. Alzheimer's Disease: Imaging and Biomarkers

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 44.02/F5

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Cortical and subcortical disruptions underlying memory deficits in early Alzheimer's disease and mild cognitive impairment

Authors: *F. G. YANG[1], M.-J. CHIU[2,3,4,5], C.-E. TSENG[1], Y.-F. CHEN[6], T.-F. CHEN[2], T.-W. TSENG[2], J.-C. CHEN[2];
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Abstract: Alzheimer's disease (AD) causes tissue loss and neuronal death, which drastically affect cognitive functions. The most remarkable signs of brain damage in AD include shrinkage of the brain volume and white matter disruption. Patients with mild cognitive impairments (MCI) also display similar but less prominent change in the brain. Although previous research has reported poor neurocognitive performance of these patients, the relationship between brain damage and cognitive impairments remains unclear. The present study employed volumetric measurement, voxel-based morphometry (VBM) and tract-based spatial statistics (TBSS) to compare the group differences. The correlations of performance in the attention, memory and language domains and indices of grey and white matter integrity were also examined. We observed significant difference in the total hippocampus volume among the three groups. The VBM analysis showed significant difference in the hippocampus, thalamus, the PFC, visual and motor cortices between the AD and control groups, but only the hippocampus, thalamus, and the visual cortex between the control and MCI groups ($p<0.001$). In terms of cortical thickness, the control group had significantly greater thicknesses in bilateral middle temporal gyrus and left parahippocampus than the AD and MCI groups. Significant difference in the right parahippocampus thickness was only seen between the MCI and control groups. The TBSS analysis only revealed significant difference in FA and MD between controls and ADs. The regions displaying significant difference included inter-commissural and association tracts. Pearson correlation analysis showed significant correlations in multiple grey and white matter ROIs with Delayed recall of Logical Memory and Semantic Verbal Fluency. The study has suggested that atrophy and cortical thinning in temporal and occipital regions are closely related with memory and verbal deficits in patients.

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Poster

044. Alzheimer's Disease: Imaging and Biomarkers

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 44.03/F6

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant TR000903

Title: MicroRNAs in human cerebrospinal fluid as diagnostic biomarkers for Alzheimers Disease

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Abstract: Alzheimer's disease (AD) is the most common form of dementia and the greatest known risk factor for AD is increasing age. Although AD is progressive, AD treatments can temporarily slow disease progression. Thus a preclinical tool that could diagnose AD earlier in stage and allow treatments to be initiated earlier in the disease would be of great clinical value. However, there are currently no biomarkers that can reliably predict the onset of AD, nor are there any that can distinguish early AD from other age-related dementias. The existence of extracellular RNAs in biofluids provides a fertile molecular landscape from which diagnostic and prognostic biomarkers may be isolated and exploited. Hence, the identification of RNAs present in cerebrospinal fluid (CSF) provides an opportunity to define important biomarkers for clinical use in neurodegenerative diseases. We examined microRNA (miRNA) expression in human CSF to evaluate their clinical utility as diagnostic biomarkers for AD. We isolated total RNA from existing, banked CSF samples isolated from healthy subjects and AD patients obtained from the Oregon Alzheimer’s Disease Center (OADC) and analyzed miRNA expression using TaqMan® Array Human MicroRNA Card Set v3.0 (Life Technologies) arrays. Initial studies included CSF samples representing 16 healthy and 16 AD subjects, with male and female equally represented. In this proof-of-principle analysis, subjects were segregated into “Group 1” and “Group 2” by disease status, and only miRNA presence/absence was considered, without regard to abundance. Analysis was restricted to 208 miRNAs, each of which was variably present in as few as 5, and as many as 25, of the 32 subjects. Results from these studies revealed that ~8% of the miRNAs

were significant at the 0.05 level by Fisher's Exact test. Five miRNAs met the 25% false discovery rate (FDR) threshold, while 2 met the 10% FDR threshold. Of the 5 with FDR 10%, 1 miRNA was preferentially expressed in Group 1, and 4 in Group 2. A classification rule combining 2 miRNAs preferentially expressed in Group 2 yielded correct classification percentages of 87% and 94% for Groups 1 and 2, respectively. These studies highlight the potential utility of miRNAs in human CSF as clinical biomarkers for AD. The presence/absence analysis used here did not rely on any form of normalization, and thus may be a robust means to identify clinical biomarkers. Further analyses will investigate the use of multiple miRNA targets, incorporating relative abundance levels, to improve the sensitivity and specificity of disease identification. Additional healthy and AD CSF samples are being processed to confirm the specificity of these results.

Disclosures: **J.A. Saugstad:** None. **T.A. Lusardi:** None. **C.A. Harrington:** None. **J.I. Phillips:** None. **B. Lind:** None. **J.A. Lapidus:** None. **J.F. Quinn:** None.

Poster

044. Alzheimer's Disease: Imaging and Biomarkers

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 44.04/F7

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Cure Alzheimer's Fund

Title: Phage display: the search for blood-base biomarkers in Alzheimer's disease

Authors: *C. CARROLL[1], E. H. KOO[3], Z. AN[4], Y. LI[2];
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Abstract: Alzheimer's disease (AD) is the sixth leading cause of death in the US, and there is currently no effective treatment or cure. Pathology in AD is thought to develop decades before symptoms present, so we set out to find a biomarker for the disease that can be used to track the progression. Current biomarkers are either invasive (cerebral spinal fluid) or expensive (brain imaging), making a blood-based biomarker an attractive alternative. We used two phage-display libraries, which display random peptide sequences on its coat, to pan antibody populations in either AD or age-matched control blood serum. We found approximately eighteen sequences that

were enriched in AD samples, of which 5 were validated. Validation studies were done on the original twenty samples using an enzyme-linked immunosorbent assay (ELISA), where higher absorbance values for AD patient samples over control samples corresponded to a positive hit. We are now testing a blinded set of serum samples to see if our panel can effectively sort AD from normal controls. This panel has the potential to serve as an effective diagnostic tool. If the disease is diagnosed earlier, therapeutics that failed earlier clinical trials could be retested for increased efficacy in this pre-symptomatic cohort.

Disclosures: **C. Carroll:** None. **E.H. Koo:** None. **Z. An:** None. **Y. Li:** None.

Poster

044. Alzheimer's Disease: Imaging and Biomarkers

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Swedish Research council grant 2012-1593

Swedish Alzheimer foundation

Åhlén foundation

Swedish Lundbeck foundation

Stohnes research foundation

Uppsala Berzelii Technology Centre for Neurodiagnostics

Title: Transferrin receptor mediated transcytosis of an antibody based radioligand for quantification of intrabrain levels of soluble amyloid-beta aggregates

Authors: *D. SEHLIN[1], X. T. FANG[1], L. M. CATO[1], J. FÄLTING[3], G. ANTONI[2,4], L. LANNFELT[1], S. SYVÄNEN[1];
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Abstract: Positron emission tomography (PET) [11C]PIB imaging detects amyloid plaques formed early in the course of Alzheimer’s disease (AD) pathogenesis. The introduction of

[11C]PIB was a game changer for diagnosis of AD as amyloid plaque imaging detects AD pathogenesis early in the course of disease and helps distinguishing AD from other types of dementia. However, the plaque pathology does not correlate well with disease progression or outcome of therapeutic interventions, limiting the usefulness of amyloid PET tracers for such applications. The aim of the present project was to develop a PET radioligand based on an antibody (mAb158) that binds selectively to soluble amyloid β (Aβ) protofibrils, which may correlate better with disease severity than amyloid plaques. Antibodies are large molecules which do not readily pass over the blood-brain barrier (BBB). To reduce the size of the molecule and to decrease the systemic half-life, a F(ab')2 fragment of the antibody was generated. F(ab')2-158, lacking Fc related effector functions, showed a pronounced reduction in half-life in mice (2 hours for F(ab')2-158 compared to 11 days for mAb158) which is advantageous for imaging as high PET radioligand concentrations in the blood of the brain would give rise to a large confounding unspecific signal. To further improve F(ab')2-158 as a PET ligand it was chemically linked to a transferrin receptor (TfR) antibody, enabling TfR mediated transcytosis to actively transport F(ab')2-158 over the BBB and bind to its target in the brain. After radiolabelling with 125I, the bispecific fusion protein was analysed with ELISA to confirm retained binding to both Aβ protofibrils and TfR. Next, APP-transgenic (tg-ArcSwe) and non-transgenic mice of different ages received a single injection of radioiodinated fusion protein or F(ab')2-158. After saline perfusion, brains were collected 2 h to 3 days post injection and the brain uptake was measured *ex vivo* and compared to brain levels of soluble Aβ protofibrils and total Aβ. The fusion protein displayed a ten-fold higher brain uptake compared to F(ab')2-158 and its brain retention increased with age, correlating closely with brain levels of soluble Aβ protofibrils. After labelling with a PET radionuclide, this fusion protein has the potential to become useful for PET-imaging of soluble Aβ aggregates in AD patients.

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Poster

044. Alzheimer's Disease: Imaging and Biomarkers

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 44.06/F9

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Distribution of autoradiography signal of [18F]T807 and [3H]T807 matches with that of tau antibody AT100 in post-mortem brain tissue of Alzheimer's patients

Authors: *F. GOMEZ[1], Y.-G. LIN[2], Q. LIANG[2], J. RYDER[3], H. WANG[3], G. ATTARDO[2], M. MINTUN[2], D. SKOVRONSKY[2];
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Abstract: The autoradiography of the PET biomarker [18F]T807 in postmortem brain tissue of one Alzheimer's (AD) patient has been reported to overlap with that of the anti PHF-tau antibody AT100. The present study is designed to extend these finding by using brain tissue from 10 AD patients that present different degrees of tau accumulation. In order to establish the correlation between tau aggregation and [18F]T807 autoradiography signal, the distribution of [18F]T807 was examined in the same cortical section of AD (n=10) or normal controls (n=2) subject using either autoradiography of the radioligand or AT100 immunohistochemistry. In a second set of slices a similar protocol was conducted using [3H]T807 in order to increase the resolution of the ARG signal and show cellular colocalization of the radioligand and the PHF-Tau antibody. We found that the pattern of both [18F]T807 and [3H]T807 signal distribution match closely with that of the PHF-Tau antibody. We are currently conducting the quantification of these results and the colocalization studies using [3H]T807 and AT100.

Disclosures: **F. Gomez:** A. Employment/Salary (full or part-time):; Avid RP. **Y. Lin:** A. Employment/Salary (full or part-time):; Avid RP. **Q. Liang:** A. Employment/Salary (full or part-time):; Avid RP. **J. Ryder:** A. Employment/Salary (full or part-time):; Eli Lilly. **H. Wang:** A. Employment/Salary (full or part-time):; Eli Lilly. **G. Attardo:** A. Employment/Salary (full or part-time):; Avid RP. **M. Mintun:** A. Employment/Salary (full or part-time):; Avid RP. **D. Skovronsky:** A. Employment/Salary (full or part-time):; Avid RP.

Poster

044. Alzheimer's Disease: Imaging and Biomarkers

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 44.07/F10

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH/NIA 1 R21 AG037843

Brigham Young University, College of Life Sciences, Start-Up Grant

Brigham Young University, School of Family Life, Gerontology Program

Title: Methodology for computing white matter nerve fiber orientation in human histological slices

Authors: *J. J. WISCO[1], A. NAZARAN[2], H. V. VINTERS[3], N. K. BANGERTER[2]; [1]Physiol. and Developmental Biol., [2]Electrical and Computer Engineering, and Neurosci. Ctr., Brigham Young Univ., Provo, UT; [3]Pathology and Lab. Med., David Geffen Sch. of Med. at UCLA, Los Angeles, CA

Abstract: INTRODUCTION We endeavor to validate Diffusion Tensor Imaging (DTI) tractography algorithms in histologically processed and stained white matter fibers of brain tissue specimens that have matching DTI data. Our initial manual segmentations attempts have been labor intensive, time consuming, and subjective. In this work, we present an algorithm for calculating the average directionality of nerve fibers across a given Region of Interest (ROI) in the white matter of Luxol Fast Blue stained sections through the medial temporal lobe. The presented method is rapid and computationally efficient, and is based on a threshold of the histological slices and 2D Fourier domain (FD) analysis. The calculation provides a central tendency of the nerve fibers (CTNF) in sample images in an ROI along with the confidence over the estimation (CE) of the orientation. METHODS We obtained one high-resolution scanned image of a histological section from 8 different subjects. In each image, we defined over 800 ROIs at various isotropic sizes ranging from 0.4x0.4 mm up to 0.8x0.8 mm for analysis. Reconstructing fiber directionality involved a multi-step, semi-automated process of 1) threshold segmentation on LAB color space, 2) transformation of segmented image into the FD, 3) high pass filtering and calculating the energy of the image in "angular window size" of the FD at each angle, and 4) calculating of CTNF based on an angular orientation histogram (AOH) of image energy in FD. To calculate CTNF, we compute the average orientation in the 180 degrees range in AOH where the standard deviation (SD) is minimum. We calculate CTNF in 180 degrees range due to conjugate symmetry in FD. We also use the minimum SD to calculate CE. An expert anatomist evaluated the determined fiber directionalities in comparison with visually inspected fiber directionality from the same ROI's. RESULTS Our semi-automated method agrees with that of the expert visualization, but also recognizes nuances of microstructure that contribute to the calculated fiber orientation in each ROI. The method is extraordinarily fast. The same region of analysis for one tissue slice takes several months to achieve using visual judgment. Our algorithm can calculate better results in hours. In addition, with increasing resolution of the ROIs, accuracy of the orientation estimates increases, particularly in the white and gray matter interface. CONCLUSION Our research team will now be able to efficiently compare anatomical fiber directionality from histological sections with matched DTI of the same post-mortem specimens. In future, we will apply the algorithm to small biopsies and to whole-brain axial, coronal, and sagittal sections.

Disclosures: **J.J. Wisco:** None. **A. Nazaran:** None. **N.K. Bangerter:** None. **H.V. Vinters:** None.

Poster

044. Alzheimer's Disease: Imaging and Biomarkers

Location: Halls A-C

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NSERC, RGPIN-2014-04659

CIHR, FRN 93603

Title: The effects of physical exercise on hippocampal microvasculature in a mouse model of Alzheimer's disease

Authors: *E. MALISZEWSKA-CYNA[1,3], J. J. OORE[1], M. THEODORE[1], L. A. M. THOMASON[2], A. DORR[2], M. M. KOLETAR[2], J. STEINMAN[5,4], J. G. SLED[5,4], B. STEFANOVIC[4,2], I. AUBERT[1,3];
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Abstract: Alzheimer's disease (AD) is associated with cerebrovascular impairments including decreased vascular density, increased tortuosity, and capillary fragmentation. Moreover, global hypoperfusion has been found to correlate with amyloid-β accumulation and exacerbated cognitive decline. Current treatment strategies for AD patients focus primarily on managing symptoms but fail to address the persistent neurovascular impairment that leads to cognitive deficits. Targeting the neurovascular unit is gaining recognition as an important therapeutic strategy for AD. The difficulty in devising such treatment lies in part in the complexity and multitude of factors involved in AD pathology. Physical exercise has the potential for broad beneficial effects to prevent or delay some pathologies associated with AD, including vascular compromise. Physical fitness triggers several protective mechanisms: it can increase perfusion and neovascularization mediated by angiogenesis and it can increase the bioavailability of growth factors stimulating formation of new vessels. We hypothesize that physical exercise has beneficial effects on brain microvasculature in an animal model of amyloidosis. After developing plaque deposits and cognitive deficits, mice with amyloid pathology and their non-transgenic

littermates entered the study. Half of these mice had access to a spinning disk on which they could run freely and the other half were housed in a standard cage with no spinning disk. After 3 months, the animals were deeply anaesthetized and perfused with Nile red enriched Mercox resin to visualize the microvasculature. The brains were then isolated, dehydrated and placed in a clearing solution for 3 days. The complete microvasculature of the hippocampus was imaged by 2-photon fluorescence microscopy at 512 x 512 nominal in-plane resolution, every 2.5 um, over 0.5 x 0.5 cm x 0.1 mm region using 780 nm excitation. The microvascular network was identified using multi-scale, semi-automated tracking algorithm. Vessel diameter, length, tortuosity, and density were quantified. Pilot data suggest a 14% increase in mean length and a 22% increase in mean diameter of the hippocampal microvasculature with running resulting in restoration of vessel density in transgenic animals with running. The importance of neurovascular health for cognitive function and for combating AD is receiving increasing interest. This research has the potential to provide new treatment options, including promotion of an active lifestyle so as to prevent or delay AD pathology, ultimately providing a better quality of life in the elderly population and in AD patients.

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Poster

044. Alzheimer's Disease: Imaging and Biomarkers

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Topic: C.02. Alzheimer's Disease and Other Dementias

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Brigham Young University, College of Life Sciences, Mentoring Environment Start-Up Grant

Brigham Young University, School of Family Life, Gerontology Program

Title: The spatial relationship between iron, tangles, and plaques in the subiculum

Authors: *B. J. HERRINGTON[1], B. BARZEE[1], S. BARLOW[1], S. ROBISON[1], M. HANSEN[1], A. SALIN[1], M. STONE[2], J. BRIDGEWATER[2], T. KAVAFYAN[2], K. STEED[1,2], M. E. STARK[2], H. DONG[3], A. W. TOGA[3], H. V. VINTERS[2], J. J. WISCO[1,2,4];
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Abstract: INTRODUCTION: Alzheimer's disease (AD) appears to show its effects at a particularly early stage within the hippocampus. More specifically, the subiculum of the hippocampus has been noted as a region that is subjected to more severe pathological changes due to the disease. Hyper-phosphorylated Tau proteins (HP-tau) and amyloid-beta plaques (Abeta) are known to be associated with AD pathology, and Abeta has been shown to spatially correlate with non-heme iron (Fe). Iron induces a signal dropout in susceptibility-weighted Magnetic Resonance Imaging (MRI). This signal dropout indicates that MRI could potentially be used to detect HP-tau and Abeta *in vivo*. Our objective is to determine the spatial correlation between Abeta, HP-tau, and Fe within the subiculum. Our hypothesis is that both proteins should exhibit spatial correlation with Fe. METHODS: Hippocampi were sectioned (6 microns thick) and serially stained using immunohistochemistry for tau, Abeta, and Fe. Each slide was digitally scanned and viewed using Leica Digital Image Hub. Images of the subiculum from each section were exported to Adobe Photoshop CS6 and pseudocolored according to each stain. Each image was then aligned together using a manual affine registration and elastic warp. We compared the spatial overlap of each stain. Our data set consisted of 5 hippocampi from deceased subjects: a 76-year-old (yo) F with cerebrovascular disease (CVD), AD Braak Stage VI and diffuse Lewy Body Disease; a 96 yo F with CVD and Braak VI; one 70 yo M with Braak VI only; an 86 yo M with Braak IV-V; and our control, an 81 yo F who suffered from scleroderma and severe pulmonary hypertension. RESULTS: A spatial correlation was observed between Abeta and Fe in the subiculum of all four of our disease subjects. HP-tau, although showing an overlap with Fe, was much more widespread throughout the subiculum in these four subjects. Our 81 yo F control did not show co-localization between Fe, Abeta, or HP-tau. CONCLUSION: Our lab previously demonstrated that in individuals with Braak VI, there exists a spatial correlation between only Abeta and Fe in the hippocampus, but in the entorhinal cortex, a co-localization between HP-tau and Fe only. This new data indicates that in the Braak VI subiculum, Abeta spatially correlates with only Fe. This has sensitivity and specificity implications for diagnostic susceptibility weighted MR imaging. Our observation of widespread HP-tau in the subiculum is consistent with the widespread HP-tau usually observed in later Braak stages (V and VI). In future studies, there should be an attempt to explore the co-localization of HP-tau with Fe in earlier Braak stages.

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Poster

044. Alzheimer's Disease: Imaging and Biomarkers

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 44.10/G1

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Detection of soluble amyloid beta and Tau proteins in the interstitial fluid of non-human primates by *in vivo* microdialysis

Authors: *A. RASSOULPOUR[1], M. HEINS[1,2], G. FLIK[1], I. VEINBERGS[1], J. FOLGERING[2], J. SUTCLIFFE[2], C. SCHLUMBOHM[2], T. CREMERS[2];
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Abstract: Alzheimer's disease (AD) is the most common cause of age-related cognitive decline, affecting more than 35 million people worldwide. Amyloid plaques of which amyloid is the main component and neurofibrillary tangles consisting of fibrillar tau aggregates are both hallmarks of Alzheimer's disease. In recent years, much attention has focused on the soluble forms of amyloid and tau proteins that are released by cells and can be found in the interstitial fluid (ISF) surrounding cells. These soluble forms of the proteins are hypothesized to have a biological role on their own, and, in addition, contribute to the pathophysiology of AD and other neurodegenerative diseases. Previous work using *in vivo* microdialysis of the rodent brain has demonstrated the ability to detect the soluble forms of these proteins by microdialysis in mouse ISF as well as to modulate their levels in the CNS, therapeutically (Cirrito el al, 2003; Yamada et al., 2011; Rassoulpour et al., 2010). In the current study we utilized push-pull microdialysis in the hippocampus and prefrontal cortex of 4-5 year old cynomologous monkeys to measure $A\beta_{42}$ and total-Tau protein in the ISF. In addition, $A\beta_{42}$ and total-Tau proteins were measured in the CSF and plasma of the same animals. We found that both $A\beta_{42}$ and total-Tau protein can be detected in the ISF from the cynomologous monkey. Analysis of tissue from the hippocampus and prefrontal cortex, CSF and plasma revealed differential expression across areas. Current studies are underway to further examine the levels of these proteins in response to pharmacological manipulation and to compare with the effects seen in rodents. Taken together with future pharmacological data in multiple species and compartments, this data may provide

greater insight into the utility of *in vivo* microdialysis evaluation of pathogenic proteins as an impactful translational method in neurological disease drug discovery.

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Poster

044. Alzheimer's Disease: Imaging and Biomarkers

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Brigham Young University, Office of Research & Creative Activities Grant NIH/NIA 1 R21 AG037843

Brigham Young University, College of Life Sciences, Start-Up Grant

Brigham Young University, School of Family Life, Gerontology Program

Title: Spatial correlation between iron, plaques, and tangles in the entorhinal cortex may present iron to be a potential biomarker for alzheimer's disease using MRI

Authors: ***S. H. BARLOW**[1], M. HANSEN[1], A. SALIN[1], B. BARZEE[1], K. STEED[1,2], J. J. WISCO[1,2,3], M. STONE[2], J. BRIDGEWATER[2], T. KAVAFYAN[2], E. STARK[2], H. V. VINTERS[2], H. DONG[4], A. W. TOGA[4], B. J. HERRINGTON[1];
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Abstract: INTRODUCTION: Although Alzheimer's Disease (AD) is one of the highest causes of death in the United States, there are still very few effective treatments and many of these treatments focus on slowing down the effects of the disease, not curing it. The effects of AD are usually not seen until AD has progressed for a long time. Finding a way to diagnose AD early is an important step in curing the disease. The entorhinal cortex is one of the first regions of the brain to exhibit AD pathology - the buildup of Amyloid-beta plaques (Abeta) and hyper-phosphorylated Tau protein tangles (HP-tau). Non-heme iron (Fe) has been shown to spatially correlate with Abeta. Since Fe causes a signal dropout in susceptibility-weighted Magnetic Resonance Imaging (MRI), this imaging modality could possibly be used as a way to detect

Abeta in the brain. The purpose of our research was to confirm that iron correlated spatially to Abeta in the entorhinal cortex. Also, we wanted to see if HP-tau also correlated to iron in the entorhinal cortex. METHODS: We stained entorhinal cortex regions that were sectioned 6 microns thick for Abeta, HP-tau, and Fe. Each section was scanned and viewed with Leica Digital Image Hub. We then exported images of the entorhinal cortex from different sections to Adobe Photoshop CS6, pseudo-colored images of the different stains, and aligned the images of the different stains on top of each other with a manual affine registration and elastic warp. We then qualitatively analyzed the spatial overlap of the three stains. We obtained our data from entorhinal cortex regions of five deceased subjects: a 76-year-old (yo) F with cerebrovascular disease (CVD), AD Braak Stage VI and diffuse Lewy Body Disease; a 96 yo F with CVD and Braak VI; one 70 yo M with Braak VI only; a 86 yo M with Braak IV-V; and our control, an 81 yo F who had scleroderma and pulmonary hypertension. RESULTS: We confirmed that Fe spatially correlates with Abeta, but much less so with HP-tau in the entorhinal cortex in the Braak IV-V subject. The 81 yo F control did not show spatial correlation between Fe, Abeta, or HP-tau as expected. However, in the Braak VI subject, this trend was reversed: we saw more correlation between Fe and HP-tau than between Fe and Abeta. CONCLUSION: A signal dropout in the entorhinal cortex due to Abeta using MRI may be a method to diagnose AD in its earlier stages. A signal dropout in the entorhinal cortex due to HP-tau could be a potential diagnosis for AD in its later stages. In the future, we want to look at the correlation of iron to Abeta and HP-tau in earlier stages of AD.

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Poster

044. Alzheimer's Disease: Imaging and Biomarkers

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 44.12/G3

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: *In vivo* investigation of brain glucose utilization in APP-PS1 mice: [18F]FDG micro-pet vs [14C]2-DG autoradiography

Authors: C. WINTMOLDERS[1], A.-M. WALDRON[2], A. BOTTELEBERGS[1], J. KELLEY[1], S. STAELENS[2], *X. LANGLOIS[1];
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Abstract: BACKGROUND: Brain glucose utilization imaging, such as FDG-PET, is proving to be a powerful tool for the detection and monitoring of neurodegenerative diseases such as Alzheimer's Disease (AD). The reduction of brain glucose utilization in patients is most likely related to AD-associated neuropathology; however the exact causality still needs to be established. Studying brain glucose metabolism in transgenic AD mouse models may help to further characterize this causality. Our previous studies in TASTPM mice showed a negative correlation between amyloid plaque load and brain glucose uptake using [18F]FDG micro-PET and [14C]2-DG autoradiography. The present study was conducted to confirm this finding in another transgenic model, the APP-PS1 mice, and to compare the sensitivity and reliability of [18F]FDG micro-PET and [14C]2-DG autoradiography by comparing the measurements of brain glucose uptake within the same animal. METHODS: Brain glucose uptake was measured in 12-month-old female APP-PS1 mice which overexpress human mutated APPswe (KM670/671NL) and PS1 (L166P). First, static µPET scans were acquired after intravenous injections of [18F]-FDG and a conscious uptake period of 45 minutes. In addition, mice were scanned with [18F]-Florbetapir to assess amyloid burden. Six weeks after the µPET scans, [14C]2-DG was administered by intraperitoneal injections. The animals were sacrificed after an uptake period of 45 minutes and brain tissue was processed for autoradiography and immunohistochemistry. RESULTS: The results demonstrated that 12-month-old APP-PS1 mice display a global hypo-metabolism compared to wild type control mice. The outcome for both of the brain glucose utilization detection methods was similar. Also, high amyloid burden could be detected with amyloid PET and immunohistochemistry. CONCLUSION: The *in vivo* [18F]FDG/[18F]-Florbetapir PET results were confirmed by the *ex vivo* [14C]2-DG autoradiography /immunohistochemistry experiments, thus demonstrating the translatability of these findings. All animal studies were ethically reviewed and carried out in accordance with European Directive 86/609/EEC Welfare and Treatment of Animals.

Disclosures: **C. Wintmolders:** A. Employment/Salary (full or part-time):; Janssen Research and Development. **X. Langlois:** A. Employment/Salary (full or part-time):; Janssen Research and Development. **A. Bottelebergs:** A. Employment/Salary (full or part-time):; Janssen Research and Development. **J. Kelley:** A. Employment/Salary (full or part-time):; Janssen Research and Development. **S. Staelens:** 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.; Janssen Research and Development. **A. Waldron:** 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.; Janssen Research and Development.

Poster

044. Alzheimer's Disease: Imaging and Biomarkers

Location: Halls A-C

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Gift from T. and P. Grossman

Title: Toward specific Aβ oligomer aptamers

Authors: *K. M. NELSON[1], S. SREEVATSAN[2], K. H. ASHE[3], M. A. WALTERS[1];
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Abstract: Alzheimer’s disease (AD) is the most common neurodegenerative disease in the United States. While the exact cause of AD remains undefined, compelling genetic evidence suggests the accumulation of neurotoxic oligomers and fibrils of the amyloid-β peptide (Aβ) trigger disease onset. It is generally accepted that oligomers are more neurotoxic than fibrils, and several oligomers have been isolated from brain tissue and characterized to varying degrees. There is no consensus, however, on which of these oligomers are the most important in disease pathogenesis. The methods used to characterize oligomers vary between laboratories, making it difficult to compare results. Recent biophysical studies of a variety of Aβ amyloid fibrils have yielded atomic-resolution structures that differ in detail but are substantially similar in specific quaternary structural motifs, which serve as an organizing principle around which fibrils may be classified. The structure of Aβ oligomers however, remains elusive. New evidence indicates that different oligomers may affect neurons through separate pathways. We hypothesize that specific neurotoxic oligomers must have distinct quaternary structures that dictate their various biological functions. We propose to decipher the distinctive structural motifs of specific Aβ oligomers using single-stranded DNA aptamers. We are building a toolkit of aptamers that bind selectively to different classes of Aβ assemblies. We will establish their basis of binding using NMR and use this information to infer the structure of specific Aβ oligomers in humans with AD and transgenic mice that model AD. Quaternary structure-specific aptamers may provide mechanistic insights into the neurotoxicity of Aβ oligomers, and aid the development of diagnostic tools and therapeutic reagents.

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Poster

044. Alzheimer's Disease: Imaging and Biomarkers

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: CIHR

Canadian Partnership for Stroke Recovery

Title: The differential effect of exercise on motor cortex vasculature in a model of Alzheimer's disease

Authors: L. A. M. THOMASON[1], E. MALISZEWSKA-CYNA[2], J. STEINMAN[3], *I. AUBERT[2], J. G. SLED[3], B. STEFANOVIC[1];
[1]Physical Sci., [2]Brain Sci. Res. Program, Sunnybrook Res. Inst., Toronto, ON, Canada; [3]Mouse Imaging Ctr., Toronto, ON, Canada

Abstract: Alzheimer’s disease (AD) is a neurodegenerative disorder that is the leading cause of dementia world-wide. While the primary pathological hallmarks of AD remain amyloid-beta plaques and neurofibrillary tangles, there is growing interest in the role of the brain vasculature in the disease etiology and progression. Exercise is known to increase angiogenesis and has been shown reduce amyloid-beta load and improve cognitive function in mouse models of AD. We were thus interested in assessing the effect of exercise on the vasculature in the motor cortex of a transgenic mouse model of AD (TgCRND8). Male and female TgCRND8 and their non-transgenic littermates were given access to a running wheel from 3-months of age until 6-months of age. At 6 -months of age, TgCRND8 mice were injected (IP) with Methoxy XO4 to label amyloid-beta and sacrificed 24 hours later. TgCRND8 and non-transgenic littermate mice were perfused with a mercox-BABB (Benzyl Alcohol-Benzyl Benzoate) solution containing Nile red to visualize the microvasculature. After fixation in 4% paraformaldehyde, the brain was dehydrated progressively with methanol and cleared by BABB. Four 0.5x0.5x1mm3, partially overlapped (by ~25%), regions in the primary motor cortex (M1) were imaged on a two photon fluorescence microscope, using 780nm excitation. Acquired volumes were deconvolved and stitched together. A multi-scale automated segmentation algorithm was used to track the

vasculature. Our preliminary results suggest that vessel density is decreased by ~20% in the motor cortex of the TgCRND8 mice compared to their non-transgenic littermates and the 3-month exercise regimen partially resolves this effect. These data support further investigation into the functional consequences of exercise as a potential regimen attenuating the progressive decrease in vascular density in AD.

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Poster

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIA NIH 5R01 AG021927

UAB Department of Neurology

Title: Functional connectivity changes associated with financial capacity impairment in prodromal and clinical Alzheimer's disease

Authors: *T. A. BARTEL[1], D. L. KERR[1], D. G. MCLAREN[2], D. C. MARSON[1]; [1]Neurol., Univ. of Alabama At Birmingham, Birmingham, AL; [2]Neurol., Massachusetts Gen. Hosp. and Harvard Med. Sch., Charlestown, MA

Abstract: INTRODUCTION: The key IADL of financial capacity (FC) clinically shows declines early in prodromal and clinical Alzheimer's disease (AD). Prior structural MRI studies have shown relationships between cortical atrophy and FC decline in patients with mild cognitive impairment due to AD (MCI) and mild dementia due to AD (AD). Here we build on this work by examining the relationship of DMN resting state functional connectivity to FC in cognitively normal older adults (CN), patients with MCI, and patients with AD. METHODS: Participants consisted of 51 CN, 27 MCI, and 31 AD patients diagnosed by study consensus conference. Participants completed the Financial Capacity Instrument (FCI) and a resting state fMRI scan. Time series data was extracted from four seed regions: (1) the posterior cingulate cortex (PCC), (2) medial prefrontal cortex (mPFC), and (3/4) left/right inferior parietal lobules (lIPL, rIPL). Seed region time courses were correlated with the whole brain. Correlations were

then converted to Z-scores using Fisher's z-transform. Two-sample t-tests assessed connectivity differences between groups. The relationship between connectivity and FCI score was assessed using multiple regression models including age and education as covariates. Results were thresholded at $p<0.01$ in at least 10 voxels. RESULTS: Significant differences in FCI score existed across groups ($p<0.001$). Consistent with prior fMRI studies, we also found DMN connectivity decreases in the MCI and AD groups relative to CN. For CN, we found positive relationships between FCI score and DMN connectivity with the right middle frontal gyrus (all seed regions). For MCI patients, we found positive relationships in the superior frontal gyrus (lILP, rIPL, mPFC) and superior parietal lobule/precuneus (lIPL, mPFC). For AD patients, we found positive relationships with insular cortex (lIPL, rIPL), superior temporal lobe (lIPL, mPFC, PCC), and posterior cingulate/precuneus (rIPL, PCC). CONCLUSIONS: This study suggests that intrinsic connectivity changes in prodromal and clinical AD are linked to impaired FC. Using cortical DMN nodes as seed regions, we found that FC, as represented by the FCI, is linked to connectivity within and between multiple brain networks in AD. The results provide initial evidence that functional connectivity metrics can augment neuroanatomical and cognitive models of FC decline, and provide insights into how network dysfunction impairs FC in AD.

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Poster

044. Alzheimer's Disease: Imaging and Biomarkers

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Brigham Young University, School of Family Life, Gerontology Program

Title: MR relaxometry of short-T2 tissues using 3D ultra-short echo time MRI in *ex vivo* brain with known Braak VI taopathy

Authors: ***A. NAZARAN**[1], N. BANGERTER[2], K. PERKINS[2], D. PARK[2], H. V. VINTERS[4], J. J. WISCO[4,3,5];

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Abstract: INTRODUCTION: The location and amount of proteins in the brain can be used to discriminate between different neurological diseases. However, it is very difficult to directly image proteins in the brain with MRI due to a very weak and rapidly decaying (short T2*) MR signal that is overwhelmed by the much stronger and longer-lasting (longer T2*) signal from other water components in the brain. Normal MRI techniques are not suitable for probing the signal of very short T2* tissues, since the signal from these tissues has typically decayed before it is sampled. Here, we explored the use of a custom 3D ultra-short TE (UTE) technique to detect signal from very short T2* tissue in an *ex vivo* brain with known Braak VI taopathy. With the technique, we were able to detect the MR signal from tissues with T2* values of less than 1ms. We were further able to quantify both the T1 and T2* of the short-T2* tissues detected. METHODS: We implemented a 3D UTE MRI sequence with a 3D cones k-space trajectory, and conducted *ex vivo* scans on a 3 Tesla scanner of a formalin fixed human temporal lobe from a subject with known Braak VI taopathy, heaviest in the hippocampus and para hippocampal gyrus. We chose an area of the brain for our study that we expect to have high volume of beta amyloid and tau proteins. We then acquired the UTE images at TEs of 0.4, 0.6, 0.8, 1, 1.2, 1.5, 2, 2.5, 3, and 3.5ms and TR of 16ms. Resolution was 1mm isotropic and the FOV was 15 cm in all directions. Each of these scans was repeated at flip angles ranging from 5 to 60 degrees. Difference images were then formed by subtracting the TE=3.5ms images from the TE=0.4ms images, effectively suppressing longer T2* tissues. We defined four regions of interest (ROIs) in areas with visible short T2* signal in the hippocampus and then calculated an average T2* for each ROI by fitting the signal from each echo to a simple monoexponential curve. T1 was then estimated for each ROI by ascertaining which flip angle yielded the largest signal for the corresponding ROI, and applying the Ernst angle formula relating maximum signal to flip angle and T1. RESULTS: We measured T2* values in the short T2* tissues at approximately 1.5 - 2.6ms for the ROIs having about 5-12 pixels. We hypothesize that these regions yielding short T2* MR signal correlate with areas with heavy Tau protein deposits. The T1 estimated in these regions was approximately 300ms. Short T2* signal is detected both in areas around the hippocampus as well as around blood vessels. CONCLUSION: A novel 3D UTE MRI sequence with a 3D cones k-space trajectory was used to image short T2* tissues in the hippocampus. Future work will seek to determine if the short T2* signal observed is water bound to protein deposits as hypothesized.

Disclosures: **A. Nazaran:** None. **N. Bangerter:** None. **K. Perkins:** None. **D. Park:** None. **H.V. Vinters:** None. **J.J. Wisco:** None.

Poster

044. Alzheimer's Disease: Imaging and Biomarkers

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 44.17/G8

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant U01 AG024904

Title: Increased rate of white matter integrity loss in Alzheimer's disease patients: A one-year follow up study from the Alzheimer's Disease Neuroimaging Initiative

Authors: *C. D. LEONARDO[1], T. M. NIR[1], N. JAHANSHAD[1], K. M. ESCHENBURG[1], A. W. TOGA[2], C. R. JACK JR.[3], M. A. BERNSTEIN[3], M. W. WEINER[4], P. M. THOMPSON[1,5,6,7,8];
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Abstract: Alzheimer's disease (AD) is a progressive neurodegenerative disease that affects one in nine people over age 65. Discovering biomarkers that allow efficient monitoring of the disease will help identify individuals at risk for AD and may serve as therapeutic outcome measures. Since 2010, the Alzheimer's Disease Neuroimaging Initiative (ADNI) has included diffusion tensor imaging (DTI) in the MR protocol to better understand how white matter integrity and connectivity change as AD progresses. In this longitudinal study, we monitored patients and cognitively healthy elderly people to evaluate changes in measures of white matter fiber integrity after one year. Baseline and one-year follow-up structural T1-weighted brain MRI and diffusion-weighted images (5 b0s and 41 DWI) were obtained from 35 cognitively normal subjects (age: 74.5 +/- 6.5 y; 18M/16F), 81 people with MCI (age: 73.3 +/- 7.3 y; 53M/28F), and 28 AD patients (age: 77.1 +/- 8.2 y; 21M/7F). Average white matter fiber integrity measures of fractional anisotropy (FA) and mean diffusivity (MD) were calculated from 16 tract-wise regions of interest and across the entire white matter at both time points. The percent differences in these DTI measures relative to baseline were obtained. We used a random effects linear regression model (to account for the various acquisition sites) and tested for statistical one-year differences between AD patients and healthy elderly, covarying for age and sex. We also related changes in white matter microstructure to changes in clinical dementia rating (CDR). We used the false discovery rate (FDR) to correct for multiple comparisons. AD patients had significantly greater

reductions in integrity (as determined by lower FA and higher MD) within the entirety of the white matter (WM). These effects were noted regionally in the superior longitudinal fasciculus, the inferior fronto-occipital fasciculus (IFO), and the corpus callosum (CC) for FA, as well as the corona radiata and the CC for MD. We also found a negative association between changes in CDR score (where higher values denote worse performance) and the average FA across the full white matter. This association was positive with respect to diffusivity (a greater increase in CDR corresponds to increased diffusivity and reduced white matter coherence). To a large extent, these significant associations were also localized to the same WM regions, including the IFO and the CC.

Disclosures: **C.D. Leonardo:** None. **T.M. Nir:** None. **N. Jahanshad:** None. **K.M. Eschenburg:** None. **A.W. Toga:** None. **C.R. Jack Jr.:** None. **M.A. Bernstein:** None. **M.W. Weiner:** None. **P.M. Thompson:** None.

Poster

044. Alzheimer's Disease: Imaging and Biomarkers

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 44.18/G9

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: ISAO Pilot grant

Title: *In vivo* PET imaging of Aβ plaques using 89Zr labeled Bapineuzumab in the 5xFAD mouse model

Authors: ***J. STEVENS**[1], P. MARTINEZ[1], A. FRANSSEN[1], L. DUBOIS[3], C. URBACH[4], B. BRANS[4], P.-J. VISSER[2], D. VUGTS[5], G. VAN DONGEN[5], M. LOSEN[1];
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Abstract: Background: Alzheimer's Disease (AD) is the most common neurodegenerative disorder amongst the elderly. Recently, a number of large Phase III clinical trials using passive immunotherapy have met with disappointing results. It is currently thought that treatment in these patients started too late and therefore early diagnosis of AD is becoming increasingly important. Recent advances in PET imaging tracers have provided means to detect amyloid

deposition in the brain. Our results show that it might also be feasible to use antibodies as a diagnostic tool to visualize brain pathology *in vivo*. Methods: Recombinantly produced bapineuzumab (hu-IgG1) or isotype controls were labeled with 89Zr. 100 µg antibody (3.7 MBq) was injected intraperitoneally into 6 and 9 months old female 5xFAD or wild type (WT) littermate controls. Mice were subsequently scanned for 1 hour using a Siemens Focus 120 microPET every second day over a period of up to 10 days. Activity in dissected brain regions was measured using a gammacounter, and autoradiography of brain slices was performed for validation of the PET imaging data. Results: 1 day after injection a specific binding could already be detected in 5xFAD mice injected with bapineuzumab compared to WT control animals. The signals remained stable over a period of 10 days. No difference between 5xFAD and WT animals was found in the isotype control group. While difference between 5xFAD and WT animals could readily be detected at 6 months of age, this difference increased at 9 months of age. Conclusion: This study shows the first proof of principle that anti-Aβ antibodies accumulate in the brain *in vivo*, and this within one day after injection using PET. The longer half-life of 89Zr compared to other isotopes also allows this method to be used in more clinics around the world for diagnosis of AD and also allows for the study of antibody dynamics in the brain. Furthermore, this new technique holds great promise for the further study of AD, by using for instance antibodies directed against different forms of Aβ.

Disclosures: **J. Stevens:** None. **P. Martinez:** None. **D. Vugts:** None. **G. van Dongen:** None. **L. Dubois:** None. **C. Urbach:** None. **B. Brans:** None. **P. Visser:** None. **M. Losen:** None. **A. Franssen:** None.

Poster

044. Alzheimer's Disease: Imaging and Biomarkers

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 44.19/G10

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Blood-brain barrier breakdown as the starting point of cerebrovascular diseases: A dynamic contrast-enhanced magnetic resonance imaging study

Authors: *A. MONTAGNE[1], S. BARNES[2], M. HALLIDAY[1], M. SWEENEY[1], A. SAGARE[1], A. AHUJA[1], M. LAW[3], H. CHUI[4], R. JACOBS[2], B. ZLOKOVIC[1];
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Caltech, Pasadena, CA; [3]Dept. of Radiology, [4]Dept. of Neurol., Keck Sch. of Med. - USC, Los Angeles, CA

Abstract: There is increasing evidence showing blood-brain barrier (BBB) compromise in individuals with vascular cognitive impairment including those with mild cognitive impairment (MCI) stage and Alzheimer's Disease (AD). Furthermore, apolipoprotein E4 (apoE4), known as the most prevalent genetic risk factor for AD, has direct toxic effect on the cerebrovascular system and has been associated with an increased susceptibility of the BBB to injury. To explore these relationships, we assessed the BBB permeability (Ktrans, blood-to-brain transfer) in different brain regions using dynamic contrast-enhanced magnetic resonance images (DCE-MRI). The DCE images were analyzed using various mathematical models including the commonly used Patlak analysis. In total, we performed neurological testing, BBB permeability measurements with DCE-MRI, and lumbar puncture to measure vascular injury biomarkers and determine the effects of apoE genotype in a cohort of 28 patients. Patients were separated clinically into young normal controls (n=6; age range 23-47), age-matched to MCI normal controls (n=11; age range 55-90) and MCI (n=11; age range 55-84). We found an age-dependent BBB leakage in the cohort of cognitively normal individuals (n=17; age range 23-90), especially within the hippocampus CA1 region and Dentate Gyrus (DG). We also found that the BBB integrity is more affected in MCI patients compared to age-matched controls, which is most pronounced in the hippocampus CA1 region ($p<0.001$). Preliminary data also suggest that apoE4 carriers (n=5) have an increased BBB permeability compared to non-carriers (n=9), once more in the above-mentioned brain regions. Our imaging data were corroborated by increased values of albumin quotient and increased cerebrospinal fluid (CSF) levels of sPDGFRβ (soluble Platelet-Derived Growth Factor Receptor-Beta), a marker of mural cells pericytes. These results show that changes to the cerebrovasculature occur in the hippocampus in an age-dependent manner and worsen in MCI patients and apoE4 carriers. The different DCE models that were used to get Ktrans gave different absolute values but nearly identical results between group comparisons, suggesting the results are robust to the specific pharmacokinetic model used and not an artifact of the modeling. This paradigm should bring further understanding regarding the mechanism of the BBB disruption and recovery, help establish the relationship between subtle BBB disruption and cognitive decline, and possibly serve as an early indicator of neurovascular dysfucntion associated with cognitive impairment.

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Poster

044. Alzheimer's Disease: Imaging and Biomarkers

Location: Halls A-C

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Program#/Poster#: 44.20/G11

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: P01 AG026276

T32AG00035

IS10RR022984-01A1

Title: Altered task-evoked fMRI activity in preclinical Alzheimer's disease

Authors: *B. A. GORDON[1], T. BLAZEY[2], T. L. S. BENZINGER[3], J. C. MORRIS[4], A. M. FAGAN[5], D. M. HOLTZMAN[5], J. ZACKS[6], D. A. BALOT[6];
[2]Div. of Biol. and Biomed. Sci., [3]Radiology, [4]Knight Alzheimer's Dis. Res. Ctr., [5]Neurol., [6]Psychology, [1]Washington Univ. In St Louis, St Louis, MO

Abstract: Background: There is a growing emphasis on examining the preclinical stages of Alzheimer's disease (AD). Such states can be defined in non-demented control individuals according to levels of AD pathology measured using amyloid imaging or cerebrospinal fluid levels of Aβ42, tau, and p-tau. Rising levels of such pathology precedes the onset of cognitive dysfunction. It is critical to understand how functional networks in the brain respond in such preclinical phases in the absence of significant cognitive impairment. In a population of cognitively normal adults we examined whether levels of pathology correlated with altered blood oxygenation levels in two tasks requiring sustained attention. Methods: Participants consisted of 66 cognitively normal individuals (mean age=63.9, females=42), with no evidence of clinical dementia as reflected by a Clinical Dementia Rating of 0. Participants performed two mixed-design fMRI tasks. Each task alternated between short rest blocks and longer task blocks, with randomly jittered inter-trial intervals within a test block. The first task required participants to make a living/nonliving judgment to presented words. The second task was a Stroop task, where participants responded to the color of words where the color information was congruent, incongruent, or had no overlap with the word information. AD biomarkers were quantified using mean cortical binding potential (MCBP) from an amyloid PET session, and levels of tau, ptau, and Aβ42 were obtained from cerebrospinal fluid. Analyses controlled for the age, gender, and education level of the participants. Results: When examining block-level effects, each task elicited robust activations in visual, dorsal attentional, and salience networks and deactivations in the default-mode network (DMN). For both tasks, higher levels of CSF tau and ptau were associated with greater block level activations distributed across dorsal attention and salience networks. There were no significant associations with measures of amyloid. In the first task, animate trials deactivated the DMN less and inanimate trials evoked more activity in motor

regions. In the Stroop task, incongruent trials elicited more activity in the anterior cingulate cortex, left lateral parietal regions, and bilaterally in the dorsolateral prefrontal cortex. There were no significant associations between trial-level effects and any AD biomarkers. Conclusions: This study demonstrates altered functional activity related to CSF markers of neurodegeneration in the presence of no behavioral impairment. The observed effects were most prominent in functional networks thought to support attentional control.

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Poster

044. Alzheimer's Disease: Imaging and Biomarkers

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 44.21/G12

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NHMRC project grant APP1024966

Title: Regiospecific loss of essential sphingolipids in the early stages of alzheimer's disease

Authors: ***N. KAIN**[1], T. COUTTAS[1], A. DON[1], B. GARNER[2];
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Abstract: Background: The greatest genetic risk factor for Alzheimer's disease (AD) is the ε4 allele of the brain lipid transporter Apolipoprotein E, which directly regulates secretion of the

potent neuroprotective signaling lipid Sphingosine 1-Phosphate (S1P) from astrocytes in the brain1. S1P synthesis is catalysed by sphingosine kinases 1 and 2 (SphK1 and 2)2. In this study, we investigated whether alterations to S1P metabolism occur early in AD pathogenesis and if the activity and protein levels of enzymes responsible for S1P formation and degradation may provide an explanation for any changes in S1P levels observed. Methods: By liquid chromatography-tandem mass spectrometry we quantified S1P levels in six brain regions that are differentially affected by AD pathology, in a cohort of 34 post-mortem brains, divided into four groups based on Braak Neurofibrillary Tangle (NFT) staging. Results: S1P levels were significantly positively correlated with SphK1 activity in the hippocampus ($P = 0.012$), providing the enzymatic basis for declining S1P with increasing NFT pathology. Hippocampal S1P levels were 2.5-fold higher in ApoE2 carriers compared to ApoE4 carriers, and multivariate regression analysis indicated that hippocampal S1P levels are significantly influenced by both Braak stage and ApoE genotype. Conclusions: This is the first report describing loss of S1P and sphingosine kinase activity in pre-clinical AD pathogenesis. Furthermore, SphK1 has been reported to positively regulate glutamate secretion, long-term potentiation, and synaptic strength in hippocampal neurons3, establishing a rationale for further exploration of S1P and its receptors as possible targets of interest in AD therapy. 1.Soliven, B., Miron, V., & Chun, J. (2011) Neurology. 76:8, S9-14 2.van Echten-Deckert, G. & Walter, J. (2012) Progress in Lipid Research. 51, 378-93. 3.Kanno, T., Nishizaki, T., Proia, R.L., Kajimoto, T., Jahangeer, S., Okada, T. & Nakamura, S. (2010) Neuroscience. 171:973-80.

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Poster

044. Alzheimer's Disease: Imaging and Biomarkers

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 44.22/H1

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Classification of Alzheimer's disease using nonlinear independent component analysis

Authors: *D. DUNCAN, T. STROHMER;
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Abstract: Objective: Alzheimer's Disease (AD) is the most common type of dementia and affects over 5 million people in the United States and over 35 million people worldwide; these numbers are expected to grow significantly. Therapeutic intervention is most likely to be

beneficial in the early stages of the disease, so it is important to identify the disease as early as possible in order to administer medication that will effectively stop the disease. The goal of this study is to classify magnetic resonance imaging (MRI) data of brains of patients with AD and those without AD and then to identify changes in brain MRI in early stages of AD. Methods: A novel approach based on the diffusion map framework, a leading manifold learning methods, is used for this classification. Diffusion mapping provides dimensionality reduction of the data and pattern recognition that can be used to distinguish brains of patients with AD from the controls. Since diffusion mapping may detect abnormal behavior in the data, it can be used to determine differences in brains of patients with AD. Diffusion mapping assumes access to the underlying process that it aims to reveal, but in MRI data, the relationship between the images and the underlying brain activity may be stochastic, and the data are assumed to be noisy due to calibration. Thus diffusion mapping is not the most suitable approach to use with the MRI data. An extension of diffusion maps that uses local principal components analysis (PCA) constructs coordinates that generate efficient geometric representations of the complex structures in the MRI. A data-driven adapted distance is used between blocks of MRI data to approximate the Euclidean distance between the features from the data that are considered noisy due to calibration differences. Results: Neuroimaging has been shown to be a powerful tool for studying changes in the progression of AD. MRI scans are useful for identifying features that can help predict which patients will develop AD. The data used are from the Alzheimer's Disease Neuroimaging Initiative (ADNI), a worldwide project in which subjects who have mild cognitive decline and memory loss problems are recruited to clinical trials for MRI scans. The algorithm is tested on MRI data from patients who developed AD and cognitively normal subjects (controls). Conclusions: The important difference in our method and other methods that have been used to classify and detect early onset of AD in patients is the nonlinear and local network approach, which is necessary to eliminate the calibration differences of MRI data of patients with different shapes and sizes of brains as well as to account for the various types of scanners and centers collecting data.

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Poster

044. Alzheimer's Disease: Imaging and Biomarkers

Location: Halls A-C

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NSF GRFP Grant No. DGE-0707424

Title: Comparison of subcortical morphometry in alzheimer's disease and HIV positive subjects

Authors: *B. S. WADE[1,2], S. H. JOSHI[3], M. REUTER[4], E. S. DAAR[5], T. B. CAMPBELL[6], G. SCHIFITTO[7], E. SINGER[8], R. COHEN[9], M. S. BROWN[10], X. HUA[2], J. R. ALGER[8], D. F. TATE[11], B. A. NAVIA[12], P. M. THOMPSON[2];
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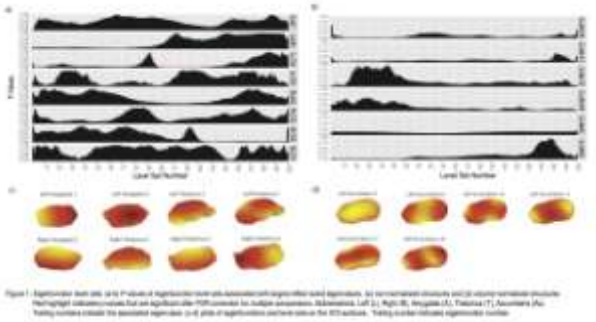
Abstract: HIV/AIDS is associated with cognitive impairment and in some cases with dementia, but the profile of atrophy differs from that seen in Alzheimer's disease (AD). Over 50% of HIV+ individuals show significant neurocognitive impairment. HIV preferentially affects white matter and several subcortical structures even in patients on combination antiretroviral therapy. The differential profiles of atrophy may be functionally relevant in these populations. Here, we compared the morphometry of several subcortical structures in AD and HIV+ participants using shape-based and volumetric analyses. We studied 210 HIV+ participants (48.6 ± 8.4 years; 175 men and 35 women) scanned by the HIV Neuroimaging Consortium and 144 AD subjects (76.4 ±7.3 years; 77 men and 67 women) from the ADNI dataset. FreeSurfer was used to segment the accumbens, amygdala, caudate, cerebellum, hippocampus, pallidum, putamen and thalamus. We studied the global intrinsic geometry and local surface topology of the structures' surfaces using the Laplace-Beltrami spectrum of eigenvalues (EVs) and eigenfunctions (EFs), respectively, using Shape-DNA. The first 20 EFs and 200 corresponding level sets were computed for each structure. Level set lengths were used as a metric of local shape variation. To test for the effect of diagnosis on local shape, a regression of level set length on diagnosis, age, sex and acquisition location was used. As expected, subcortical volumes are generally smaller in AD than in HIV. In the shape domain, the left amygdala (EVs 1 and 3), left thalamus (EVs 2 and 5), right amygdala (EV 2), right thalamus (EVs 2, 3 and 5) and left accumbens (EVs 3, 5, 10, 11, 17 and 18) were significantly different. Only the lengths of level sets 104 – 131 along the second EF of the left thalamus were significant after FDR (Figure 1). We report differential patterns of atrophy in AD and HIV which may add to our understanding of these diseases. Future work will determine whether these shape differences can determine the functional relevance of these differences and predict longitudinal outcomes in HIV and AD.



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Poster

044. Alzheimer's Disease: Imaging and Biomarkers

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Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 44.24/H3

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: grant from the Ministry of Science, ICT, and Future Planning, Republic of Korea (grant no. 2013M3C7A1069644)

SNUH-SK Telecom Grant (grant no: 2013-0405)

Title: Distinct patterns of structural brain network in mild cognitive impairment with high and low amyloid-beta burden: Graph-theoretical analysis of diffusion tensor imaging

Authors: ***D. LEE**[1], E. SEO[2], J. PARK[3], J. HAN[1], D. YI[1], B. SOHN[4], Y. CHOE[1], M. BYUN[1]; [1]Jongno-gu, Seoul Natl. Univ. Hosp., Seoul, Korea, Republic of; [2]Chosun Univ., Kwangju, Korea, Republic of; [3]Hanyang Univ., Seoul, Korea, Republic of; [4]Boramae Med. Ctr., Seoul, Korea, Republic of

Abstract: *Background:* Amnestic mild cognitive impairment (aMCI) has been considered as a prodromal state of Alzheimer disease (AD). Approximately 40% of aMCI individuals, however, show very low beta-amyloid protein (Aβ) deposition, not sufficient for being regarded as AD. Very little is known about the differences of brain alterations between aMCI individuals with very low Aβ burden and those with high Aβ burden.*Objectives:* This study aimed to compare the characteristics of structural brain networks between aMCI with high and low Aβ burden using graph theoretical analysis of diffusion tensor imaging (DTI). *Methods:* We included 25 aMCI and 23 cognitively normal (CN) elderly. They underwent clinical evaluation, carbon-11-labelled Pittsburgh compound B (^{11}C-PiB) positron emission tomography (PET), DTI, and neuropsychological tests. Based on ^{11}C-PiB uptake values, aMCI group was divided into low Aß burden group, so called PiB-negative aMCI (aMCI_PiB-, n=11) and high Aß burden group, so called PiB-positive aMCI (aMCI_pib+, n=14). All CN elderly had very low Aß burden. Brain regions except cerebellum were parcellated into 90 regions of interest (ROIs) which served as nodes. Edges were determined by DTI tractography with a threshold of four fibers. WM networks were constructed for each subject and analyzed using graph theoretical approaches. *Results:* The overall MCI group showed reduced small-world index compared with CN elderly group (p=0.049). Subgroup analysis revealed that aMCI_PiB+ showed no significant change compared with CN. In contrast, aMCI_PiB- showed reduced local clustering compared with CN (p=0.049) and aMCI_PiB+ (p=0.019). In terms of neural substrates of cognitive deficits, animal fluency was associated with path length (r=-0.655, p=0.040) and local clustering (r=-0.694, p=0.026), respectively, in aMCI_PiB+, while no relation was found in a MCI_PiB-. *Conclusions:* Our results suggest that, in spite of clinical similarity, aMCI_pib+ and aMCI_pib- have distinct patterns of brain network alteration and their relation to cognitive impairments.

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Poster

044. Alzheimer's Disease: Imaging and Biomarkers

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Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 44.25/H4

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Medical Research Council Grant

NIHR Cambridge Biomedical Resource Centre Grant

Down's Syndrome Association Grant

Title: Investigating white matter alterations in people with Down syndrome

Authors: *L. R. WILSON[1], T. ANNUS[1], G. WILLIAMS[2], Y. T. HONG[2], T. FRYER[2], P. J. NESTOR[3], S. H. ZAMAN[1], A. J. HOLLAND[1];
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Abstract: Down syndrome (DS) is caused by a third copy of chromosome 21, and is the most commonly identified cause of intellectual disability. Furthermore, chromosome 21 contains the amyloid precursor protein gene and people with DS, who consequently have three copies of this gene, typically develop Alzheimer's disease (AD) in middle age. It is important to understand how amyloid accumulation leads to neurodegeneration in order to prevent dementia in DS, but also because it may inform our understanding of sporadic AD. Gaining a detailed understanding of how the structure of the DS brain differs from typical development before the accumulation of amyloid is vital, so that changes due to neurodegeneration in the presence of amyloid can be accurately mapped. Thus, the aim of this study was to determine how white matter tracts in DS may differ from the typically developing population, prior to the development of amyloid pathology. Seventeen adults with DS (7 male) and 15 typically developing controls (6 male) matched for age underwent diffusion weighted 3Tesla MRI (2mm isotropic resolution; 63 non-colinear directions). Participants with DS also underwent positron emission tomography (PET) using [11C]-Pittsburgh compound-B (PiB) to rule out the presence of fibrillar Aβ. Fractional anisotropy (FA) was compared between the two groups using tract based spatial statistics. FA in the DS group was reduced across the brain ($p < 0.001$), most extensively in tracts of the frontal and superior temporal lobes. Significant alterations were also seen in the anterior thalamic radiation; the anterior corpus callosum; the posterior cingulum; and the cerebellum, with smaller clusters in the corticospinal tracts. These results may suggest altered connectivity in frontal regions in people with DS prior to Aβ pathology. This is interesting because unlike sporadic AD, the dementia that arises in DS often manifests with changes in behaviour and personality, which are suggestive of a frontal lobe disturbance. The present results suggest that a possible explanation for this may be that developmental alterations in frontal connections in DS make these regions selectively vulnerable to accumulation of amyloid later in life.

Disclosures: **L.R. Wilson:** None. **T. Annus:** None. **G. Williams:** None. **Y.T. Hong:** None. **T. Fryer:** None. **P.J. Nestor:** None. **S.H. Zaman:** None. **A.J. Holland:** None.

Poster

044. Alzheimer's Disease: Imaging and Biomarkers

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 44.26/H5

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: WRIISC / VA Office of Public Health

Title: Mid-life precuneus hypometabolism association with traumatic brain injury history

Authors: *S. SOMAN[1,3], J. CHENG[3], J. KONG[3], J. K. FAIRCHILD[3], S. CHAO[3], L. KINOSHITA[4], M. M. ADAMSON[3], J. W. ASHFORD[3], A. J. FURST[2];
[1]Stanford Univ. / Palo Alto VA, Menlo Park, CA; [2]War Related Illness and Injury Study Ctr. (WRIISC), Stanford Univ. / Palo Alto VA, Palo Alto, CA; [3]War Related Illness and Injury Study Ctr. (WRIISC), Palo Alto VA, Palo Alto, CA; [4]Palo Alto VA, palo Alto, CA

Abstract: OBJECTIVE: To investigate whether TBI severity is associated with AD like metabolic decline independent of age in a cohort of mid-life veterans **BACKGROUND**: There is increasing evidence suggesting that a history of TBI is increasing the risk for dementia and may fasten its onset. However, it is unclear to what extent there is a specific link between TBI and AD. **DESIGN/METHODS**: A convenience sample of mid-life veterans was selected from our study center. Diagnosis of TBI severity and Post-traumatic stress disorder (PTSD) was established using research criteria. All patients underwent FDG imaging within 1 week of the examination. FDG scans were spatially normalized to the MNI FDG template in SPM8 and smoothed with at 6mm kernel. The scans were next entered into a multiple regression analysis with TBI severity as variable of interest and Age, CAPS scores, and presence of memory complaints as nuisance variables. Whole brain T-maps exploring negative correlations between TBI severity and metabolic decline were performed using family wise error (FWE) at $p < 0.05$. **RESULTS**: Our sample consisted of 50 Veterans seen at the CA WRIISC. 6 Cases were excluded due to no available CAPS scores despite positive PTSD diagnosis, and 1 subject was excluded due to extensive brain changes related to history of MS. Whole brain multiple regression analysis controlling for Age, PTSD, and Memory complaints demonstrated Glucose metabolism decreased significantly with increasing TBI severity in the right precuneus and left angular gyrus. **Conclusions:** TBI severity is associated with AD like metabolic decline independent of age.

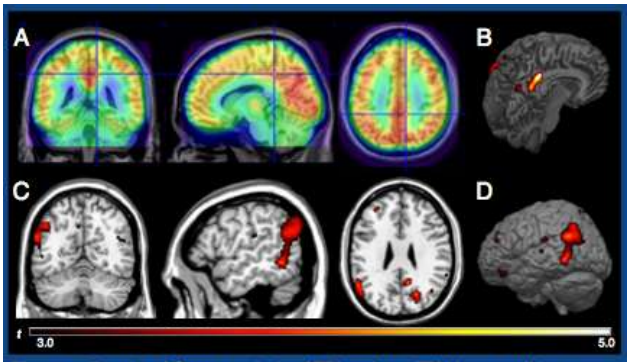

Figure Results of Group analysis of TBI and non TBI Veterans (Mean age 47) evaluated at the CA War Related Illness and Injury Study Center (CA WRIISC). (A) Average FDG-PET images for group demonstrating no clear areas of hypometabolism at the individual subject level. Cross hairs indicate area of right precuneus, with no clear hypometabolism. (B, C, D) Multiple regression analysis controlling for age, PTSD level (using CAPS score) and presence of memory complaints in same cohort of patients, demonstrating areas of hypometabolism in the right precuneus and left angular gyrus. Data is thresholded at $t > 3$ for illustration purposes (peak in the precuneus, $t = 5.45$). Results are presented in neurological orientation. The observed hypometabolic pattern is noted to be similar to that seen in Alzheimer's Disease.

Disclosures: **S. Soman:** None. **J. Cheng:** None. **J. Kong:** None. **S. Chao:** None. **M.M. Adamson:** None. **J.W. Ashford:** None. **A.J. Furst:** None. **J.K. Fairchild:** None. **L. Kinoshita:** None.

Poster

044. Alzheimer's Disease: Imaging and Biomarkers

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 44.27/H6

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIA K08 AG34628 (jointly sponsored by NIA, AFAR, the John A. Hartford Foundation, the Atlantic Philanthropies, the Starr Foundation and an anonymous donor)

NIA RC1 AG035878

NIA P50 AG016570

NIA U19 AG032438

Easton Consortium for Drug and Biomarker Discovery

NCRR/NCATS UL1 TR000124

UCLA CTSI

Title: Brain and plasma neuronal pentraxin receptor levels in a transgenic APP/PS1 rat model of cerebral amyloidosis

Authors: E. TENG[1], T. BILOUSOVA[2], K. TAYLOR[2], R. GYLYS[2], *S. A. FRAUTSCHY[3], J. M. RINGMAN[2], G. M. COLE[1];
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Abstract: Neuronal pentraxin receptor (NPR) is a synaptic protein that is almost exclusively produced in the brain and has been implicated in AMPA receptor trafficking and long-term depression. Proteomic studies indicate that NPR can be detected in cerebrospinal fluid and plasma, and suggest that it may serve as a biomarker for Alzheimer's disease (AD). However, the mechanistic association between AD pathology and brain and plasma NPR levels remains uncertain. We examined age-associated NPR expression in brain and plasma in a transgenic APP/PS1 rat model of AD in which Aβ plaque deposition begins at 9 months of age and becomes widespread by 18-20 months of age. Western blotting revealed similar levels of full-length (55 kD and 65 kD) NPR species in wild-type (Wt) and APP/PS1 rats in cortical homogenate at 3 months of age (prior to Aβ plaque deposition), but significantly increased levels of both species in APP/PS1 rats at 9 and 18-20 months of age (p's<0.02). Subsequent analyses indicated that these age-dependent differences were driven by relative increases in NPR in membrane-associated fractions, and relative decreases in NPR in soluble fractions. Genotype-related differences in NPR expression in membrane-associated fractions were also seen in the hippocampus, which exhibits significant Aβ pathology, but not in the cerebellum, where Aβ pathology is not evident. Western blotting conducted on plasma samples showed relative elevations of a 26 kD NPR fragment in APP/PS1 rats relative to Wt rats at 9 and 18-20 months of age (p's<0.02), but not at 3 months of age. In 18-20 month-old animals, plasma levels of the 26 kD NPR fragment correlated with cortical [$r(11)=0.66$, $p=0.03$] and hippocampal [$r(14)=0.67$, $p=0.01$] levels of the 65 kD full-length NPR species. We subsequently examined human plasma samples obtained from presymptomatic *PSEN1* mutation (A431E) carriers or related at-risk controls. Levels of the 26 kD NPR fragment were significantly higher with increased age ($p=0.007$) and in mutation carriers ($p=0.012$). Taken together, our findings from APP/PS1 rat brain suggest that NPR and Aβ accumulate in similar temporal and regional patterns. Aβ pathology may increase membrane-associated NPR levels via TACE inactivation. Furthermore, age- and genotype-related differences were seen in rat and human plasma levels of a 26 kD NPR fragment, which raise the possibility that this fragment may represent a peripheral biomarker of cerebral NPR levels and Aβ accumulation that may be particularly sensitive to early stages of disease progression.

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Poster

044. Alzheimer's Disease: Imaging and Biomarkers

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 44.28/H7

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Magnetic resonance spectroscopy (MRS) in CNS disease models

Authors: K. LEHTIMÄKI, *L. TAHTIVAARA, T. LAITINEN, T. AHTONIEMI, A. NURMI; Charles River Discovery Res. Services Finland Ltd, Kuopio, Finland

Abstract: Magnetic resonance imaging (MRI) is used routinely in clinical studies and imaging is becoming the only truly translatable, non-invasive biomarker to human central nervous system (CNS) diseases. MRI is applied, for example, in clinical Alzheimer's and Huntington's disease for diagnosis, and in clinical trials to assess therapeutic effects. Same imaging tools can be used as translational markers for preclinical CNS research. In addition to anatomical MRI, magnetic resonance spectroscopy (MRS) can be used to study metabolic changes in the brain to determine the relative concentrations of key brain metabolites in disease and during therapeutic interventions. In order to study brain metabolite changes in CNS disease indications and to find common nominators, various rodent CNS disease models were analyzed with *in vivo* 1H-MRS to detect changes in the following metabolites: creatine (Cr), phosphocreatine (PCr), GABA, glutamate (Glu), glutamine (Gln), myo-inositol (INS), N-acetyl-aspartate (NAA), N-Acetyl-aspartyl-glutamic acid (NAAG), taurine (Tau), and choline (Cho). CVN mouse model (APPSweDI/NOS2-/-) for Alzheimer's disease and aged rats for cognitive decline were analyzed for hippocampal metabolites, G93A-SOD1 model for ALS was analyzed for brainstem metabolites, and R6/2 and Q175 models for Huntington's disease were analyzed for striatal metabolites. In the CVN model for Alzheimer's disease and aged rat model for cognitive decline, 1H-MRS analysis of *in vivo* metabolites showed significant changes associated to aging: INS had increased whereas NAA had decreased in hippocampus. SOD1 model for ALS showed decrease in markers related to neuronal health and neurotransmission, including derecrease in NAA and GABA in brainstem. The Huntington's disease models showed typically increased glutamine and creatine, and decreased NAA in striatum. Relevant changes in brain metabolites associated to neuronal health, neurotransmission and energy metabolism can be detected with MR spectroscopy. As similar findings have also been reported in clinical studies, the results obtained highlight the importance of non-invasive *in vivo* 1H-MR spectroscopy as a translational marker to study CNS diseases.

Disclosures: **K. Lehtimäki:** None. **L. Tahtivaara:** None. **T. Laitinen:** None. **T. Ahtoniemi:** None. **A. Nurmi:** None.

Poster

044. Alzheimer's Disease: Imaging and Biomarkers

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 44.29/H8

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Polish National Science Centre grant NN401 596840

JPND grant 2/BIOMARKAPD/JPND/2012

Title: Lymphocytes from sporadic Alzheimer's disease patients display alterations in apoptosis associated with increased p21 levels

Authors: ***U. WOJDA**[1,2], J. WOJSIAT[2], K. LASKOWSKA-KASZUB[2], J. KUZNICKI[1]; [1]IIMCB, Warsaw, Poland; [2]Nencki Inst. of Exptl. Biol., Warsaw, Poland

Abstract: Despite intensive research, the pathogenesis of Alzheimer's disease (AD) is poorly understood so that an effective cure and reliable early markers are missing. There are data showing that in AD alterations in cellular processes occur not only in neurons, but also in peripheral cells such as lymphocytes. Recently, we have demonstrated in sporadic AD (SAD) lymphocytes G1 phase arrest and increased levels of p21 protein, the key regulator of G1/S cell cycle checkpoint and apoptosis, and the p53 target (1). Our current work aimed at comparing apoptotic response to oxidative stress evoked by 2-deoxy-D-ribose (2dRib) treatment in EBV-immortalized B-lymphocytes from healthy individuals, 8 patients with familial AD (FAD) bearing 8 different mutations in PS1: P117R, M139V, L153V, H163R, S170F, F177L, I213F, E318G, and 16 patients with sporadic form of AD. Using MTT assay, we found that 24h after 40 mM 2-deoxy-D-ribose (2dRib) treatment, the percentage of surviving SAD lymphocytes was significantly decreased comparing to the age-matched controls and FAD cells. In agreement with these data, early apoptosis measured by AnnexinV staining was higher in SAD lymphocytes than in control and FAD cells. Also measurements of mitochondrial membrane potential (MMP) using cationic dye JC-1 showed differences in the response to 2dRib between SAD and FAD cells: MMP of SAD lymphocytes was significantly decreased comparing to control and FAD cells. Accordingly, SAD lymphocytes showed increased percentage of cells with fragmented DNA in SubG1-phase, when compared to control and FAD lymphocytes. Comparing the

response in two control groups age-matching SAD and FAD, we found that the differences between SAD and FAD cells are not due to aging. Moreover, higher apoptotic response in SAD lymphocytes was correlated with increased levels of p21 protein. Altogether, our results showed that SAD lymphocytes are more vulnerable to 2dRib than age-matched control and FAD cells and that mechanism of apoptosis in SAD differs from FAD. Our data indicate that lymphocytes may be useful for further studies on the molecular aspects of AD pathology and for the development of new diagnostic methodologies targeting apoptotic proteins such as p21. 1. Bialopiotrowicz E, Kuzniewska B, Kachamakova-Trojanowska N, Barcikowska M, Kuznicki J, Wojda U. Cell cycle regulation distinguishes lymphocytes from sporadic and familial AD patients. Neurobiol Aging 2011 32(12):2319.e13-26. This research was supported by the Polish National Science Centre grant NN401 596840 to UW and the JPND grant 2/BIOMARKAPD/JPND/2012 to JK and UW.

Disclosures: **U. Wojda:** None. **J. Kuznicki:** None. **J. Wojsiat:** None. **K. Laskowska-Kaszub:** None.

Poster

044. Alzheimer's Disease: Imaging and Biomarkers

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 44.30/H9

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH NS077049

NIH P51RR165

Title: *In vivo* and *ex vivo* detection of lesions by MRI in an animal model of cerebral amyloid angiopathy

Authors: *E. D. HEUER[1,2], J. JACOBS[1], R. DU[2], X. ZHANG[2], S. WANG[2], O. KEIFER[3], A. CINTRON[2], J. DOOYEMA[2], L. C. WALKER[2,3];
[1]Univ. of Hawaii At Hilo, Hilo, HI; [2]Yerkes Natl. Primate Res. Ctr., [3]Dept. of Neurol., Emory Univ., Atlanta, GA

Abstract: Recent advances in Magnetic Resonance Imaging (MRI) have greatly enhanced the ability of researchers and clinicians to assess the structural and functional integrity of the aging brain. An important source of age-related cognitive dysfunction is cerebrovascular disease.

However, while high field-strength magnets and advanced neuroimaging protocols (e.g. T2* and Susceptibility Weighted Imaging (SWI)) have increased the sensitivity of diagnostic imaging, they simultaneously highlight the relative paucity of information on the precise nature of vascular-related abnormalities. Attempts to refine the interpretation of MRI anomalies have relied on immediate histological analysis of tissue samples imaged postmortem, which may not fully reflect the signal from the living brain, or on the necessarily delayed analysis of tissue samples from patients who had been imaged during life, often months or years previously. The current investigation examined the correspondence between *in vivo* MRI, *ex vivo* MRI, and post-mortem histology in a nonhuman primate model of cerebral β-amyloid (Aβ) angiopathy (CAA). Four aged squirrel monkeys (*Saimiri sciureus*), which naturally develop CAA in old age, and a younger control subject were imaged *in vivo* in a Siemens 3T MRI. Following euthanasia, the brains were fixed and then re-scanned at 3T and in a Bruker 7T MRI. Histological analysis indicated that no major lesions were missed by T2, T2* and/or SWI, i.e., no unexpected lesions were revealed microscopically in tissue sections. T2*/SWI scans consistently detected hemosiderin deposition both *in vivo* and *ex vivo*. Despite significant, widespread Aβ deposition in the brains of the aged animals, the deposits were not associated consistently with MRI signal anomalies. However, in the occipital lobe of one aged animal, a large, parenchymal, T2-TSE hyperintensity was detected *in vivo* that also included focal hypointensities revealed by T2*/SWI sequences. Histologically, this region was marked by inflammation and reactive gliosis associated with large-vessel CAA, hemosiderin, and vascular cuffing. While overall there was reasonably good correspondence between the *in vivo* and *ex vivo* images, there were occasional discrepancies. Establishing the histological basis of signal irregularities in MRI scans will be critical to the accurate interpretation of vascular-related brain changes in the elderly.

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Disclosures: **E.D. Heuer:** None. **L.C. Walker:** None. **R. Du:** None. **J. Jacobs:** None. **O. Keifer:** None. **S. Wang:** None. **A. Cintron:** None. **J. Dooyema:** None. **X. Zhang:** None.

Poster

045. Huntington's Disease Animal Models and Therapeutic Strategies

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 45.01/H10

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: MJ Murdock Grant

Title: Viral vector mediated expression of mutant huntingtin in the dorsal raphe produces neuropathology but does not induce depressive-like behaviors in wildtype mice

Authors: *M. R. PITZER[1,2], A. WARDEN[1], J. LUERAS[1], S. WEBER[1], J. MCBRIDE[2];
[1]Univ. of Portland, Portland, OR; [2]Oregon Natl. Primate Res. Cntr, Beaverton, OR

Abstract: Huntington's disease (HD) is a neurodegenerative disorder caused by the mutation in the huntingtin gene(HTT). An expansion in the gene's repeat length for the glutamine codon (CAG) renders a misfolded, dysfunctional protein that often incorporates into inclusion bodies. HD is characterized by motor and cognitive decline as well as mood disorders, with depressive symptoms being particularly common. Approximately 50% of the HD population suffers from depressive symptoms. Because these symptoms often manifest a decade or more prior to the knowledge that the person is at risk for the disease, we hypothesize that some portion of the early depression in HD is a consequence of the pathology arising from the mutant gene. While not wholly effective, depression in HD is often treated with drugs that increase serotonin availability. With this in mind, the current mouse study was designed to investigate whether the over-expression of mutant HTT in the serotonin-producing neurons of the dorsal raphe of wildtype mice can produce depressive-like behaviors. Consequently, we stereotaxically injected adeno-associated virus (AAV) encoding mutant HTT containing either a toxic (AAV-82Q) or control (AAV-16Q) CAG repeat length into the dorsal raphe of wildtype mice. Depressive-like behaviors and motor behaviors were assessed for 12 weeks following surgery. Post-necropsy, quantitative PCR and immunohistochemistry (IHC) verified AAV transduction in the dorsal raphe nucleus. IHC also demonstrated microgliosis and mutant huntingtin-positive inclusions in serotonergic neurons in animals injected with AAV-82Q but not controls. Importantly, AAV-82Q mice showed a 75% reduction in the cells stained positively for the serotonin synthesis enzyme, tryptophan hydroxylase-2 compared to controls ($p<0.05$). Despite mutant HTT-mediated pathology in the midbrain dorsal raphe neurons, no significant changes in motor or depressive-like behavior were detected. Consequently, we conclude that 11 weeks of mutant HTT expression in the dorsal raphe of wildtype mice is sufficient to reduce serotonin enzyme staining but is not sufficient to elicit depressive-like behaviors. Ongoing studies are investigating if a longer timecourse of mutant HTT expression might be necessary to elicit depressive-like behaviors or if mutant HTT expression in other regions of the brain, such as the hippocampal dentate gyrus, may play a role in HD depression. Together, these results may be helpful in addressing which therapeutic and/or pharmacological strategies could have the highest impact when treating depressive symptomology in patients suffering from HD.

Disclosures: **M.R. Pitzer:** None. **A. Warden:** None. **J. Lueras:** None. **S. Weber:** None. **J. McBride:** None.

Poster

045. Huntington's Disease Animal Models and Therapeutic Strategies

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 45.02/H11

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NIDA IRP Funds

Title: Adenosine as a biomarker of Huntington Disease

Authors: *X. GUITART[1], W. REA[1], M. ORRU[1], L. CELLAI[2], I. DETTORI[2], C. LLUIS[3], A. CORTES[3], V. CASADO[3], F. PEDATA[4], S. FERRE[5];
[1]NIDA, IRP, Baltimore, MD; [2]Univ. of Firenze, Firenze, Italy; [3]Univ. de Barcelona, Barcelona, Spain; [4]Univerity of Firenze, Firenze, Italy; [5]NIDA IRP, Baltimore, MD

Abstract: Results from genetic studies and from experiments with animal models have suggested the involvement of adenosine A_{2A} receptor ($A_{2A}R$) in the pathogenesis of Huntington Disease (HD). An $A_{2A}R$ gene polymorphism has been found to be significantly associated with a decrease in the age at onset in HD, and some controversial abnormalities of $A_{2A}R$ expression or function have been found in transgenic mouse HD models. We recently reported a complete insensitivity of the $A_{2A}R$ antagonist, KW-6002, at inducing locomotor activity in a transgenic HD rat model[5]. When trying to discover a possible $A_{2A}R$ abnormality, we found no evidence for differences in the striatal density of $A_{2A}R$ (with saturation experiments with the $A_{2A}R$ antagonist [^{3}H]ZM-241385), or in the ability of the $A_{2A}R$ agonist CGS 21680 to produce locomotor depression. Significantly, transgenic rats did not respond to the locomotor activating effects of the adenosine A_1 receptor (A_1R) antagonist CPT either. Again no evidence for differences were seen in the striatal density of A_1R (with saturation experiments with the $A_{2A}R$ antagonist [^{3}H]DPCPX) or in the ability of the A_1R agonist CPA to produce locomotor depression. The results therefore indicate the existence of a decreased striatal adenosinergic tone, which was confirmed with *in vivo* microdialysis experiments, with a significantly lower extracellular concentration of adenosine in transgenic HD rats compared to controls. These results support a switch in the focus of research in the pathogenesis of HD from the receptor ($A_{2A}R$) to the neurotransmitter (adenosine) and from the neuron to the astrocyte.

Disclosures: **X. Guitart:** None. **W. Rea:** None. **M. Orru:** None. **L. Cellai:** None. **I. Dettori:** None. **C. Lluis:** None. **A. Cortes:** None. **V. Casado:** None. **F. Pedata:** None. **S. Ferre:** None.

Poster

045. Huntington's Disease Animal Models and Therapeutic Strategies

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 45.03/H12

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: Ministerio de Ciencia e Innovación (SAF2012-39142 to S.G., SAF2011-29507 to J.A)

Cure Huntington's Disease Initiative (CHDI

Centro de Investigaciones Biomédicas en Red sobre Enfermedades Neurodegenerativas (CIBERNED CB06/05/0054 and CB06/05/0042)

Fondo de Investigaciones Sanitarias Instituto de Salud Carlos III (RETICS: RD06/0010/0006)

Title: Increased p75NTR mediates hippocampal synaptic and cognitive dysfunction in Huntington´s disease

Authors: V. BRITO[1,2,3], A. GIRALT[1,2,3], L. ENRIQUEZ-BARRETO[4], M. PUIGDELLÍVOL[1,2,3], N. SUELVES[1,2,3], A. ZAMORA-MORATALLA[5], J. J. BALLESTEROS[5], E. D. MARTIN[5], N. DOMINGUEZ-ITURZA[4], M. MORALES[4], J. ALBERCH[1,2,3], *S. GINES-PADROS[1,2,3];
[1]Med. School, Univ. of Barcelona, Barcelona, Spain; [2]Inst. d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona, Spain; [3]Ctr. de Investigación Biomédica en Red sobre Enfermedades Neurodegenerativas (CIBERNED), Barcelona, Spain; [4]Structural Synaptic Plasticity Lab, Dept. of Neurodegenerative Diseases,, Ctr. de Investigación Biomédica de la Rioja, La Rioja, Spain; [5]Inst. for Res. in Neurolog. Disabilities (IDINE), Univ. of Castilla-La Mancha, Albacete, Spain

Abstract: Learning and memory deficits are common clinical features of Huntington's disease (HD) and the most distressing symptoms at early disease stages. These cognitive impairments have been mainly associated to fronto-striatal HD pathology. However, accumulating evidence point to an important role of the hippocampus in cognitive and synaptic dysfunction in HD. The negative role of the p75NTR neurotrophin receptor in spine density, learning and memory prompted us to explore whether disturbed p75NTR function could contribute to hippocampal-dependent cognitive decline in HD. In this study, we show that the levels of p75NTR are

significantly increased in the hippocampus of two distinct HD mouse models and HD patients. Genetic normalization of hippocampal p75NTR levels in novel HD mutant mice heterozygous for p75NTR (KI:p75+/-) prevented cognitive and synaptic plasticity deficits and ameliorated dendritic spines abnormalities likely by normalization of the GTPase RhoA activity. We also demonstrate that viral-mediated over-expression of p75NTR in the wild-type hippocampus reproduced HD learning and memory deficits while knockdown of p75NTR in the hippocampus of HD mice prevented cognitive decline. Moreover normalization of p75NTR levels in KI;p75+/- mice do not recover striatal function and strongly underscore the hippocampus as the specific brain region involved in recognition, spatial and associative memory deficits in KI;p75+/- mice and agree with our hypothesis that aberrant p75NTR expression in the hippocampus of KI mice underlie HD hippocampal-memory deficits. Together, these findings provide new evidence for hippocampal-dependent cognitive deficits in HD and reveal a novel mechanism for p75NTR on mediating synaptic, learning and memory dysfunction in HD suggesting the possibility of treating synaptic and memory impairments in HD by pharmacological or genetic modulation of p75NTR function.

Disclosures: **V. Brito:** None. **A. Giralt:** None. **L. Enriquez-Barreto:** None. **M. Puigdellívol:** None. **N. Suelves:** None. **A. Zamora-Moratalla:** None. **J.J. Ballesteros:** None. **E.D. Martin:** None. **N. Dominguez-Iturza:** None. **M. Morales:** None. **J. Alberch:** None. **S. Gines-Padros:** None.

Poster

045. Huntington's Disease Animal Models and Therapeutic Strategies

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 45.04/I1

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: CHDI Foundation

Title: Neonatally transplanted human glial progenitors improves motor skills and survival of R6/2 mouse model of Huntington’s disease

Authors: ***A. BENRAISS**, J. MAUCERI, T. J. MICHAEL, H. B. BURM, D. CHANDLER-MILITELLO, S. WANG, S. A. GOLDMAN;
Univ. of Rochester Med. Ctr., ROCHESTER, NY

Abstract: Huntington's disease (HD) is caused by an expansion of the CAG tri-nucleotide repeat of the first exon of the gene encoding huntingtin (Htt). Although striatal neuronal death is a hallmark of HD, the extent to which neuronal death is cell-autonomous or instead dependent upon interactions with glia has been unclear. To address the specific role of both glial progenitor cells (GPCs) and their derived astrocytes in the pathogenesis of HD, we investigated the effect of chimerizing the striata of newborn immunodeficient rag1-/- mice with GPCs generated from HD patient-derived embryonic stem cells. In this model, the striatally-injected hGPCs expand over time, either remaining as progenitors or differentiating into astrocytes. By 12 weeks of age, the brains of these mice reveal extensive chimerization, with predominantly human striatal glia. We found that mice engrafted with HD hESC GPCs (48 CAGs) manifested poor motor coordination relative to controls engrafted with normal hESC GPCs (18 CAG), as assessed by rotarod testing. On that basis, we then performed the converse experiment, and asked whether normal glia might rescue aspects of the Huntington Disease phenotype; we did so by engrafting normal human GPCs into the striata of R6/2 (120Q) HD mice. For this experiment, GPCs were isolated from 18-22 week g.a. fetal human brain using CD44-based magnetic activated cell sorting (MACS), then transplanted into the striata of the newborn R6/2 mice. We found that the engrafted mice displayed slower motor deterioration than did their untreated controls, as assessed by rotarod. Linear regression revealed that the rate of motor deterioration was significantly slowed in the human GPC engrafted mice, relative to untreated controls ($F = 4.8$ [2, 124 df]; $P<0.001$). In addition, the normal human glial chimeric R6/2 (120Q) x rag1-/- mice survived longer than untreated mice, with a mean increase in lifespan of 12 days (hGPC-engrafted, n=29; untreated, n=28; $P<0.01$ by ANOVA). Together, these data suggest that glial pathology may contribute significantly to the motor decrements and neurodegeneration associated with HD, and that targeting glial pathology in HD may provide significant therapeutic benefit.

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Poster

045. Huntington's Disease Animal Models and Therapeutic Strategies

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 45.05/I2

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: CIHR MOP 74465

NSERC

Title: Expression and colocalization of somatostatin and NMDA receptor subtypes in huntington transgenic mice brain

Authors: S. PAIK, *R. K. SOMVANSHI, C. SINGH, S. ZOU, U. KUMAR;
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Abstract: In Huntington's disease (HD), medium sized aspiny neurons expressing somatostatin (SST) are selectively preserved whereas projection neuron positive to N-methyl-D-aspartate receptors (NMDARs) are significantly lost. Our recent studies also revealed that SSTR1/5 double knockout mice exhibit neurochemical and biochemical changes comparable to HD transgenic (tg) mice brain. However, the distributional pattern of SSTR and NMDAR subtypes at the levels of mRNA and protein as well as their colocalization in HD tg mice brain is not known. Accordingly in the present study, we determined the expression and colocalization of SSTR and NMDAR subtypes in wt and HD tg mice brain. Experimental Procedure: Receptor expression in mice brain was accomplished by using qRT-PCR and colocalization by double labeled immunofluorescence immunocytochemistry in brain sections from wt and HD tg mice. Results: Our results showed significant changes in receptor expression in cortical and striatal brain regions of wt and HD tg mice. mRNA expression of SST in striatum was lower in comparison to cortex of wt mice whereas in HD tg mice SST mRNA levels decreased significantly in both cortex and striatum. SSTRs mRNA expression in striatum was in the order of SSTR1>SSTR2>SSTR3>SSTR4>SSTR5. In contrast, receptors mRNA expression in cortex was in the order of SSTR1>SSTR3/SSTR4>SSTR2>SSTR5. HD tg mice exhibited significant changes in receptor specific manner in cortex and striatum. SSTR1 mRNA was increased whereas SSTR2, SSTR4 and SSTR5 mRNA levels were decreased with no significant changes in SSTR3 mRNA levels in cortex. In case of striatum, mRNA expression of SSTR1 and SSTR2 was decreased with no significant changes in SSTR3, SSTR4 and SSTR5. NMDARs mRNA expression in cortex and striatum was relatively higher for NR2B followed by NR1 and NR2A in wt mice brain and decreased significantly in HD tg mice. Also, the protein expression of receptors in striatum and cortex was comparable to mRNA levels. The sub-cellular colocalization of SSTR with NMDAR subtypes was receptor and region specific in wt and HD tg mice. Conclusions: Our results suggest that significant loss of SST and SSTR subtypes in cortex might be responsible for the activation of striatal NMDARs due to the withdrawal of inhibitory effect of corticostriatal glutamatergic input to the striatum. Receptor specific colocalization of SSTR with NMDAR subtypes in projection neurons may account for selective survival of these neurons in excitotoxicity.

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Poster

045. Huntington's Disease Animal Models and Therapeutic Strategies

Location: Halls A-C

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Program#/Poster#: 45.06/I3

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: Canadian Institutes of Health Research

Title: Role of palmitoylation in synaptic and extra-synaptic NMDAR localization in striatal neurons: Implications for Huntington disease

Authors: R. KANG[1], *L. A. RAYMOND[2];
[1]Univ. of British Columbia, vancouver, BC, Canada; [2]Dept Psychiatry and Brain Res. Ctr., UBC, Vancouver, BC, Canada

Abstract: Previous studies have shown NMDA receptor (NMDAR) activity is altered in YAC transgenic mouse models of Huntington disease (HD), prior to onset of motor deficits. In YAC72 early postnatal striatal neuron cultures, the NMDAR subunit GluN2B was shifted from internal pools to the plasma membrane and showed a significantly faster rate of NMDAR insertion to the surface (Fan, et al., 2007). In acute cortical-striatal brain slices from 1 month-old YAC128 mice, elevated striatal NMDAR current and GluN2B surface expression was found localized to extrasynaptic sites (Milnerwood, et al, 2010**)**. Here, we investigated a potential mechanism for the increased NMDAR extra-synaptic localization in YAC HD mice. Palmitoylation is a post-translational protein modification increasingly recognized as an important regulator of neuronal development, synaptic function and plasticity. Recently, NMDARs have been reported to be palmitoylated, and palmitoylatoin on different clusters of cysteine residues results in distinct effects on NMDAR surface expression in cultured cortical neurons (Hayashi, et al., 2009). We found that palmitoylation of GluN2B was reduced in striatum of YAC128 mice starting at one month of age. Furthermore, NMDARs containing the cluster II palmitoylation-resistant mutant GluN2B (GluN2B(5CS)) showed significantly enhanced surface expression in striatal neurons from FVB/N mice in striatal-cortical embryonic co-cultures, mimicking the increase observed for wild-type GluN2B in YAC128 co-cultures. Notably, the enhanced surface expression of GluN2B(5CS) in FVB/N SPNs was not observed at synaptic sites, but instead was restricted to extra-synaptic surface membrane. Interestingly, the palmitoylation of GluN2B was reduced by 4h treatment with 100μM 2-bromopalmitate in cultured cortical neurons, and synaptic activity-dependent palmitoylation of GluN2B was regulated differentially in FVB/N vs. YAC128 striatal neurons. These results suggest that reduced palmitoylation on cluster II of GluN2B contributes to the enhanced extra-synaptic surface expression of GluN2B in YAC128 striatal neurons, and that

the palmitoylation of GluN2B plays a critical role in the pathological mechanism of Huntington disease. 5 *Funded by the Canadian Institutes of Health Research.*

Disclosures: **R. Kang:** None. **L.A. Raymond:** None.

Poster

045. Huntington's Disease Animal Models and Therapeutic Strategies

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 45.07/I4

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: CHDI 340202

Title: Aberrant tracer coupling between striatal medium spiny neurons in a mouse model of Huntington's disease

Authors: B. KADRIU, C. ROZAS, M. A. CHACON, *S. S. DELLAL, D. S. FABER; Neurosci., Albert Einstein Col. of Med., Bronx, NY

Abstract: Electrical transmission between neurons via gap junctions is present throughout the brain and is necessary for synchronizing neural circuits. Furthermore, gap junctions permit the diffusion of small signaling molecules and metabolites between connected cells. There is also evidence that, like their chemical counterparts, electrical synapses can undergo plasticity. Specifically, the neurotransmitter dopamine has been demonstrated to both weaken and enhance coupling between cells in the retina through its actions at D1 and D2 receptors, respectively. Huntington's disease (HD) is a hereditary neurodegenerative disease characterized by severe motor impairment that involves the loss of striatal medium spiny neurons (MSNs) and cortical projection neurons. Dopamine (DA) is a critical factor for the normal operation of the basal ganglia, and during the early stages of HD in humans, DA levels are increased and DA receptor expression is decreased. We were interested in whether this dopaminergic dysfunction in the striatum in HD leads to aberrant electrical connectivity between MSNs. To begin to address this question, we performed whole-cell patch-clamp recordings on MSNs with the diffusible morphological tracer neurobiotin (NB), which is small enough to cross-gap junctions. We then performed immunohistochemistry (IHC) on these brain slices using a fluorophore-tagged streptavidin, collected z-stacks via confocal laser-scanning microscopy, and then counted the number of tracer coupled cells. We found that on average, the number of coupled cells was threefold greater for MSNs from R6/2 mice (a rapidly progressing HD model), as compared to

their wild type (WT) littermates. This difference was observed at both the early and late time points of the disease (5 and 12 weeks). We also found that when brain slices were treated with the gap junction blocker meclofenamic acid (100 μM), there was significantly less tracer coupling and there was no longer a difference in tracer coupling between WT and HD, indicating that the tracer coupling was due primarily to passage of the NB from the recorded cell across gap junctions. In agreement with this observation, we found that there was increased expression of connexin 36 in HD striatum (R6/2, 5 weeks), but not in cortex, as assessed by Western blot analysis. These results complement prior findings that HD pathology is characterized by significant changes in both chemical and electrical transmission in the striatum. Future work will aim to determine whether the increases in cell coupling in striatum result from a dysregulated dopaminergic system and whether it contributes to the motor symptoms of HD.

Disclosures: **B. Kadriu:** None. **M.A. Chacon:** None. **S.S. Dellal:** None. **C. Rozas:** None. **D.S. Faber:** None.

Poster

045. Huntington's Disease Animal Models and Therapeutic Strategies

Location: Halls A-C

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Program#/Poster#: 45.08/I5

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Title: Inhibition of Class IIa HDACs as a therapy for Huntington's disease

Authors: O. AZIZ[1], C. A. LUCKHURST[1], D. YATES[1], K. L. MATTHEWS[1], G. CREIGHTON-GUTTERIDGE[1], R. WILLIAMS[1], D. ALLCOCK[1], A. F. HAUGHAN[1], P. BRECCIA[1], A. VAN DE POEL[1], E. STONES[1], H. MCNEIL[1], W. BLACKABY[1], G. MCALLISTER[1], I. MUNOZ-SANJUAN[2], *C. DOMINGUEZ[2], M. MAILLARD[2], V. BEAUMONT[2];
[1]BioFocus, Saffron Walden, United Kingdom; [2]CHDI Managment Inc, LOS ANGELES, CA

Abstract: Histone deacetylase (HDAC) inhibition is proposed as a therapeutic strategy for Huntington's disease (HD)[1-2] . Genetic knockdown of HDAC4 in the R6/2 HD mouse model, a transgenic overexpresser of exon 1 *HTT* with an expanded CAG repeat, ameliorates behavioural as well as cortico-striatal neurophysiological deficits and improves survival. Aggregation of huntingtin protein (HTT) in CNS tissue is also reduced on HDAC4 knockdown in the R6/2 model, and also in HdhQ150 knock-in mice, which carries the full length mutant CAG expanded *HTT* allele[3]. These data suggest that an HDAC4-selective inhibitor has therapeutic potential in

HD. Previously we have reported on highly selective compounds for HDAC class IIa enzyme inhibition over class I, IIb and III enzymes with potent cellular activity[4], however, these compounds demonstrated limited phenotypic improvements in an HD mouse model, R6/2, which was attributed to a relatively low predicted free fraction in brain. Here we describe the profiles of a novel compound series of catalytic-site small-molecule inhibitors of the class IIa HDACs, as well as attempts to develop an associated biomarker strategy for the assessment of target engagement in the brain. The improved brain exposure and free fraction in brain of these compound gives us greater confidence for their assessment of efficacy in rodent models of HD. *1. Sadri-Vakili G and Cha JH., Curr. Alzheimer Res. (2006) Sep;3(4):403-8 2. Butler R and Bates GP., Nat. Rev. Neurosci. (2006) Oct;7(10):784-96. 3. Mielcarek M et al., PLoS Biology **11**, e1001717, doi:10.1371/journal.pbio.1001717 (2013) 4. Bürli, RW et al., JMedChem, **23** (2013) 6598-6603*.

Disclosures: **O. Aziz:** None. **C.A. Luckhurst:** None. **D. Yates:** None. **K.L. Matthews:** None. **G. Creighton-gutteridge:** None. **R. Williams:** None. **D. Allcock:** None. **P. Breccia:** None. **A.F. Haughan:** None. **E. Stones:** None. **H. McNeil:** None. **W. Blackaby:** None. **G. McAllister:** None. **I. Munoz-Sanjuan:** None. **C. Dominguez:** None. **M. Maillard:** None. **V. Beaumont:** None. **A. Van De Poel:** None.

Poster

045. Huntington's Disease Animal Models and Therapeutic Strategies

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 45.09/I6

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: CHDI Grant #340202

Title: Potential mechanisms of altered excitability in the striatum of a mouse model of Huntington's disease

Authors: *M. A. CHACON, C. ROZAS, D. BARTOLOME-MARTIN, Y. CAO, D. S. FABER; Albert Einstein Col. of Med., Bronx, NY

Abstract: We are interested in biophysical mechanisms underlying altered excitability of medium spiny neurons (MSNs) in dorsal striatum of mouse models of Huntington’s Disease (HD). Here we describe two potential complementary mechanisms. First, we recently demonstrated a build up of accommodation of spike firing rate in MSNs called fast-activity

dependent homeostasis (fADH). fADH is induced by repetitive impulse activity and is manifest by a progressive attenuation in the induced firing rate. Interestingly, fADH is impaired in the R6/2 mouse model of Huntington's disease at pre-or early-symptomatic stages. Here we asked if this phenomenon occurs in the BACHD transgenic models of HD and if it is displayed selectively by MSNs of the direct (D1) and/or indirect (D2) pathways. Using whole-cell recordings we found that at 2 and 3 months of age fADH is decreased in BACHD MSNs, as compared with wild type (WT) littermates, while there is no difference at 1 and 6 months. The impaired fADH can be rescued by activators of the voltage-gated potassium channel Kv7.2/3, which is involved in the regulation of excitability and spike frequency accommodation. Analysis of D1- and D2-expressing MSNs did not show any significant difference in fADH, although we found differences in intrinsic properties of both D1 and D2, as reported previously. In agreement with the observed fADH, WT D1-MSNs exhibit an increased M-current activation in resting conditions, as compared with BACHD D1-MSNs which did not exhibit any enhanced activation current. Surprisingly, no differences were found in D2-MSNs. Second, an increased input resistance (Rin), which could also contribute to hyperexcitability, was found in 2 and 3 month old BACHD mice, as observed in other HD mouse models. This increased resistance is most not likely due to a change in rectification, since the slopes of I-V curves were increased in both the depolarizing and hyperpolarizing directions in BACHD MSNs. To study this in more detail we use BaCl2, a blocker of Kir channels. Membrane voltage responses to hyperpolarizing and depolarizing current injections in the presence of BaCl2 show a significant loss of rectification. However, after BaCl2 superfusion, the difference in slope between WT and BACHD mice was maintained, again suggesting that a loss of rectification does not account for the increased Rin in BACHD mice. These results demonstrate an age-dependent appearance of fast-activity dependent homeostasis in striatal MSNs of WT mice, which is not present in BACHD mice at the same age, and suggest that reduced activation of both Kv7.2/7.3 channels and a leak conductance can underlie the hyperactivity of MSNs in BACHD mice.

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Poster

045. Huntington's Disease Animal Models and Therapeutic Strategies

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: JAE predoc fellowship from CSIC

Ministry of Economy and Competitiveness Grants SAF2011-22855

Ministry of Economy and Competitiveness Grants SAF2011-22506

GVA Prometeo/2012/005

Title: Characterization of a potential novel therapeutical tool to reverse histone hypoacetylation in neuropathologies

Authors: *D. M. GUIRETTI, L. M. VALOR, A. BARCO;
Inst. de Neurociencias (UMH-CSIC), Sant Joan d´Alacant, Spain

Abstract: It has been proposed that alteration in the level of neuronal histone acetylation in the brain may underlie cognitive decline and other neurological symptoms associated with aging, neurodegenerative diseases (e.g., Huntington's and Alzheimer's diseases) and congenital intellectual disability disorders (e.g., Rubinstein-Taybi syndrome). Consistent with this view, inhibitors of histone deacetylases (HDACi) ameliorate neuropathological traits in animal and cellular disease models for these conditions. However, the chronic treatment with compounds may cause significant side effects because the indiscriminate promotion of acetylation can interfere with cell division and have other deleterious consequences. To minimize the adverse effects of HDACi and overcome the highly transient impact of HDACi administration, we have generated a recombinant lentivirus that overexpresses the KAT domain of the CREB-binding protein (CBP) selectively in neurons. This strategy for the genetic enhancement of neuronal histone acetylation in neurons may represent a novel therapeutic approach in the neuropathologies referred above. We believe that the comparison of the consequences of the infection with this lentivirus and HDACi treatments will contribute to a better understanding of the pleiotropic effects associated with HDACi-induced hyperacetylation and the refinement of related therapeutic strategies by improving targeted delivery to affected brain regions. We first demonstrate the effectiveness of the viral vector to increase the acetylation level of the four-nucleosome histones in primary hippocampal cultures. In addition, we also observed the hyperacetylation of several non-histone substrates. Moreover, these biochemical changes correlated with an increase in the number of dendritic spines in infected hippocampal neurons. Importantly, unlike the chronic pharmacological treatment, the genetic manipulation did not compromise neuronal survival. Additional experiments to investigate the impact on gene expression of this chronic increase of KAT activity are in progress, as well as the evaluation of this genetic tool in cellular and animals models of neuropathology.

Disclosures: **D.M. Guiretti:** None. **L.M. Valor:** None. **A. Barco:** None.

Poster

045. Huntington's Disease Animal Models and Therapeutic Strategies

Location: Halls A-C

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

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FCT postdoctoral fellowship SFRH/BPD/91811/2012 (M.N. Laço)

Title: Mitochondrial-based neuroprotective effects of resveratrol and nicotinamide in *in vitro* and *in vivo* Huntington's disease models

Authors: *L. NAIA[1,2], T. R. ROSENSTOCK[1,3], A. OLIVEIRA[1], S. I. OLIVEIRA-SOUSA[1], G. L. CALDEIRA[1,3], M. N. LAÇO[1,2,3], M. R. HAYDEN[4], C. R. OLIVEIRA[1,2,3], A. C. REGO[1,2,3]; [1]Ctr. For Neurosci. and Cell Biol., Coimbra, Portugal; [2]Fac. of Medicine, Univ. of Coimbra, Coimbra, Portugal; [3]Inst. for Interdisciplinary Research, Univ. of Coimbra (IIIUC), Coimbra, Portugal; [4]Dept. of Med. Genet., Ctr. for Mol. Med. and Therapeutics, Child and Family Res. Institute, Univ. of British Columbia, Vancouver, BC, Canada

Abstract: Mammalian sirtuins are a conserved family of class III NAD^+-dependent deacetylases, which have been postulated to be beneficial for targeting mitochondrial abnormalities. Being a neurodegenerative disease that selectively affects the striatum and the cortex, Huntington's disease (HD) has been commonly linked to mitochondrial dysfunction as one of the main pathological mechanisms. Therefore, we hypothesized that modulation of sirtuins might be beneficial in HD. In this study we tested the influence of resveratrol (RESV) *versus* nicotinamide (NAM, a Sirt inhibitor) in counteracting mitochondrial dysfunction in HD cells expressing full-length human mutant huntingtin, namely striatal and cortical neurons isolated from YAC128 transgenic mice embryos and HD human lymphoblasts. Moreover, we analyzed the effect of RESV and NAM in an *in vivo* HD model. We observed a slight decrease in histone acetylation with RESV and increased histone acetylation with NAM in HD cell models. Moreover, HD lymphoblasts exhibited a decrease in PGC-1alpha and Tfam protein levels, linked to a small reduction in the number of mitochondrial DNA copies. Functionally, both HD models displayed

a deregulation in mitochondrial respiration and mitochondrial membrane potential, implicating a decline in mitochondrial function. Interestingly, both RESV and NAM completely restored most of the evaluated parameters, providing a positive add on mitochondrial function in HD. In the *in vivo* study 1 mg/kg/day RESV and 250 mg/kg/day NAM were administered to 9 month-old YAC128 *versus* wild-type (WT) mice during 28 days. We found that RESV increased the latency to fall off in the rotarod test; moreover, RESV and NAM greatly increased histone acetylation in both YAC128 and WT striatal and cortical samples. Additionally, NAM completely restored the levels of mitochondrial-encoded complex IV subunit in YAC128 striatal samples. These data suggest that RESV and NAM are able to modulate protein acetylation and ameliorate mitochondrial function in HD, which may partially control HD-related motor disturbances.

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Poster

045. Huntington's Disease Animal Models and Therapeutic Strategies

Location: Halls A-C

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: John G. Kulhavi Professorship in Neuroscience

College of Medicine

Ronald E. McNair Scholars Program

Title: Transplantation of genetically altered mesenchymal stem cells over expressing human and mouse brain derived neurotrophic factor in the R6/2 transgenic mouse model of Huntington’s disease

Authors: *A. C. MOORE[1,2], A. CRANE[1,2], M. LU[1,2,3], G. DUNBAR[1,2,3,5], J. ROSSIGNOL[1,2,4]; [2]Program in Neurosci., [3]Dept. of Psychology, [4]Col. of Med., [1]Central Michigan Univ., Mount Pleasant, MI; [5]Field Neurosciences Inst., Saginaw, MI

Abstract: Huntington’s disease (HD) is an incurable, progressive neurodegenerative disorder caused by an expanded CAG trinucleotide repeat on chromosome four. In HD, degeneration of

medium spiny neurons begins in the putamen and caudate nucleus and progresses to the cerebral cortex, as its severity increases. Symptoms include cognitive dysfunction and chorea. After its onset, life expectancy of victims of HD is about 20 years. Although there is currently no known cure for HD, stem cell therapies offer viable potential for replacing or protecting affected cells in animal models of HD. Recent research suggests that bone marrow derived mesenchymal stem cells (MSCs) exert neuroprotective effects through expression of brain derived neurotrophic factor (BDNF), a protein that supports survival of existing neurons and differentiation of developing stem cells, which is diminished in those afflicted with HD. To counter its depletion, the goal of this study was to genetically modify MSCs with a permanently integrative lentivirus to over-express either human or mouse BDNF. Lentiviral vectors containing green fluorescent protein (GFP) and human and mouse BDNF were constructed from E. coli plasmids and verified with PCR and restriction assay. Following verification, the vectors were then introduced in a culture medium of packaging cells for virus purification and collection. After purification, bone-marrow-derived MSCs from the R6/2 transgenic mouse model of HD were separately transfected with the human BDNF lentivirus and the mouse BDNF lentivirus. Fluorescent microscopy imaging indicated that MSCs express GFP, which suggests BDNF expression. Enzyme linked immunosorbent assay (ELISA) was used to quantify BDNF levels in cells and in the cell culture media of both BDNF-transfected MSCs and MSCs with an empty vector. Western blotting was used to confirm the presence or absence of BDNF in all groups (MSCs transfected with human BDNF, MSCs transfected with mouse BDNF, and empty vector). Cells from each of these groups were transplanted into the striatum of R6/2 mice and motor performance was measured using the rotarod apparatus. Results suggest that genetically altered BDNF-secreting MSCs have the potential to promote behavioral sparing. Support for this project was provided by funding from the Ronald E. McNair Scholars Program (to AM), the college of Medicine (to JR), and the John G. Kulhavi Professorship and Field Neurosciences Institute (to GLD).

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Poster

045. Huntington's Disease Animal Models and Therapeutic Strategies

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 45.13/I10

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Title: The influence of gaze on static postural control in individuals with Huntington’s disease

Authors: *L. COOKE[1], L. MURATORI[2], K. BEERS[1], C. FISCHER[1], E. RABIN[1];
[1]NYIT Col. of Osteo, Old Westbury, NY; [2]Physical Therapy, Stony Brook Univ., Stony Brook, NY

Abstract: It is possible that increased postural sway in Huntington's disease (HD) is related to poor ocular control. Posture is controlled with visual feedback, among other modalities. In order to interpret retinal feedback, corresponding feedback about eye position is required to distinguish eye movement from external movement. One of the first presenting symptoms of HD is abnormal ocular motor control including impairments in voluntary eye movements (saccades) and the vestibulo-ocular reflex (VOR) used to stabilize gaze during head movement. HD patients show impairment of fixation, difficulty suppressing saccades toward novel visual stimuli, and an inability in making saccades without head movement. Inability to suppress reflexive glances to novel stimuli and delayed voluntary saccades may be due to damage of the frontal lobes and basal ganglia in the earliest stages of the disease. The purpose of this study was to determine whether impaired ocular control in individuals with HD contributes to increased postural sway. Seven individuals with HD and seven healthy controls participated in all conditions. To determine if changing gaze is disruptive to postural control, participants were initially asked to stand as still as possible with feet side-by-side for 25 seconds while watching several different conditions of stationary and moving targets on a screen. Head and trunk position were captured using a Vicon camera system and eye position was recorded via Iscan video-based eye tracking system. Results: (1) Participants with HD swayed with greater amplitude in all conditions; (2) Individuals with HD had greater sway power at frequencies related to visual stimuli changes; (3) Participants with HD moved their head and eyes in phase of one another. These findings support the notion that postural sway in people with HD is indeed influenced by changes in gaze, and that some of those postural movements may be related to gaze control.

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Poster

045. Huntington's Disease Animal Models and Therapeutic Strategies

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: University of Wyoming Neuroscience COBRE (5P20RR015640-10)

NIH R21 NS07237202

Title: Thioltransferases TXN1 and TXNDC10 protect against neuronal atrophy in a lentiviral mouse model of Huntington's disease

Authors: *Z. LU[1,2], L. BARROWS[1], J. CHEN[1,2], J. MOLINE[1], J. FOX[1,2];
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Abstract: Huntington's disease (HD) is a progressive neurologic disorder caused by polyglutamine-expanded mutant huntingtin protein (mhtt). Mutant hungtingtin protein forms oxidative and thiol-dependent oligomers whose rate of degradation is slower than monomeric mhtt. We have hypothesized that a thiol transferase exists that converts oxidized mhtt oligomers to monomeric protein and may thereby decrease mhtt levels. We screened a number of thiol transferase enzymes by co-transfection with plasmids encoding N171-40Q huntingtin and thioltransferases into COS1 cells and measured mhtt levels 48 hours later by Western blot analysis. The primary screen revealed that the thioltransferases thioredoxin 1 (TXN1) and thioredoxin domain-containing protein 10 (TXNDC10) decreased total soluble mhtt. In a secondary screen we expressed enzymatically active / inactive versions of TXN1 and TXNDC10 with N171-40Q huntingtin. We were able to confirm that TXN1 and TXNDC10 decreased N171-40Q huntingtin levels in COS1 cells. We subcloned these two genes separately into a lentiviral vector that uses the phosphoglycerate kinase promoter. We tested their potential protective effects in mouse HD by co-injection of lentiviruses expressing the following combinations of genes: WT control (N171-18Q + inactive TXN1 or TXNDC10), HD control (N171-82Q + inactive TXN1 or TXNDC10) and HD treated (N171-82Q + active TXN1 or TXNDC10). Lentivirus was delivered by unilateral intra-striatal injection at 8 weeks of age in B6/C3H F1 female mice; mice were sacrificed at 16 weeks of age. There was no effect of the treatments on behavioral outcomes. However, the HD control group had significantly smaller striatal neuronal cell bodies than the WT control group as determined by confocal stereology; this effect was reversed in the HD treated groups by both TXN1 and TXNDC10. Therefore, TXN1 and TXNDC10 provide therapeutic benefit in this lentiviral model of HD.

Disclosures: **Z. Lu:** None. **L. Barrows:** None. **J. Chen:** None. **J. Moline:** None. **J. Fox:** None.

Poster

045. Huntington's Disease Animal Models and Therapeutic Strategies

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 45.15/I12

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Title: Xyloketals protect polyQ-induced toxicity in *C. elegans*

Authors: *Z. PEI, Y. ZENG;
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Abstract: Huntington's disease (HD) is an autosomal dominant inherited neurodegenerative disorder characterized by progressive motor dysfunction, including chorea and dystonia, emotionl disturbances, memory, and weight loss. Unfortunately, no treatment is available to prevent the progression of the disease.The protein aggregation is the pathological hallmark of HD, while inhibition of protein aggregation has shown beneficial effects against HD. Model organisms, such as *C. elegans* are powerful tools in drug development. Marine environments are rich sources of novel and unusual secondary metabolites, many of which show considerable promise as therapeutic agents. Xyloketals are a series of novel natural compounds from marine mangrove fungi. We previously demonstrated that xyloketal B has neuroprotective effects in different cell models via its antioxidant properties. In the present study, we generated HD elegans model by expressing different repeats (Q16, Q60 and Q150) in muscle cells under the control of the unc-54 myosin heavy-chain promoter. We found that expression of HD repeats-dependently induced protein aggregation. The expression of Q 16 was diffuse while the expression of Q60 or Q150 was focal distribution corresponding to protein aggregates. Moreover, the formation of protein aggregates was associated with the dysfuntion of mobility. Q60 or Q150 but no Q16 severely impaired the mobility of animals. Xyloketals significantly reduced polyQ aggregation and alleviated the associated dysfunction of mobility. Thus, xyloketals may represent an attractive candidate for treatment of HD.

Disclosures: **Z. Pei:** None. **Y. Zeng:** None.

Poster

045. Huntington's Disease Animal Models and Therapeutic Strategies

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 45.16/J1

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: AHA Predoctoral Fellowship 12PRE10410000

CIRM Training Grant TG2-01152

NIH U24 NS078370

Title: Modeling defects in the Huntington's Disease neurovascular unit and blood-brain barrier using iPS Cells

Authors: *R. G. LIM[1], C. QUAN[2,1], M. CASALE[1], J. STOCKSDALE[1], A. R. KING[1], L. SALAZAR[1], S. WINOKUR[1], .. HD IPSC CONSORTIUM[3], L. M. THOMPSON[1];
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Abstract: Huntington's disease (HD) is a devastating, neurodegenerative disease that typically strikes in mid-life and is caused by a CAG repeat expansion within the coding region of the HD gene. HD primarily induces degeneration of medium spiny neurons within the striatum and atrophy of the cortex, but emerging data suggests that the neurovascular unit (NVU) and blood-brain barrier (BBB) also contribute to disease pathogenesis. Vascular abnormalities and aberrant gene regulation of the BBB in HD patients and animal models warrant further investigation of this system in HD. Two critical pathways implicated in HD, Shh and Wnt signaling, are utilized by astrocytes and brain endothelial cells (BEC) in the NVU to control BBB development and maintenance. We therefore hypothesize that abnormal signaling exists in and between BECs, astrocytes and neurons, which causes abnormal expression of BBB proteins and contributes to neuronal dysfunction and pathogenesis in HD. To test this hypothesis, we have used a panel of induced pluripotent stem cell (iPSCs) with varying numbers of CAG repeat units to generate *in vitro* models of HD neurons and other cells of the NVU, including BECs and astrocytes. Preliminary data shows functional and transcriptional changes in these cells. RNA-Seq and qPCR reveal a number of BBB/NVU genes that are differentially expressed in HD and these HD BECs exhibit disruption of tight junction organization, reduced TEER, and decreased resistance to puromycin treatment. Furthermore, iPSC-derived NSCs and BECs recapitulate BBB pathology seen in HD animal models, such as abnormal WNT/SHH signaling. Ongoing studies will determine if aberrant signaling from astrocytes or cell autonomous defects lead to BBB disruption and if alterations in signaling and transcription exist between the cellular subunits of the NVU, affecting BBB maintenance and subsequent neuronal damage. Understanding these changes in signaling and the effects on the BBB in HD will allow us to better understand the contributions of BBB breakdown to the progression of HD and other CNS diseases where the BBB is disrupted. These studies will provide a resource for therapeutic assay development applicable to a broad spectrum of neurodegenerative diseases.

Disclosures: **R.G. Lim:** None. **C. Quan:** None. **M. Casale:** None. **J. Stocksdale:** None. **A.R. King:** None. **L. Salazar:** None. **S. Winokur:** None. **.. HD iPSC Consortium:** None. **L.M. Thompson:** None.

Poster

045. Huntington's Disease Animal Models and Therapeutic Strategies

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 45.17/J2

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NRF Grant 2011-0012728

Title: Investigating effects of extracts of human adipose-derived stem cell in Huntington's disease

Authors: *M. LEE[1], W. IM[2];
[1]neurology, Seoul Natl. Univ. Hosp., Seoul, Korea, Republic of; [2]Seoul national university hospital, seoul, Korea, Republic of

Abstract: Stem cell therapy is a perspective remedy for permanent terminal disorders including Huntington's disease (HD). Adipose-derived stem cell (ASC) is as an abundant and easily available source of stem cells with multipotent characters suitable for regenerative medical applications. Stem cells secrete various factors which can modulate a hostile microenvironment of diseases. ASCs also express multiple growth factors and could be used to treat disease via secretion factors, called paracrine mechanism. We have previously showed that extracts isolated from human ASCs (hASC-extracts) slow the progression of Huntington's disease (HD). Here, we investigated the mechanism of effects of hASCs-extracts and found that small vesicles isolated in hASCs-extracts show the similar effects *in vitro* HD model.

Disclosures: **M. Lee:** None. **W. Im:** None.

Poster

045. Huntington's Disease Animal Models and Therapeutic Strategies

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 45.18/J3

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NS-28721

The Cure Huntington's Disease Initiative Foundation

The Methodist Hospitals Endowed Professorship in Neuroscience

Title: The treatment benefit of the Group 2 metabotropic glutamate receptor agonist LY379268 in R6/2 mice stems from a neuroprotective rather than a symptomatic effect

Authors: *A. J. REINER, Y. DENG, N. DEL MAR, H. REN, J. T. ROGERS;
Anat. & Neurobio., The Univ. of Tennessee Hlth. Sci. Ctr., Memphis, TN

Abstract: We have found that daily subcutaneous injection with a maximum tolerated dose of the mGluR2/3 agonist LY379268 (20mg/kg sc) beginning at 4 weeks of age dramatically improves the phenotype in R6/2 mice, especially in males, who we found show a slightly more aggressive R6/2 phenotype than females. For example, we found that daily 20mg/kg LY379268 in male R6/2 mice prevents a 20% striatal neuron loss at 10 weeks, and significantly improves motor parameters such as rotarod performance and distance traveled in open field. In the present study, we examined if the LY379268 benefit is due to an acute palliative effect of the drug on behavior, or if it is due to a cumulative neuroprotective effect from the daily delivery of the drug. To test the acute effects, we examined rotarod and open field performance in 10 week old male R6/2 mice before and 6, 12 and 24 hours after a single sc administration of 20mg/kg LY379268. We found that the mice showed substantial and significant deficits at 6 and 12 hours after drug on both rotarod (40% reduction) and open field distance traveled (50-75% reduction), but recovered by 24 hours. No such treatment-related deficits were seen when these same mice were injected with vehicle on a subsequent day. In a second line of study, we determined if chronic treatment with 20mg/kg LY379268 beginning at 4 weeks of age would still produce a benefit, even if drug injections were curtailed (and replaced by vehicle injections) for the last three days prior to testing at 10 weeks of age. We found that both those chronically treated male R6/2 mice receiving the 3-day LY379268 holiday prior to behavioral testing, and those receiving drug through to the test day, performed significantly better on rotarod and open field than did R6/2 males not receiving LY379268 (2-fold improvement). As R6/2 males receiving drug and then vehicle during the 3-day drug holiday performed somewhat better than did R6/2 males receiving drug throughout, the results suggest that despite the benefit of chronic treatment, the drug retains an acute depressive effect. Our overall results are consistent with a neuroprotective effect of daily LY379268 for treating R6/2 HD mice, which our evidence suggests is in large part mediated by a cortical and thalamic BDNF boosting effect of the drug (Reiner et al., Brain Res., 2012). Modifying dose and treatment frequency may make it possible to achieve the neuroprotective effect without the acute suppressive effect of LY379268.

Disclosures: **A.J. Reiner:** None. **Y. Deng:** None. **N. Del Mar:** None. **H. Ren:** None. **J.T. Rogers:** None.

Poster

045. Huntington's Disease Animal Models and Therapeutic Strategies

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 45.19/J4

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NINDS (NS082338)

CHDI

Title: The protective effect of metformin on the mutant huntingtin induced toxicity

Authors: ***J. JIN**[1], M. TAO[2], Q. PENG[1], M. JIANG[1], W. DUAN[1,3,4];
[1]Dept. of Psychiatry and Behavioral Sci., Johns Hopkins Univ. Sch. of Med., Baltimore, MD; [2]Dept. of Psychological and brain science, Johns Hopkins Univ., Baltimore, MD; [3]Departments of Neurosci., Baltimore, MD; [4]Program in Cell. and Mol. Med., Baltimore, MD

Abstract: Huntington's disease (HD) is a devastating neurodegenerative disease caused by the pathological elongation of the CAG repeats in the huntingtin gene. Caloric restriction (CR) has been a most reproducible environmental intervention to improve health and prolong lifespan. We have demonstrated that CR delayed onset and slow disease progression in a mouse model of HD. Metformin, an anti-diabetic drug, mimics CR acting on cell metabolism at multiple levels. A recent study has shown that long-term administration of metformin improved health span and lifespan in mice. In addition, administration of metformin in drinking water improved motor function and extended survival in an HD mouse model. These studies provided compelling evidence that metformin may be an ideal repositioning drug for HD treatment. To determine whether metformin directly modulates mutant huntingtin-induced neurotoxicity, striatal cells expressing mutant huntingtin were treated with metformin, we found that metformin rescued cell toxicity induced by mutant huntingtin, indicated by reduced LDH release and increased ATP levels. Furthermore, metformin activated AMPK in normal striatal cells and attenuated the inactivation of AMPK in mutant huntingtin expressing striatal cells, pharmacological blocking AMPK activation partially abolished the protective effects of metformin on mutant huntingtin, indicating that AMPK and other signaling pathways are involved in the protective effect. We are

investigating molecular mechanisms underlying the protective effect of metformin in HD. Our ultimate goal is to develop a fast track therapeutic strategy for HD.

Disclosures: **J. Jin:** None. **M. Tao:** None. **Q. Peng:** None. **M. Jiang:** None. **W. Duan:** None.

Poster

045. Huntington's Disease Animal Models and Therapeutic Strategies

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 45.20/J5

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: Ripples of Hope Trainee Award in Rare Diseases

Teva Pharmaceuticals

Title: Toward developing a panel of antisense oligonucleotide drugs targeted to single nucleotide polymorphisms enriched within mutant huntingtin genes to provide an allele-specific gene silencing treatment option for the majority of the Huntington disease population

Authors: *N. S. CARON, A. L. SOUTHWELL, N. H. SKOTTE, C. KAY, Y. XIE, E. B. VILLANUEVA, E. PETOUKHOV, C. N. DOTY, M. R. HAYDEN;
Med. Genet., Ctr. for Mol. Med. and Therapeut., Vancouver, BC, Canada

Abstract: Huntington disease (HD) is a devastating neurodegenerative disorder for which there is no effective treatment. HD is caused by a CAG trinucleotide repeat expansion in the HTT gene (>35 repeats), resulting in an elongated polyglutamine tract within the huntingtin protein. The expansion of the polyglutamine tract in mutant huntingtin results in gain of toxic cellular functions leading to the pleiotropic symptoms of HD. Therefore, reducing levels of mutant huntingtin in patients is an attractive therapeutic strategy for the treatment of HD. Others have demonstrated that non-selective reduction of huntingtin levels through a variety of gene-silencing modalities improves behavioural and neuropathological phenotypes in rodent models of HD. However, the wild type protein is essential for neuronal health, and the reduction of both wild type and mutant huntingtin may not be well tolerated over the long treatment duration required in humans. Thus, selectively reducing levels of mutant huntingtin represents a more favourable therapy for the treatment of HD. One strategy for the selective reduction of mutant huntingtin is the use of RNase-H activating antisense oligonucleotides (ASOs) targeted to single nucleotide polymorphisms (SNPs) within HTT that are in linkage disequilibrium with the CAG

expansion. Our lab has previously evaluated ASOs targeted to one such HD-associated SNP and identified potent, selective, and well tolerated candidates currently undergoing pre-clinical validation. However, targeting a single HD-associated SNP can maximally provide a treatment option for ~50% of the HD population. Therefore, we are pursuing a combinatorial approach by developing a panel of ASOs targeting 3-5 HD-associated SNPs heterozygous in minimally overlapping patient populations to increase population coverage for the majority of those with HD.

Disclosures: **N.S. Caron:** None. **A.L. Southwell:** None. **N.H. Skotte:** None. **C. Kay:** None. **Y. Xie:** None. **E.B. Villanueva:** None. **E. Petoukhov:** None. **C.N. Doty:** None. **M.R. Hayden:** A. Employment/Salary (full or part-time):; Teva Pharmaceutical Industries Ltd..

Poster

046. Huntington's Disease Mechanisms I

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 46.01/J6

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: CSUPERB New Investigator S12 Grant

NIH/NIGMS Grant 1SC3GM096945

MBRS-RISE NIH Grant 2 R25 GM061190-05A2

Title: Age-dependence of chloride and potassium channel dysfunction in Huntington’s disease skeletal muscle

Authors: *D. R. MIRANDA, M. WONG, C. M. MCKEE, M. MENDIZABAL, R. J. TALMADGE, A. A. VOSS;
California State Polytechnic Univ., Pomona, CA

Abstract: Huntington’s disease (HD) is a genetic disorder that causes a gradual impairment of movement, a progressive decline in cognition and increasingly severe behavioral irregularities. Although typically considered a primary neuropathy, we recently found skeletal muscle defects in HD that may contribute to the motor defects. In the R6/2 mouse model of HD, currents through the skeletal muscle chloride channels (ClC-1) and inward rectifying potassium channels (Kir) are significantly reduced compared to wild type at the end-stage of the disease (10 to 12 weeks of age). This reduction is correlated with aberrant splicing of *Clcn-1* (ClC-1 gene) mRNA

and decreased total *Clcn-1* and *Kcnj2* (Kir2.1 gene) mRNA in HD skeletal muscle. Because ClC-1 and Kir channel currents help maintain the resting membrane potential, their reduction causes the muscle to become hyperexcitable and may help explain the chorea, dystonia, and muscle rigidity that characterize HD. In this study, we determined the time course over which these channel defects develop. We measured ClC-1 and Kir currents in individual *flexor digitorum brevis* and *intraosseous* muscle fibers from male and female R6/2 mice using two-electrode voltage clamp. We also examined *Clcn-1* mRNA processing and total amounts of *Clcn-1* and *Kcnj2* mRNA. Measurements were taken from both HD and wild type muscle obtained from mice ranging from 20 to 76 days of age. Our results show an early and progressive dysfunction in ClC-1 channels in HD muscle that parallels the developing muscle defects. For example, at 5 weeks of age, the earliest time point that motor symptoms are observed in R6/2 mice, we found a significant decrease in the ClC-1 conductance in HD (1.62 ± 0.14 mS/uF, n=11) compared to wild type (2.27 ± 0.16 mS/uF, n=8) muscle fibers (values shown as mean ± SEM with a P=0.01). These functional defects were associated with changes in *Clcn-1* and *Kcnj2* mRNA. The results show muscle defects that can help explain the motor symptoms observed throughout the lifetime of the HD mice.

Disclosures: D.R. Miranda: None. **M. Wong:** None. **R.J. Talmadge:** None. **A.A. Voss:** None. **C.M. McKee:** None. **M. Mendizabal:** None.

Poster

046. Huntington's Disease Mechanisms I

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 46.02/J7

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: CDRV Strategic pilot grant

Title: Huntington disease and olfactory dysfunction: Structural abnormalities of the olfactory system and early caspase activation in the olfactory bulb are observed in HD mouse models

Authors: *R. K. GRAHAM[1,2], M. LAROCHE[1,2], M.-J. DEMERS[1,2], M. LESSARD-BEAUDOIN[1,2], M. GARCIA-MIRALLES[3,4], C. KREIDY[3,4], S. FRANCIOSI[5], M. R. HAYDEN[5], M. A. POULADI[3,4];
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[4]Translational Lab. in Genet. Med., Singapore, Singapore; [5]Med., Ctr. for Mol. Med. and Therapeut., Vancouver, BC, Canada

Abstract: Olfactory dysfunction and altered neurogenesis are observed in several neurodegenerative disorders including Huntington disease (HD). These deficits are an early symptom and correlate with decline in global cognitive performance, depression and degeneration of olfactory regions in the brain. The olfactory dysfunction observed in neurodegenerative diseases is often accompanied by structural abnormalities of the olfactory epithelium, the olfactory bulb (OB) and the olfactory cortices in human brain. Despite evidence demonstrating early olfactory dysfunction in HD patients, only limited details are available in murine models. Preliminary data demonstrate a decrease in OB weight (YAC128, p=0.027; BACHD, p=0.023) and the volume of the piriform cortex (YAC128, p=0.004; BACHD, p=0.013) is observed in HD vs. WT mice. Furthermore, a decrease in piriform neuronal counts (p=0.003) and enhanced immunostaining of EM48 is observed in the olfactory regions of YAC128 vs. WT brain. We also examined odor investigation behaviors in the mice using the habituation/dishabituation test. YAC128 mice investigate trial 1 odors longer than WT littermates (p=0.04).In order to determine whether apoptotic events are associated with the olfactory dysfunction and altered neurogenesis in HD we are assessing caspase activation and TUNEL. Preliminary data demonstrate that a significant decrease in the proform of caspase-6, suggesting activation, and an increase in levels of the proform of caspase-8 are observed in pre-symptomatic YAC128 OB vs. WT. No change in caspase-3 or -9 was detected. At the present time there is a serious lack of biomarkers for either HD or Alzheimer disease. Identification of early markers of the disease will help inform therapeutic approaches for these diseases and will clarify the utility of olfactory function tests in patients with these disorders and in the aging population.

Disclosures: **R.K. Graham:** None. **M. Laroche:** None. **M. Demers:** None. **M. Lessard-Beaudoin:** None. **M. Garcia-Miralles:** None. **C. Kreidy:** None. **S. Franciosi:** None. **M.R. Hayden:** None. **M.A. Pouladi:** None.

Poster

046. Huntington's Disease Mechanisms I

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 46.03/J8

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: Oberlin College Office of Foundation, Government, and Corporate Grants

Oberlin College Research Fellowship

Title: Acute exposure to Chlorpyrifos generates oxidative stress and mitochondrial dysfunction in a striatal cell model of Huntington's disease

Authors: G. A. DOMINAH, *G. F. KWAKYE;
Oberlin Col., Oberlin, OH

Abstract: In spite of the genetic cause of Huntington's disease (HD), emerging evidence strongly suggests environmental influence on the age of onset, progression, and severity of the disease. However, the identity of the environmental risk factor is currently unknown. Recognizing the similarities in the pathophysiological mechanisms between HD and pesticide neurotoxicity, we hypothesized that the common agricultural pesticide chlorpyrifos (CPF) would exhibit disease-toxicant interaction and reveal the influence of CPF in HD neuropathophysiology. We investigated the effects of acute CPF toxicity and its principal metabolites chlorpyrifos oxon (CPO) and 3,5,6-trichloropyridinol (TCP) in an established murine striatal ST*Hdh* cell model of HD by assessing cell viability, reactive species production, mitochondrial membrane potential, antioxidant buffering capacity, and energy homeostasis, as well as antioxidant mediated neuroprotection. Following a 48-hour exposure to the metabolites CPO and TCP, we observed no significant dose and genotypic differences in cell survival. Interestingly, expression of mutant HD resulted in increased dose-dependent susceptibility to CPF exposure and production of reactive species compared to wild-type cells. Furthermore, we report that the mutant HD induced vulnerability to CPF exposure is mediated through diminished antioxidant buffering capacity, enhanced production of free radicals, decreased mitochondrial function and energy production. To further investigate the possible neuroprotection of HD cells against CPF neurotoxicity, we treated ST*Hdh* cells with N-acetylcysteine (NAC) and observed that the CPF induced toxicity was significantly ameliorated in mutant HD cells. These results strongly suggest that mutant HD and CPF exhibit a disease-toxicant interaction to cause enhanced striatal neurotoxicity via oxidative stress and mitochondrial dysfunction that could exacerbate the neurodegenerative processes in HD.

Disclosures: **G.A. Dominah:** None. **G.F. Kwakye:** None.

Poster

046. Huntington's Disease Mechanisms I

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 46.04/J9

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NIH

CHDI

Title: Polyamine modulates mutant huntingtin toxicity

Authors: M. JIANG[1], J. ZHENG[1], S. ABADALI[1], Q. PENG[1], J. JIN[1], *W. DUAN[1,2,3]; [1]Dept Psychiatry, Johns Hopkins Univ., BALTIMORE, MD; [2]Dept. of Neurosci., [3]Program in Cell. and Mol. Med., Johns Hopkins Univ., Baltimore, MD

Abstract: Polyamines such as putrescine, spermidine, and spermine are small organic polycationic molecules that are present in almost all-living organisms. The regulation of these polyamines is required for cells to grow and function in an optimal manner. Dysregulation of cellular polyamines is associated with aging and various pathological conditions, including Huntington's disease (HD) (Vivo et al., Neurosci Lett 2001). In addition, cellular polyamine levels decreased along with aging. Consequently, dietary supplement of spermidine efficiently induced autophagy and ameliorated age-induced memory impairment, increased life span in low organisms, and reduced age-associated protein oxidation in mice. These data imply the important role of polyamines in aging and age-associated neurodegenerative diseases. In this study, we tested the effects of spermidine on mutant huntingtin-induced toxicity in a cell model of HD. Striatal cells expressing mutant huntingtin were treated with spermidine for 24 hrs. We found that spermidine induced autophagy, and dose-dependently attenuated mutant huntingtin toxicity, indicated by reduction of lactate dehydrogenase release and maintainence of ATP levels in striatal cells expressing mutant huntingtin under serum withdrawal condition. The molecular mechanisms by which spermidine protects cells against mutant huntingtin toxicity and the contribution of polyamine pathways to HD pathogenesis are under investigation. We expect that our results will provide a new understanding of the polyamine pathway in HD pathogenesis and a potential novel therapeutic strategy for HD. Acknowledgement: We acknowledge financial support from NINDS (NS082338) and CHDI foundation (to WD).

Disclosures: **M. Jiang:** None. **W. Duan:** None. **J. Zheng:** None. **S. Abadali:** None. **Q. Peng:** None. **J. Jin:** None.

Poster

046. Huntington's Disease Mechanisms I

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 46.05/J10

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: Hereditary Disease Foundation

NIH Grant R01 GM089903

Title: Epigenetic and transcriptional dysregulation in prodromal Huntington's disease

Authors: *F. YILDIRIM, C. W. NG, Z. R. CROOK, D. E. HOUSMAN, E. FRAENKEL; MIT, Cambridge, MA

Abstract: Transcriptional dysregulation is an early and pivotal feature of Huntington's disease that is strongly reflected in mouse models of this disorder. We have recently demonstrated in *in vitro* and mouse models genome-wide changes in DNA methylation and histone H3K4 trimethylation patterns that may underlie transcriptional dysregulation in HD (1, 2). In the study reported here, centering on the prodromal disease stage, we discovered that the pathogenic transcriptional and epigenetic alterations in the brain in mouse models in fact precede the overt onset of disease symptoms. Genome-wide analysis of transcription by RNA sequencing (RNA-Seq) revealed many key neuronal genes such as Drd1a and Drd2, Penk and Adora2a whose disruption are significant in HD pathogenesis to be down-regulated in the R6/1 mouse striatum as early as 8 weeks of age. RNA-Seq in a second mouse model that is the full length Huntingtin model of CHL2 [Hdh(CAG)150] heterozygous knock-in mice validated the ongoing transcriptional dysfunction in the striatum during prodromal HD and confirmed a highly significant overlap of differentially-expressed genes between the R6/1 and CHL2 models even at this early disease stage (142 overlapping genes, $p < 3e\text{-}128$). Comparison with a human HD study (3) showed that gene expression changes in our mouse models were consistent in the caudate nucleus of postmortem brains from HD patients (162 genes in R6/1 ($p < 3e\text{-}54$) and 51 genes in CHL2 ($p < 1.3e\text{-}10$)). This provides strong evidence that these mice models faithfully recapitulate the transcriptional dysfunction in human HD and that a significant portion of the early transcriptional changes persist throughout the later disease stages. Extension of our studies to genome-wide analysis of histone H3K27 acetylation, an active transcription and enhancer mark, by chromatin immunoprecipitation followed by sequencing (ChIP-Seq) revealed highly coordinated ($p< 1.7e\text{-}10$) histone acetylation changes along with the detected transcriptional changes in the striatum of R6/1 mice at 8 weeks of age. By studying the DNA sequences that lie within the altered H3K27 acetylation regions, we identified transcriptional regulators whose activity may be most proximally-disrupted by mutant Huntingtin consequently leading to the earliest transcriptional changes that we detected by RNA-Seq. The potential therapeutic value of targeting these predicted transcriptional regulators is currently under evaluation by functional experiments in mouse models of HD. 1.M. Vashishtha et al., Proc Natl Acad Sci U S A 110,

E3027 (Aug 6, 2013). 2.C. W. Ng et al., Proc Natl Acad Sci U S A 110, 2354 (Feb 5, 2013). 3.A. Kuhn et al., Hum Mol Genet 16, 1845 (Aug 1, 2007).

Disclosures: **F. Yildirim:** None. **C.W. Ng:** None. **Z.R. Crook:** None. **D.E. Housman:** None. **E. Fraenkel:** None.

Poster

046. Huntington's Disease Mechanisms I

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 46.06/J11

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NIH Director's transformative award

Roberson Family

Team KJ

Title: Allele-specific modification of the mutant huntingtin gene with transcription activator-like effectors

Authors: ***K. FINK**[1], P. DENG[2], W. CARY[1], A. TORREST[1], S. KALOMOIRIS[1], C. NACEY[1], H. STEWART[1], K. POLLOCK[1], K. PEPPER[1], W. GRUENLOH[1], G. ANNETT[1], T. TEMPKIN[3], V. WHEELOCK[3], D. J. SEGAL[2], J. A. NOLTA[1];
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Abstract: Huntington’s disease (HD) is an autosomal dominant neurodegenerative disorder caused by an abnormal expansion of CAG repeats encoding a polyglutamine sequence in the N-terminal region of the HD gene. It has been suggested that postnatal reduction of mutant huntingtin through protein interference or conditional gene knockout could prove to be an effective therapy for patients suffering from HD. The current study explored novel methods to reduce or silence expression of the mutant Huntingtin allele using transcription activator-like effectors (TALE) in primary human HD fibroblasts. In this study, human HD and control fibroblasts with varying CAG repeat lengths were characterized by the ratio of mutant to healthy huntingtin protein using homogeneous time-resolved fluorescence, levels of reactive oxygen species (ROS) via immunocytochemistry, the amount of poly-ubiquitinated proteins using

Western blots, and size of the CAG repeats through polymerase chain reaction. For allele-specific targeting, three TALE designed to target single-nucleotide polymorphisms (SNP) in the mutant allele were packaged into a vector backbone containing a Krüppel associated box (KRAB) domain to promote transcriptional repression of the disease-associated allele. Each SNP site was carefully selected based on proximity to the promoter region, global minor allele frequency (MAF) score, and specificity of the TALE to its target region. Three additional TALE used in unique combinations with each other and designed to target specific sites in the expanded CAG regions were packaged into a vector backbone containing the nuclease FokI to create double stranded breaks in the mutant allele. This strategy was implemented to shorten the expanded CAG repeats by gene collapse and non-homologous end-joining repair. The efficacy of each gene targeting strategy was quantified by transfecting primary human HD or control fibroblasts with either a TALE designed for gene suppression or correction. The rate of change pre- and post-treatment of the mutant protein ratio, ROS, poly-ubiquitinated proteins, and expression of the expanded CAG repeats was then quantified. This study demonstrates the potential of gene modification using TALE and provides a foundation for personalized treatment for individuals suffering from Huntington's disease. *Support for this project was provided by the NIH (Nolta), and philanthropic donors from the HD community, including the Roberson family and TeamKJ.*

Disclosures: **K. Fink:** None. **P. Deng:** None. **W. Cary:** None. **A. Torrest:** None. **S. Kalomoiris:** None. **C. Nacey:** None. **H. Stewart:** None. **K. Pollock:** None. **K. Pepper:** None. **W. Gruenloh:** None. **G. Annett:** None. **T. Tempkin:** None. **V. Wheelock:** None. **D.J. Segal:** None. **J.A. Nolta:** None.

Poster

046. Huntington's Disease Mechanisms I

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 46.07/J12

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: Fellowships from the Ph.D. School for Genetic Medicine, University of Copenhagen, Denmark

Stadslæge Svend Ahrend Larsen og grosserer Jon Johannesons Fond

Pfizer Ripples of Hope

Title: Insulin-like growth factor 1 signalling in huntington disease

Authors: *N. H. SKOTTE[1], M. A. POULADI[2], K. HUYNH[1], T. T. NIELSEN[3], R. GRAHAM[4], A. NØRREMØLLE[3], M. R. HAYDEN[1];
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Abstract: Huntington disease (HD) is a hereditary, fatal neurodegenerative disorder caused by a CAG repeat expansion in the huntingtin gene, which results in an elongated polyglutamine stretch causing a toxic gain of function in the mutant huntingtin (mHTT) protein. At this time, there is no way to stop or reverse the course of the disease. The pathogenesis of HD is complicated and multiple pathways are compromised. Cleavage of mHTT at the caspase-6 cleavage site (aa586), which generates toxic N-terminal cleavage fragments containing the expanded polyglutamine repeat, is a crucial step in the pathogenesis of HD. The mechanisms causing the excessive activation of caspase-6 in HD are not completely clear, but recent evidence suggests that regulation happens at both transcriptional and posttranslational steps. It has be shown that insulin-like growth factor 1 (IGF-1) signaling can induce caspase-6 phosphorylation and prevent its activation. This finding coupled with several studies showing reduced plasma and tissue IGF-1 levels in HD patients as well as in cellular and animal models of HD, makes the IGF-1 pathway an interesting target for HD therapy. In this study, we examined if there is a relationship between reduced IGF-1 levels and aberrant caspase-6 activation in HD. Using striatal cell lines derived from knock-in mice containing HTT with 7 or 111 glutamines (Q7/Q111 cells), we confirm that Q111 cells expressing mHTT secrete reduced levels of IGF-1 compared to wtHTT expressing Q7 cells. We show that the decrease in secreted IGF-1 levels in Q111 cells is associated with enhanced activation of caspase-6 and increased cell death after serum deprivation. IGF-1 supplementation of Q111 cells reduces serum deprivation-induced caspase-6 activation, prevents the generation of the toxic mHTT 586 fragment, and ameliorates cell death compared to non-supplemented Q111 cells. Lastly, transcriptional analysis demonstrate that the HD mutation may impair the IGF system at multiple levels by affecting the expression of IGF receptors and binding proteins. Our findings suggest that dysregulated IGF-1 signalling may contribute to the excessive activation of caspase-6 in HD and that modulation of the IGF system may be an alternative approach to reduce mHTT cleavage.

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Poster

046. Huntington's Disease Mechanisms I

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

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NIH Grant 3NS039074

NIH Grant 2NS045091

NIH U24 Grant NS078370

Title: Regulation of mitochondrial autophagy by mutant huntingtin

Authors: *J. MARGULIS[1,2], D. M. ANDO[1,2], S. M. FINKBEINER[1,2];
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Abstract: Huntington's disease is an inherited and incurable neurodegenerative disorder caused by an abnormal polyglutamine (polyQ) expansion in huntingtin. The mechanisms of mutant huntingtin-mediated neurotoxicity remain unclear; however, dysregulation of protein quality control is known to be a key event in Huntington's disease pathogenesis. Here, we tested whether mutant huntingtin also affects mitochondrial quality control via dysregulation of mitochondrial autophagy, or mitophagy. In order to monitor autophagy-mediated flux of mitochondria in live neurons, we developed a sensitive and quantitative image-based technique utilizing automated microscopy and photoswitchable fluorescent proteins. The rate of mitochondrial degradation measured using this technique is sensitive to pharmacological inducers and inhibitors of mitophagy in primary neurons as well as neuronal cell lines. We found that expression of mutant huntingtin in both primary neurons and human induced pluripotent stem cell (iPSC)-derived neurons reduces mitochondrial half-life, and that this effect is dependent on the presence of the first 17 amino acids of huntingtin. The presence or absence of mutant huntingtin inclusions also affects mitochondrial function and half-life. Thus, mutant huntingtin-mediated regulation of mitophagy may represent a novel target for therapeutic intervention in Huntington's disease.

Disclosures: **J. Margulis:** None. **D.M. Ando:** None. **S.M. Finkbeiner:** None.

Poster

046. Huntington's Disease Mechanisms I

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Program#/Poster#: 46.09/K2

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: Canadian Institutes for Health Research

Cure Huntington Disease Initiative

Title: Differences in synaptic scaling in striatal-cortical co-cultures from wild-type and YAC128 mice

Authors: *A. I. SMITH-DIJAK[1,2], J. B. MAU[1], L. A. RAYMOND[1];
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Abstract: Huntington disease (HD) is a neurodegenerative disorder caused by a polyglutamine expansion in the huntingtin protein, producing mutant huntingtin (mHtt). Many pre- and postsynaptic proteins interact with mHtt, and the function of at least some of these proteins is affected by the disease-causing mutation. One of the consequences of these changes in protein function is alterations in synaptic signaling and plasticity. For example, there are changes in the localization of the NMDA receptor subunit GluN2B, which have consequences for cell signaling and survival. Particularly affected are the cortico-striatal synapses, especially those between cortical neurons and striatal spiny projection neurons (SPNs). We set out to examine changes in synaptic scaling, a form of homeostatic plasticity in which the strength of synapses in a network is increased or decreased in response to the overall level of activity in the network, in excitatory synapses onto striatal SPNs from the YAC128 HD mouse model. We used immunocytochemical analyses and electrophysiological measurements to determine the effect of 48 hours of treatment with either tetrodotoxin (TTX), bicuculline (BIC) or vehicle (water) on synaptic strength and organization in striatal-cortical co-cultures from embryonic wild-type (WT) and YAC128 mice; experiments were done after 3 weeks in culture. We found that both the degree and direction of scaling was changed in YAC128 compared to WT cultures. Specifically, we assessed: co-localization of the (postsynaptic) GluA2 AMPA receptor subunit and the (presynaptic) VGLUT1 glutamate transporter; the density of dendritic spiny protrusions; and the amplitude and frequency of miniature excitatory postsynaptic currents (mEPSCs). Of the changes observed in these metrics in response to treatment with TTX or BIC versus the vehicle control, some were conserved between the two genotypes, while others differed in size and/or direction of change. These findings suggest that homeostatic plasticity, in particular synaptic scaling, is impaired in HD in ways which could contribute to the neuronal dysfunction and eventual neurodegeneration which take place during the HD disease process.

Disclosures: **A.I. Smith-Dijak:** None. **J.B. Mau:** None. **L.A. Raymond:** None.

Poster

046. Huntington's Disease Mechanisms I

Location: Halls A-C

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NSC100-2321-B-001-009

AS-100-TP2-B02

Title: Elevated oxidative stress exacerbates neuronal atrophy via activation of AMPK-α1 in Huntington's disease

Authors: *Y. CHERN[1], T.-C. JU[2], H.-M. CHEN[2];
[1]Inst. Biomed Sci., Taipei, Taiwan; [2]Inst. of Biomed. Science, Academia Sinica, Taipei, Taiwan

Abstract: Huntington's disease (HD) is an autosomal dominant neurological disorder induced by a CAG trinucleotide expansion in the exon 1 of Huntingtin (HTT) gene. We previously reported that the abnormal activation of AMPK-α1 contributes to neuronal degeneration in HD. We showed here that the elevated oxidative stress evoked by mutant HTT (mHTT) caused the abnormal activation of AMPK-α1 and resulted in neurotoxicity in a striatal progenitor cell line (STHdhQ109) and in the striatum of a transgenic mouse model of HD (R6/2). Administration of an antioxidant (N-acetyl-cysteine, NAC) to R6/2 mice normalized the activation of AMPK-α1, reduced neuronal toxicity, ameliorated ventricle enlargement, and improved motor dysfunction. Given that NAC also reduced the oxidative stress-induced AMPK-α1 activation and the death of STHdhQ109 cells, the beneficial effect of NAC *in vivo* is likely to be direct. Interestingly, the activation of AMPK elevated the level of oxidative stress in STHdhQ109 cells and in the striatum of R6/2 mice, whereas the suppression of AMPK lowered the level of oxidative stress. In summary, we present evidence to show a detrimental, positive feedback regulation between the elevated oxidative stress and the activation of AMPK-α1 in HD.

Disclosures: **Y. Chern:** None. **T. Ju:** None. **H. Chen:** None.

Poster

046. Huntington's Disease Mechanisms I

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Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 46.11/K4

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: CHDI Research Project A6409

Title: Effects of post-translational modifications sites mutations of full-length huntingtin on neuronal toxicity

Authors: *N. ARBEZ, T. RATOVITSKI, L. NUCIFORA, J. STEWART, A. CHAUDHARY, C. ROSS;
Div. of Neurobio., Johns Hopkins Univ., Baltimore, MD

Abstract: Huntington's disease (HD) is a fatal progressive neurodegenerative disorder involving movement, cognitive and emotional symptoms. Post-translational modifications (PTMs) of expanded huntingtin (Htt) are likely to be important mediators or modulators of HD pathogenesis. Phosphorylation has been shown to have a significant impact on expanded Htt-mediated cellular toxicity. While these studies established the involvement of some N terminal phosphorylation sites, they were carried out using the N terminal fragments of Htt. In this study, aimed to functionally validate PTMs of Htt, we established cellular and biochemical assays using full length Htt. We investigated the effects of alterations of PTM sites of Htt on itsneuronal toxicity. In parallel, we studied some biochemical and structural properties ofHtt with PTM alterations. A series of constructs expressing full length Htt with PTM alterations were created. Sites were selected from previously established important phosphorylation sites (like S13 and S16) as well as potential new sites previously identified in the laboratory. We established nuclear condensation cell toxicity assay for full-Length-Htt expressed in primary neurons. Over the time of the experiment, full length Htt can strongly induce cell death. The levels of toxicity achieved with full length Htt are however lower than toxicity observed with Htt fragments (N586-82Q Htt or Exon1-82Q). Using our PTM mutants, we have confirmed the protective effects of T3D and S13/16D alterations. We have also confirmed the protective effect of S421D alteration, and also observed protection with S421A and K444R mutations within full-length Htt. Since the nuclear localization of Htt or its fragment is important in the pathology of HD, we developed an assay for quantitative analysis of nuclear/cytoplasmic localization of FL-Htt expressed in primary neurons by using confocal analysis. We found no difference between the FL-Htt constructs tested so far. Since the aggregation and the structure of Htt are important for its toxicity, we established a limited proteinase K digestion assay using our FL Htt mutants. We also established an *in vitro* aggregation assay using purified Htt-N586 proteins.Using these two assays, .we demonstrated differences between full-length normal, expanded Htt and PTM mutants. These new and

confirmed PTMs sites of Htt that modulate the cellular toxicity represent potential new therapeutical targets for the treatment of HD.

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Poster

046. Huntington's Disease Mechanisms I

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Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 46.12/K5

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: KAKENHI 24390220

Title: Differential effect of HDAC3 on cytoplasmic and nuclear polyglutamine aggregates

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Abstract: Histone deacetylases (HDACs) are one of the therapeutic targets of polyglutamine (pQ) diseases possibly through fixing aberrant translational deactivation caused by mutant polyglutamine proteins. Among various HDACs, HDAC3 is a unique class 1 HDAC that localizes in both cytoplasm and in the nucleus. However, precise functions of HDAC3 in those two compartments are vaguely known so far. HDAC3 directly binds to short pQ and the interaction is important in suppressing HDAC3's neurotoxicity, which is broken with long pQ chain and supposedly promotes neuronal death (Bardai, 2013). Therefore, HDAC3 can be a good therapeutic target for pQ diseases. However, other study using heterozygote knockout mouse did not show any efficacy on Huntington's disease pQ model mouse (Moumn**é**, 2012). Thus, the role of HDAC3 in the pathogenesis in pQ diseases is yet to be fully understood. We tried to resolve this issue by focusing on different role of HDAC3 on cytoplasmic and nuclear pQ aggregates. In addition to previous findings, we found that HDAC3 preferably binds to nuclear pQs compared to cytoplasmic ones. Specific HDAC3 inhibitors increased the amount of total pQ aggregates by increasing the amount of nuclear aggregates, whereas decreasing the amount of cytoplasmic aggregates. Selective HDAC3 inhibitors impaired nuclear proteasome activity, but quite interestingly promoted cytoplasmic proteasome activity. Our findings suggest that HDAC3 has

differential functional role in the cytoplasm and in the nucleus in terms of pQ aggregate degradation.

Disclosures: **T. Mano:** None. **T. Suzuki:** None. **A. Iwata:** None.

Poster

046. Huntington's Disease Mechanisms I

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 46.13/K6

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: CHDI Grant ID A-5954

Title: The role of complement-microglia interactions in driving synaptic loss and disease progression in Huntington's disease

Authors: D. K. WILTON[1], A. FROUIN[1], A. DAGGETT[2], W. YANG[2], *B. A. STEVENS[1]; [1]F. M Kirby Neurobilogy Ctr., Childrens Hosp. Boston, Boston, MA; [2]UCLA, Los Angeles, CA

Abstract: Huntington's disease (HD) is an inherited neurodegenerative disorder, characterized by motor, cognitive, behavioral and psychological dysfunction. In common with many neurodegenerative disorders one of the earliest events in HD pathology is dysfunction and loss of synapses (Graveland et al., 1995; Ferrante et al., 1991). Our laboratory recently identified components of the classical complement cascade as mediators of synapse elimination during developmental synaptic refinement (Schafer et al., 2012). Our data suggests a model in which less active synapses are selectively labeled with complement and then engulfed by microglia that express complement receptors (Ie CR3/Cd11b). In this study we have investigated whether aberrant reactivation of this pruning mechanism occurs in HD and whether it is responsible for the synaptic dysfunction observed. We find that in two mouse models of HD (BACHD and the knock-in model zQ175) levels of complement cascade components are elevated in disease affected regions and increase in line with disease progression. Using high resolution microscopy we show that at early time points complement localizes specifically to vulnerable cortico-striatal synapses prior to their loss from the striatum. Interestingly microglia in these regions adopt a more phagocytic phenotype at this time point suggesting they may be acting to engulf these synapses in a similar manner to that seen in the developing visual system. These results suggest that aberrant interactions between the complement system and microglia may drive early loss of

cortico-striatal synapses and contribute to behavioral deficits and neurodegenerative pathology in HD.

Disclosures: **D.K. Wilton:** None. **A. Daggett:** None. **W. Yang:** None. **B.A. Stevens:** None. **A. Frouin:** None.

Poster

046. Huntington's Disease Mechanisms I

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 46.14/K7

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Title: Development and validation of a high throughput screen to detect small molecule modulators of mutant huntingtin protein levels in HD patient-derived cells

Authors: G. MCALLISTER[1], O. LAZARI[1], I. GOWERS[1], G. CREIGHTON-GUTTERIDGE[1], J. BATE[1], R. JARVIS[1], W. BLACKABY[1], I. MUNOZ-SANJUAN[3], R. SCHOENFELD[4], S. KWAK[4], *J. A. BARD[4], M. VROUWE[2], D. F. FISCHER[2], D. MACDONALD[3];
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Abstract: Despite the identification of the pathogenic poly-CAG expansion to the huntingtin (*Htt*) gene as the cause of Huntington's Disease (HD) over 20 years ago, there remains no effective treatment for the disease. Numerous studies have been performed to identify molecules or genes which modulate the toxic effects or expression of mutant HTT (mutHTT). These studies have often been based around a single hypothesis (e.g. reducing aggregation or preventing caspase cleavage). Often, an exogenous promoter driving expression of a HTT fragment with a polyQ length well beyond a clinically relevant range is required to generate a sufficient assay signal suitable for reliable screening. This may have contributed to the lack of translatability of hit genes or molecules from one screen to another, and to higher *in vivo* models of HD. Recent advances in HTT protein detection by HTRF and MSD assays have allowed us to develop an HTRF assay detecting the expression of native mutHTT and total HTT proteins in immortalized HD patient lymphoblasts, chosen due to their clinical relevance. Cells are plated into 384-well plates and exposed to compounds for 48 h before being lysed, after which mutHTT and total HTT levels are quantified by specific antibody combinations. Toxicity is monitored by ATP determination as a surrogate for cell number. As these assays are able to measure changes in

HTT protein levels, they are not biased to identify molecules which only affect one hypothetical method of HTT toxicity (*e.g.* aggregation). As the *Htt* gene is expressed in an endogenous genomic context and with an HD-patient relevant Q-length, the assay may pick up a wide range of molecules affecting transcription, translation, and/or changes to protein fate within the cells. A pilot screen of 7000 compounds (tested n=2) from the CHDI collection comprising of both hypothesis-based and diverse compounds has revealed that the assay is suitable for HTS (Z' >0.3; S:B>2), and hit matter can be reliably detected and efficiently translated from single point to IC_{50} curves. Molecules with validated activity against the PI3K and mTOR family were identified and show good translation into a rat neuronal co-culture assay, where they both promote survival and lower HTT at similar concentrations as those identified in the primary assay. Further validation of these and other hit compounds to establish an extended screening cascade, including validation in alternative cell lines (e.g. stem cell derived neuronal precursors or fibroblasts) and determination of mechanism of action of hit compounds (i.e. do hits work via transcription, translation, autophagy pathways etc.) will be described.

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Poster

046. Huntington's Disease Mechanisms I

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: Huntington Society of Canada Grant

NSERC Discovery Grant

Title: Mutant Huntingtin mediated repression of antioxidant gene expression is rescued by a novel Nrf2 activating agent

Authors: L. TINDALE, *R. C. CUMMING;
Dept. of Biol., Univ. of Western Ontario, London, ON, Canada

Abstract: Mitochondrial dysfunction and elevated reactive oxygen species (ROS) levels are strongly implicated in various neurodegenerative disorders, including Huntington's disease (HD). Expression of the mutant Huntingtin protein (mHtt) containing an expanded polyglutamine repeat is associated with oxidative stress and toxicity in striatal neurons. We previously demonstrated that overexpression of mHtt in PC12 cells leads to elevated ROS production and a concomitant decrease in expression of the antioxidant protein peroxiredoxin1 (Prx1). Interestingly, treatment with the compound dimercaptopropanol (DMP) prevents mHtt-mediated inhibition of antioxidant gene expression and neurotoxicity. Nuclear factor erythroid 2-related factor 2 (Nrf2) is a transcription factor responsible for regulating expression of a diverse array of antioxidant genes under the control of cis-acting antioxidant response elements (AREs), including Prx1. Nrf2 is maintained at very low levels by its negative regulator Kelch-Like ECH-associated Protein 1 (Keap1), which facilitates the ubiquitination and subsequent degradation of Nrf2 by the proteasome. Post-translational modification of Keap1 and/or Nrf2 by electrophiles and oxidants disrupts the Keap1-Nrf2 interaction, resulting in the stabilization and nuclear translocation of Nrf2. There is currently great interest in identifying Nrf2 activating compounds for the treatment of neurodegenerative diseases. Here we demonstrate that mHtt prevents Nrf2 nuclear translocation and activation of antioxidant enzyme expression in PC12 cells and in an immortalized striatal cell line (STHdhQ111). In contrast, DMP exposure decreases the levels of Keap1, thereby allowing activation of Nrf2 even in the presence of mHtt. Preliminary findings indicate that DMP promotes the degradation of Keap1, possibly via an autophagic/lysosomal process. The identification of DMP as a neuroprotective agent that facilitates the degradation of Keap1 and promotes Nrf2 activation is highly novel and suggests that alternative modes of Nrf2 activation exist. In addition, DMP, also known as British anti-Lewisite (BAL), was shown to attenuate disease progression in a long term study of two HD patients conducted in 1955. The current study highlights previously unknown intracellular targets of DMP and indicates that this FDA approved compound may have relevance for the treatment of HD and other neurodegenerative disorders.

Disclosures: **L. Tindale:** None. **R.C. Cumming:** None.

Poster

046. Huntington's Disease Mechanisms I

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ANR

FUI

LECMA/Alzheimer Forschung Initiative

France Alzheimer

Portuguese Fundação para a Ciência e a Tecnologia

EMBO Installation Grant

Title: Mutant huntingtin alters Tau phosphorylation and subcellular distribution

Authors: *D. BLUM[1], F. HERRERA[2], T. MENDES[2], L. FRANCELLE[3], M. BASQUIN[1], H. OBRIOT[1], D. DEMEYER[1], N. SERGEANT[1], E. GERHARDT[4], E. BROUILLET[3], L. BUEE[1], T. OUTEIRO[4,2];
[1]Inserm U837, Alzheimer & Tauopathies, Lille, France; [2]IMM, Lisbon, Portugal; [3]CEA, Fontenay-aux-roses, France; [4]Ctr. for Nanoscale Microscopy and Mol. Physiol. of the Brain, Univ. Med. Ctr., Dept. of Neurodegeneration and Restorative Res., Goettingen, Germany

Abstract: Tau abnormalities play a central role in several neurodegenerative diseases, collectively known as tauopathies. In the present study, we examined whether mutant huntingtin, which causes Huntington's disease (HD), modifies Tau phosphorylation and subcellular localization using cell and mouse HD models. Initially, we used novel bimolecular fluorescence complementation (BiFC) assays in live cells to evaluate Tau interactions with either wild type (25QHtt) or mutant huntingtin (103QHtt). While 25QHtt and Tau interacted at the level of the microtubule network, 103QHtt and Tau interacted and formed "knot-like" inclusions localized in the vicinity of the microtubular organizing center (MTOC). Fluorescence recovery after photobleaching experiments also indicated that, whereas homomeric 103QHtt/103QHtt pairs rapidly re-entered into inclusions, heteromeric 103QHtt/Tau pairs remained excluded from the "knot-like" inclusions. Interestingly, *in vitro* Tau relocalization was associated to Tau hyperphosphorylation. Consistent with this observation, we found strong Tau hyperphosphorylation in brain samples from two different mouse models of HD, R6/2 and 140CAG knock-in. This was associated with a significant reduction in the levels of Tau phosphatases (PP1, PP2A and PP2B), with no apparent involvement of major Tau kinases. Thus, the present study strongly suggests that expression of mutant huntingtin leads to Tau hyperphosphorylation, relocalization, and sequestration through direct protein-protein interactions in inclusion-like compartments in the vicinity of the MTOC. Likewise, our data also suggests that Tau alterations may also contribute to HD pathogenesis.

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Poster

046. Huntington's Disease Mechanisms I

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NIH R01 NS079450-01

Title: Elevated neonatal iron intake potentiates progression in the R6/2 mouse model of Huntington's disease

Authors: ***K. L. BERGGREN**[1,2], J. CHEN[1,2], J. MILLER[1], J. H. FOX[1,2];
[1]Vet. Sci., [2]Interdepartmental Grad. Neurosci. Program, Univ. of Wyoming, Laramie, WY

Abstract: Huntington's disease (HD) is an autosomal dominant neurodegenerative disorder resulting from expression of polyglutamine-expanded huntingtin protein. Brain iron accumulation is implicated in potentiating the neurodegeneration in HD, Parkinson's disease, amyotrophic lateral sclerosis and Alzheimer's disease. Iron levels are also elevated in the brains of HD mice. The purpose of this study was to test the hypothesis that elevated dietary iron intake potentiates HD in the R6/2 mouse model. We tested the effect of nutritionally relevant levels of elevated iron intake during the neonatal period (post-natal days 10-17) and in adult life (5-12 weeks of age). In study 1, neonatal wild-type (WT) and R6/2 HD mouse pups were dosed with vehicle or 120μg/g body weight carbonyl iron/day. HD mice demonstrated poorer performance in spontaneous in-cage wheel running with disease progression. Neuronal cell body size in the striata and cortices of R6/2 mice at 12 weeks were significantly decreased compared to WT controls; there were further decreases in both regions of HD mice with iron supplementation. Levels of oxidized glutathione and lactate, markers of oxidative stress and energetic dysfunction, were increased in the cortices and striata of iron-treated HD mice compared to HD-control mice. In study 2, HD and WT adult mice were fed diets containing 50, 150 or 500 ppm iron from 5-12 weeks. In contrast to the neonatal study we have not found any effect of these different dietary iron levels on HD outcomes. Taken together, our findings show that the neonatal mouse HD brain is vulnerable to elevated iron intake. Early-life iron nutrition may influence onset and progression of human HD.

Disclosures: **K.L. Berggren:** None. **J. Chen:** None. **J. Miller:** None. **J.H. Fox:** None.

Poster

046. Huntington's Disease Mechanisms I

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Program#/Poster#: 46.18/L2

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NS78633

NS41574

Title: Optogenetic control of parvalbumin-expressing interneurons in the R6/2 and Q175 mouse models of Huntington's disease

Authors: *L. GALVAN, C. CEPEDA, M. LEVINE;
IDDRC, Semel Inst. For Neurosci. and Human Behavior, BRI, UCLA, Los Angeles, CA

Abstract: Huntington's disease (HD), a neurodegenerative disorder caused by a mutation in the IT15 gene, is characterized by dysfunction and ultimate degeneration of striatal medium-sized spiny neurons (MSNs) and cortical pyramidal neurons (CPNs). Although it was thought that interneurons were relatively spared in HD, recent studies have shown a significant loss of parvalbumin (PV)-expressing interneurons that increases with severity of the disease. PV-expressing interneurons display fast-firing properties and mediate feed-forward inhibition in the striatum as well as limit CPN excitability. MSNs display an increase in GABAergic activity whereas CPNs show an increase in glutamatergic activity in several HD mouse models as the phenotype progresses. We hypothesized that PV-expressing interneurons may contribute to MSN and CPN dysfunction in HD. Previously we demonstrated significant changes in PV-expressing interneuron-evoked inhibitory responses in MSNs in symptomatic R6/2 mice using optogenetics. Here, using a similar optogenetic paradigm, we further examined alterations in PV-expressing interneuron inputs to MSNs and to CPNs in the R6/2 model. Additionally, we examined PV-expressing interneuron evoked responses in another HD mouse model, the Q175 knock-in, which has a more protracted course of phenotype progression. R6/2 and Q175 mice were crossed with PV-Cre mice and subsequently injected with a Cre-dependent channelrhodopsin-2 (ChR2) construct using viral delivery. MSNs or layer II/III CPNs were recorded in slices in voltage clamp mode. In symptomatic R6/2 mice, MSN recordings showed that activation of striatal PV-expressing interneurons induced significantly larger evoked GABAergic responses with faster

kinetics than responses from wildtype controls (WTs). GABAergic responses in CPNs from R6/2 mice exhibited a significant increase in charge and different kinetics compared to those from WTs. In presymptomatic Q175 mice activation of PV-expressing interneurons in the striatum showed a trend to evoke larger MSN GABAergic responses with faster rise times than occurred in WTs, an effect similar to observations in R6/2 MSNs. Preliminary results from CPNs in Q175 mice suggested a different trend than that observed in R6/2 CPNs and findings from symptomatic Q175 mice indicated that these dysfunctions may not be maintained in the late stage of the disease in this model. Taken together these findings indicate that PV-expressing inhibitory interneurons uniquely affect MSNs and CPNs in each mouse model and can contribute to neuronal microcircuit alterations in HD.

Disclosures: **L. Galvan:** None. **C. Cepeda:** None. **M. Levine:** None.

Poster

046. Huntington's Disease Mechanisms I

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 46.19/L3

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Title: Comparison of phosphodiesterase 10A, dopamine receptors D1 and D2 and dopamine transporter ligand binding in the striatum of the R6/2 and BACHD mouse models of Huntington's disease

Authors: *S. MILLER[1], G. HILL DELLA PUPPA[1], J. REIDLING[2], L. M. THOMPSON[3], J. TREANOR[1];
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Abstract: Huntington's disease is an autosomal-dominant inherited neurodegenerative disorder characterized by motor dysfunction, cognitive decline and emotional and psychiatric disturbance. The genetic mutation is characterized by a CAG expansion resulting in formation of a mutant huntingtin protein with an expanded poly-glutamine repeat region. The medium spiny neurons of the striatum are particularly vulnerable to the toxicity of this protein and striatal degeneration is believed to underlie many of the motor symptoms that are typical of the disease. Phosphodiesterase (PDE) 10A is expressed at high levels in the medium spiny neurons of the striatum and may therefore represent a biomarker for disease progression. Our study investigated

changes in PDE10A binding using the selective PDE10A tracer ^{3}H-7980 at representative stages of Huntington's disease in two different mouse models, R6/2 and BACHD. R6/2 mice carry a transgene encoding an amino terminal fragment of the HD gene and show robust transcriptional and behavioral changes. BACHD mice express a full length transgene and display later behavioral dysfunction, aggregation and selected transcriptional abnormalities. Changes in PDE10A binding were compared to binding of radioligands for dopaminergic markers expressed by medium spiny neurons, e.g. dopamine transporter (DAT) labelled with ^{3}H-WIN 35,428 and dopamine receptors D2 and D1 labelled with ^{3}H-raclopride and ^{3}H-SCH23390, respectively. We show that in the R6/2 model binding of all ligands is significantly decreased at 8 and 12, but not 6 weeks of age. In contrast, no changes were detectable in the BACHD model at 8, 10 or 12 month of age. These findings suggest that radioligands can successfully detect neurodegeneration of the striatum of R6/2. Since decreases in D2 and PDE10A binding have been demonstrated in patients, our data suggests that this approach using the R6/2 mouse model is well suited for translational biomarker studies in Huntington's disease that may be applicable to human clinical trials of PDE10A targeted treatments.

Disclosures: **S. Miller:** A. Employment/Salary (full or part-time):; Amgen Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Amgen Inc. **G. Hill della Puppa:** A. Employment/Salary (full or part-time):; Amgen Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Amgen Inc. **J. Reidling:** A. Employment/Salary (full or part-time):; University of California at Irvine. **L.M. Thompson:** A. Employment/Salary (full or part-time):; University of California at Irvine. **J. Treanor:** A. Employment/Salary (full or part-time):; Amgen Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Amgen Inc..

Poster

046. Huntington's Disease Mechanisms I

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 46.20/L4

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NIH Grant AG019206

NIH Grant AG31153

Title: Oligodendrocyte dysfunction in Huntington's disease

Authors: *W. WEI[1,2], B. HUANG[1], X.-J. LI[1], S. LI[1];
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Abstract: Huntington's disease (HD) is a fetal, progressive neurodegenerative disorder caused by an abnormal expansion of the CAG repeat that encodes an expanded polyglutamine tract in the N-terminal region of mutant huntingtin (htt) protein. Despite the ubiquitous expression of mutant htt, HD shows selective neurodegeneration. Numerous studies have focused on neurons for pathogenesis studies, but growing evidence also indicates non-neuronal cell toxicity of htt, such as glial htt toxicity that is evidenced by white matter abnormalities in HD. However, how and why mutant htt affects oligodendrocyte is not investigated yet. To address this issue, we generated PLP-150Q and PLP-23Q-htt transgenic mice, which express mutant and wild type htt, respectively, in oligodendrocytes. PLP-150Q mice displayed progressive neurological symptoms and demyelination. To further investigate the pathogenic role of mutant htt in oligodendrocytes at endogenous level, full length mutant htt knock-in mice that express GFP exclusively in oligodendrocytes were generated. By sorting GFP-positive cells, we were able to enrich oligodendrocytes that express full-length mutant htt at the endogenous level. RNA-Seq was performed on these cells to obtain the transcriptome profiling. Our study will provide insights into the pathogenesis and therapeutic targets associated with oligodendrocytes in HD.

Disclosures: **W. Wei:** None. **B. Huang:** None. **X. Li:** None. **S. Li:** None.

Poster

046. Huntington's Disease Mechanisms I

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 46.21/L5

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: CHDI, Inc

NIH NS41574

Title: Differential and region-specific contributions of GABAergic interneurons in mouse models of Huntington's disease

Authors: *S. M. HOLLEY, K. N. RUDBERG, C. CEPEDA, M. S. LEVINE;
IDDRC, Semel Inst. for Neurosci. and Human Behavior, BRI, UCLA, Los Angeles, CA

Abstract: Throughout the course of Huntington's disease (HD), patients experience a loss in motor coordination and disturbances in mood, partly as a dysfunction of the corticostriatal pathway. In the R6/2 model of HD, we previously have shown alterations in inhibition in both cortex and striatum when compared to WT mice (Cepeda et al., 2013, Cummings et al., 2009). Here, we used optogenetics to investigate the specific contribution of GABAergic somatostatin (SOM) and neuropeptide Y (NPY) interneurons in striatum and cortex of HD mouse models. In striatal medium-sized spiny neurons (MSNs) from symptomatic R6/2 mice, optically activating SOM interneurons expressing channelrhodopsin produced inhibitory postsynaptic currents (IPSCs) that did not differ in amplitude but displayed faster kinetics compared to responses evoked in MSNs from wildtypes (WTs). Similar results were obtained when optically evoking IPSCs in symptomatic Q175 mice (12-14 month old). To further examine the contribution of striatal SOM interneurons, we optically silenced these interneurons with halorhodopsin and observed decreased frequencies of spontaneous (s)IPSCs in a subset of R6/2 MSNs with more R6/2 MSNs exhibiting such frequency reduction compared to WTs. This finding suggests that SOM-expressing interneurons may contribute to the increased inhibition observed in striatal MSNs of R6/2 mice. Activating NPY interneurons in the striatum resulted in very large IPSCs in both WT and R6/2 MSNs that displayed similar amplitudes and kinetics. Interestingly, average MSN IPSC responses from NPY interneurons were 10x larger in amplitude than SOM activated IPSCs suggesting a larger inhibitory contribution from these interneurons in the striatum. In the cortex of R6/2 mice, optically evoked IPSCs in pyramidal neurons, via SOM interneuron activation, were smaller in amplitude compared to WT responses but displayed no significant differences in kinetics. Thus, the reduced inhibition from SOM interneurons in the cortex may contribute to hyperexcitability of pyramidal neurons. Furthermore, average striatal IPSC responses through SOM interneuron activation in R6/2 and Q175 MSNs were smaller in magnitude than responses observed in cortical pyramidal neurons indicating a difference in the inhibitory contribution of SOM interneurons in the cortex and striatum. Taken together, the findings show differential inhibitory contributions from SOM and NPY interneurons in cortex and striatum in mouse models of HD.

Disclosures: **S.M. Holley:** None. **K.N. Rudberg:** None. **C. Cepeda:** None. **M.S. Levine:** None.

Poster

046. Huntington's Disease Mechanisms I

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 46.22/L6

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NIH Grant NS41574

Title: Altered spontaneous synaptic currents in external globus pallidus neurons in the R6/2 mouse model of Huntington's disease

Authors: *J. BARRY, G. AKOPIAN, C. CEPEDA, M. S. LEVINE;
IDDRC, Semel Inst. For Neurosci. and Human Behavior, BRI, UCLA, Los Angeles, CA

Abstract: Huntington's disease (HD) is a hereditary neurological disorder characterized by chorea, cognitive deficits and psychiatric symptoms. In HD there is a loss of striatal medium-sized spiny neurons (MSNs). In particular, MSNs of the indirect output pathway, which project primarily to the external segment of the globus pallidus (GPe), are believed to be affected earlier in the progression of HD. Electrophysiological changes in GPe and other striatal output regions have not been characterized in detail. In the present study we examined spontaneous inhibitory (sIPSC) and excitatory (sEPSC) postsynaptic currents in the GPe of symptomatic R6/2 mice and their wildtype (WT) littermates *in vitro*. sIPSC recordings were made from 29 neurons in WTs and 26 in R6/2 mice. There were no significant differences in average sIPSC amplitude, frequency or interevent intervals. However, GPe neurons from both R6/2 and WT mice could be subdivided in two groups with respect to event amplitude-frequency histograms. One population of cells (Type 1) displayed amplitude-frequency histograms with one peak and relatively small average amplitudes of events (48±6.6 pA, n=21 in WT and 49±6.4 pA, n=17 in R6/2), while the second population (Type 2) exhibited two peaks and had higher average amplitudes of events (124±18.3 pA, n=8 in WT and 105±16.6, n=9 in R6/2). For Type 1 neurons average sIPSC frequency was significantly higher for cells from R6/2 compared to WTs (25.8±2.1 for R6/2 vs 19.1±1.3 for WT; p=0.01) while for Type 2 neurons the average frequency was lower for cells from R6/2 compared to WTs (19.4±2.0 for R6/2 vs. 27.2±2.5 for WT; p=0.03). Average sIPSC amplitudes for Type 1 or Type 2 GPe neurons were similar for R6/2s and WTs. sEPSC recordings were made in 18 GPe neurons in WTs and 16 neurons in R6/2s. There was a significantly lower amplitude of sEPSCs in R6/2 mice (8.9±2.5 pA in R6/2 vs. 14.1±5.8 pA in WTs; p=0.0022). Overall the frequency of sEPSCs in both groups was low compared to sIPSCs and differences in frequency were not observed between R6/2 and WT mice. Assuming that large sIPSCs are associated with action potential-dependent inputs to GPe neurons, the change in sIPSC frequency of Type 1 and Type 2 cells may reflect altered properties of different synaptic connections in symptomatic R6/2 mice. We have now begun to use optogenetics to more specifically examine inhibitory inputs from the striatum to the GPe. Taken together these findings indicate that an imbalance in inhibition in conjunction with reduced amplitude of

excitatory events underlies alterations in firing patterns and function of GPe neurons in R6/2 mice.

Disclosures: **J. Barry:** None. **G. Akopian:** None. **C. Cepeda:** None. **M.S. Levine:** None.

Poster

046. Huntington's Disease Mechanisms I

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 46.23/L7

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: CHDI Grant A-5490

Title: Role of p75 neurotrophin receptor signaling in pathogenesis of Huntington's Disease

Authors: ***A. WEHNER**[1], R. L. ALBIN[2], B. A. PIERCHALA[1];
[1]Biol. and Materials Sci., [2]Neurol., Univ. of Michigan-Ann Arbor, Ann Arbor, MI

Abstract: Huntington's disease (HD) is a dominantly-inherited neurodegenerative disorder characterized by a constellation of motor, cognitive, and psychiatric symptoms. HD is caused by a CAG expansion in the *huntingtin (HTT)* gene. While the mutant protein is ubiquitously expressed, the striatum is affected earlier than most other brain regions. Striatal neurons are dependent on brain-derived neurotrophic factor (BDNF) from the cortex for proper function and survival. Non-mutant Htt has been shown to enhance BDNF expression by binding to the BDNF promoter and is also important for transport of BDNF to the striatum. In HD post-mortem tissue, there is a decrease in BDNF levels in the striatum, which has also been observed in animal models of HD. BDNF functions through its high affinity receptor, TrkB, to promote survival and maintain normal neuronal function. BDNF, however, can also bind to a lower affinity neurotrophin receptor, p75. Recent studies suggest both TrkB and p75 are improperly regulated in the striatum of HD patients and mouse models of HD. While BDNF-TrkB signaling almost exclusively promotes survival and metabolic function, p75 signaling is able to induce survival or apoptosis depending on its available ligand and associated co-receptor. In this study, we investigated the role of p75 in the Q175 knock-in mouse model by examining the level and activation of various downstream signaling molecules, as well as receptor components and effectors that associate with p75. Additionally, we examined these same signaling pathways in $Q175^{+/+};p75^{+/+}$, $Q175^{+/-};p75^{+/+}$, $Q175^{+/-};p75^{-/-}$, and $Q175^{+/+};p75^{-/-}$ mice. Electrophysiological and pathological differences were also investigated. We observed increased p75 levels in the

striatum of $Q175^{+/-}$ mice compared to WT littermates at 6 weeks of age, but not at 5 or 10 months of age. We also discovered an interaction between p75 and TrkB in the striatum at all three ages, but this interaction was reduced in the $Q175^{+/-}$ mice at 6 weeks and 5 months of age. Interestingly, the association between p75 and its apoptotic receptor components increases in $Q175^{+/-}$ mice at 10 months of age. Several downstream pathways are also dysregulated. These data suggest that p75 may play an early role in augmenting pro-survival signaling through TrkB during striatal development, and disruption of this signaling complex may be a pathological feature of HD. Conversely, at later stages of disease, p75 may play an opposing pro-apoptotic role in the striatum. This potential dual role of p75 in the pathogenesis of HD is intriguing and suggests further studies are necessary to determine the extent to which p75 and TrkB are useful therapeutic targets.

Disclosures: **A. Wehner:** None. **R.L. Albin:** None. **B.A. Pierchala:** None.

Poster

046. Huntington's Disease Mechanisms I

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NIH Grant NS41574

Title: Electrophysiological alterations of striatal cholinergic interneurons in the R6/2 mouse model of Huntington's disease

Authors: A. PARIEVSKY, S. M. HOLLEY, P. R. JOSHI, J. Y. CHEN, *C. CEPEDA, M. S. LEVINE;
IDDRC, Semel Inst. for Neurosci. and Human Behavior, BRI, UCLA, Los Angeles, CA

Abstract: Huntington's disease (HD) is a neurodegenerative disorder characterized by striatal, cortical, and thalamic degeneration. Medium-sized spiny neurons (MSNs), the projection neurons of the striatum, make up ~90-95% of the striatal population, and show significant degeneration in HD. Large cholinergic interneurons (LCI), which constitute only ~2% of the striatal neuronal population, are relatively spared but show functional deficits such as decreased acetylcholine release. We examined electrophysiological properties of LCIs to elucidate the role of these neurons in the progression of HD. Experiments were conducted in the R6/2 HD fragment mouse model, which expresses exon 1 of the human huntingtin gene and shows a

severe and rapidly progressing phenotype. Striatal LCIs were identified by somatic size, spontaneous action potential firing, and membrane properties. Previous findings have shown that the majority of excitatory glutamatergic inputs to LCIs arise from the centromedian-parafascicular (CmPf) nuclear complex of the thalamus and less so from the cerebral cortex. Evoked excitatory currents in LCIs were examined by expressing channel rhodopsin (ChR2) in the CmPf or cortex. Evoked inhibitory currents were examined by expressing ChR2 in striatal somatostatin (SOM)-containing interneurons. Spontaneous excitatory and inhibitory postsynaptic currents were also compared. LCIs from symptomatic R6/2 mice (~65 days) displayed reduced membrane capacitance and higher input resistance, consistent with reduced somatic size, as well as more irregular firing patterns and bursts of actions potentials. Due to minimal innervation of LCIs by corticostriatal projections, cortical stimulation rarely resulted in postsynaptic evoked responses, and when responses were evoked, they were very small. Both thalamostriatal evoked amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) and N-methyl-D-aspartate (NMDA) receptor-mediated currents were minimally affected in the R6/2s. Spontaneous excitatory currents, mediated by AMPA receptors, were unchanged in frequency. GABAergic inhibitory currents evoked by stimulation of SOM-expressing interneurons were increased in amplitude and spontaneous GABAergic inhibitory currents were increased in frequency. As cell death of MSNs has been explained by increased excitatory inputs to these neurons, unaltered excitatory inputs to LCIs is in marked contrast to changes seen in MSNs and may explain the selective sparing of LCIs. Increased inhibitory currents displayed by LCIs provide a potential mechanism to explain the impaired ability of these neurons to fire regularly and release acetylcholine.

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Poster

046. Huntington's Disease Mechanisms I

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 46.25/L9

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: PG was supported by NIMH (R01MH101198), VA Merit Award BX001524, and the Whitehall 2012-05-83.

Title: Altered calcium dynamics of cortical microcircuits in the R6/2 mouse model for Huntington's disease

Authors: *A. M. ESTRADA SANCHEZ[1], T. INDERSMITTEN[1], M. HAIEM[2], C. CEPEDA[1], P. GOLSHANI[2], M. S. LEVINE[1];
[1]IDDRC, Semel Inst. For Neurosci. and Human Behavior, BRI, UCLA, [2]Dept. of Neurology, David Geffen Sch. of Med., UCLA, Los Angeles, CA

Abstract: Huntington's disease (HD) is an inherited neurodegenerative disorder, characterized by dysfunctional neuronal processing along the corticostriatal pathway, which is believed to set the stage for components of its behavioral phenotype. Although changes in electrophysiological properties of cortical pyramidal neurons have been described, little is known about changes in cortical microcircuits that will impact striatal activity. Here, we used two-photon microscopy to evaluate calcium dynamics in cortical neuronal microcircuits in the R6/2 transgenic mouse model of HD. When mice were 4 weeks of age adeno-associated virus-1 (AAV1), containing the genetically-encoded calcium indicator GCaMP6, was injected into motor cortex. Two weeks after the injection, a craniotomy was performed and a glass window was implanted over the cortical injection area. Cortical calcium dynamics in layers 2/3 were then evaluated using two-photon laser microscopy in mice freely running on a spherical treadmill. Cortical calcium activity was assessed during resting and running epochs. The results showed that, based on the amplitude of change in calcium transients ($\Delta F/F$), cortical neurons in both wildtype (WT) and R6/2 mice fell into two categories: neurons showing either low or high amplitude average calcium transients. Relative to WT, a larger proportion of neurons in the R6/2 mice displayed reduced amplitude calcium transients. There was a corresponding increase in the proportion of neurons with high average amplitude calcium transients in WT mice. Interestingly, the increased proportion of neurons with reduced amplitude calcium transients observed in R6/2 mice occurred during both running and resting. We also analyzed the proportion of cell pairs with significantly correlated calcium signals as a function of distance between pairs. There was an increase in the proportion of cell pairs with significantly correlated calcium transients in R6/2 mice compared to WT mice. This increase was more evident when animals were running on the treadmill. Together, these findings indicate that neuronal activity patterns in cortical circuits in HD become altered. Future studies will shed light on the effect of these changes on striatal activity and its role in triggering the HD phenotype.

Disclosures: **A.M. Estrada Sanchez:** None. **T. Indersmitten:** None. **M. Haiem:** None. **C. Cepeda:** None. **P. Golshani:** None. **M.S. Levine:** None.

Poster

046. Huntington's Disease Mechanisms I

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 46.26/L10

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: United States Public Health Service grant NS059921

Title: Investigation of symptoms of Huntington's Disease transgenic mice reveals prevalence of a high anxiety phenotype possibly due to abnormal serotonergic and noradrenergic neurotransmission

Authors: *S. K. ALSELEHDAR, E. D. ABERCROMBIE;
Ctr. for Mol. and Behavioral Neurosci., Rutgers, The State Univ. of NJ, Newark, NJ

Abstract: Mouse models of Huntington's Disease (HD) are useful in translational studies of HD because the models well encapsulate the progressive nature of HD at both behavioral and neuropathological levels. To further address the face validity of these models, we characterized the progression of the motor phenotype of two full-length transgenic HD models (YAC128 and BACHD) using multiple tests. In our approach we monitored the mice's general activity levels weekly in a photobeam cage rack system using clear Plexiglas activity chambers and testing motor coordination on a fixed-speed rotarod task monthly. As expected, HD mice were more active in the activity chambers than wildtype (WT) mice at young ages (6 weeks through 12 weeks). Previously, this has been correlated to the early choreic phase of the human disease. However, the behavior exhibited by both WT and HD mice in the activity chamber was highly variable, which masked the differences between genotypes. To determine if this variability was due to test environment, we measured general locomotion of a second cohort of WT and HD mice in a familiar home cage environment. Post hoc tests revealed that the YAC and BAC models expressed hyperkinesis in both test environments but this effect was more robust in the familiar home cage as evident by less within-group variability in activity levels. We propose that these findings reveal an underlying anxiety profile in these mice that was enhanced by exposure to a novel environment. Heightened levels of anxiety and depression are prevalent characteristics of HD in humans and many studies have observed this in several HD mouse models as well. It is important to investigate these symptoms since they are highly expressed by individuals with HD long before any of the hallmark motor deficits. Abnormal serotonergic and noradrenergic function is thought to underlie several of the psychiatric symptoms of HD. To show the prevalence of these behaviors, we will first characterize the anxiety and depressive phenotype of BACHD mice using multiple tests. We selected BACHD mice because they showed more robust anxiety compared to YAC128 mice. Next, we will use *in vivo* microdialysis to investigate if serotonergic and noradrenergic neurotransmission are affected in HD, particularly in hippocampus. The hippocampus receives dense projections from both serotonergic raphe nuclei

and noradrenergic locus coeruleus. Furthermore, changes in hippocampal neurotransmission are thought to underlie certain abnormal behaviors such as depression in a non-disease state. We propose that a similar pathology may be occurring in HD, ultimately driving the same behavioral abnormalities. This work is in progress.

Disclosures: **S.K. Alselehdar:** None. **E.D. Abercrombie:** None.

Poster

046. Huntington's Disease Mechanisms I

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 46.27/L11

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NIH USPHS NS78633

NIH USPHS NS41574

Title: Optogenetics reveal alterations in dopamine neurotransmission in the YAC128 mouse model of Huntington's disease

Authors: J. Y. CHEN[1], *G. K. AKOPIAN[2], C. J. WANG[1], L. GALVAN[1], V. M. ANDRÉ[1], C. CEPEDA[1], M. S. LEVINE[1];
[1]IDDRC, Semel Inst. for Neuroscience, Brain Res. Institute, David Geffen Sch. of Med., [2]Semel Inst. for Neurosci., UCLA, LOS ANGELES, CA

Abstract: Huntington's disease (HD) is a progressive and fatal neurodegenerative disorder caused by a mutation in the HTT gene producing an expansion of glutamine repeats. The principal neuropathology of HD is a loss of striatal and cortical projection neurons, leading to a progressive disconnection between the cortex and striatum. Dopamine (DA) modulation of glutamatergic inputs also is disrupted in HD mouse models. Previous studies using the YAC128 mouse model of HD have provided evidence that excess DA may be present early in disease progression (2 months of age) whereas DA is depleted in the late stages (11 months). The present study used channelrhodopsin (ChR2) to selectively activate DA inputs to identified striatal medium-sized spiny neurons (MSNs) in triple transgenic YAC128 mice and control littermates (WT) expressing Cre recombinase under the tyrosine hydroxylase promoter and DsRed fluorescence under the DA D1 receptor promoter, which identifies direct-pathway MSNs. ChR2 was stereotaxically injected into the substantia nigra pars compacta of mice at 2 and 11 months

of age. Fast-scan cyclic voltammetry demonstrated no significant differences in amplitude of DA release between WT and YAC128 mice at 2 months of age. However, the decay time of DA release was significantly increased in slices from YAC128 mice, suggesting that alterations in the DA transporter occur. Addition of quinpirole, a D2 agonist, had smaller effects on DA release in YAC128 mice indicating a possible reduction of autoreceptor function. Furthermore, inhibiting DA reuptake with GBR 12935 produced smaller effects on release and decay time in 2 month-old YAC128 mice. In 11 month-old mice, the alterations in kinetics of DA release in YAC128 were less evident. Whole-cell patch clamp electrophysiological recordings in 2 month-old mice showed that ChR2-evoked release of DA had differential effects on synaptic transmission of D1 and D2 receptor-expressing MSNs. Release of DA increased the frequency of spontaneous excitatory postsynaptic currents (sEPSC) of D1-MSNs from WT mice but had no effect on sEPSC frequency of D1-MSNs from YAC128 mice. In contrast, DA release decreased EPSC frequency of D2-MSNs from both WT and YAC128 mice. Together, these data demonstrate that DA neurotransmission, particularly DA transporter and modulatory function, are altered early in disease progression. Slower decay times of DA release may contribute to increases in stereotypies observed in HD mice. Furthermore, treatments aimed at reducing the presence of DA at the synaptic cleft might help normalize behavioral alterations.

Disclosures: **J.Y. Chen:** None. **G.K. Akopian:** None. **C.J. Wang:** None. **L. Galvan:** None. **V.M. André:** None. **C. Cepeda:** None. **M.S. Levine:** None.

Poster

046. Huntington's Disease Mechanisms I

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 46.28/L12

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: Vaccinex Inc.

Title: Anti-semaphorin 4D immunotherapy ameliorates neuropathology and some cognitive impairment in the YAC128 mouse model of Huntington disease

Authors: A. L. SOUTHWELL[1], S. FRANCIOSI[1], E. B. VILLANUEVA[1], Y. XIE[1], L. A. WINTER[2], J. VEERARAGHAVAN[2], A. JONASON[2], B. FELCZAK[1], W. ZHANG[1], V. KOVALIK[1], S. WALTL[1], G. HALL[1], M. A. POULADI[3], E. S. SMITH[2], W. J. BOWERS[2], M. ZAUDERER[2], *M. R. HAYDEN[1];

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Abstract: Huntington disease (HD) is an inherited neurodegenerative disease with no disease-modifying therapy currently available. In addition to characteristic motor deficits and atrophy of the caudate nucleus, signature hallmarks of HD include behavioral abnormalities, immune activation, and cortical and white matter loss. The identification and validation of novel therapeutic targets that contribute to these degenerative cellular processes may lead to new interventions that slow or even halt the course of this insidious disease. Semaphorin 4D (SEMA4D) is a transmembrane signaling molecule that modulates a variety of processes central to neuroinflammation and neurodegeneration including glial cell activation, neuronal growth cone collapse and apoptosis of neural precursors, as well as inhibition of oligodendrocyte migration, differentiation and process formation. Therefore, inhibition of SEMA4D signaling could reduce CNS inflammation, increase neuronal outgrowth and enhance oligodendrocyte maturation, which may be of therapeutic benefit in the treatment of neurodegenerative diseases. A high affinity neutralizing antibody to SEMA4D has been described that prevents the interaction between SEMA4D and all of its known receptors, PlexinB1, PlexinB2 and CD72. Anti-SEMA4D treatment inhibits development of experimental autoimmune encephalomyelitis in multiple rodent models and promotes remyelination following chemically-induced demyelination. Considering the convergent pathogenic pathways of neuroinflammatory and neurodegenerative diseases, anti-SEMA4D treatment may have benefits in other indications, including HD. We have evaluated the preclinical therapeutic efficacy of anti-SEMA4D treatment in the YAC128 transgenic HD mouse model. Anti-SEMA4D treatment ameliorated neuropathological signatures, including striatal, cortical, and corpus callosum atrophy and prevented testicular degeneration in YAC128 mice. In parallel, a subset of behavioral symptoms were improved in anti-SEMA4D treated YAC128 mice, including reduced anxiety-like behavior and rescue of cognitive deficits. There was, however, no discernible effect on motor deficits. The preservation of brain grey and white matter and improvement in behavioral measures in YAC128 mice treated with anti-SEMA4D suggest that this approach could represent a viable therapeutic strategy for the treatment of HD. Importantly, this work provides *in vivo* demonstration that inhibition of pathways initiated by SEMA4D constitutes a novel approach to moderation of neurodegeneration.

Disclosures: **A.L. Southwell:** None. **M.R. Hayden:** A. Employment/Salary (full or part-time):; Teva Pharmaceuticals. **S. Franciosi:** None. **E.B. Villanueva:** None. **Y. Xie:** None. **B. Felczak:** None. **W. Zhang:** None. **V. Kovalik:** None. **S. Waltl:** None. **G. Hall:** None. **L.A. Winter:** A. Employment/Salary (full or part-time):; Vaccinex Inc. **J. Veeraraghavan:** A. Employment/Salary (full or part-time):; Vaccinex Inc. **A. Jonason:** A. Employment/Salary (full or part-time):; Vaccinex Inc. **E.S. Smith:** A. Employment/Salary (full or part-time):; Vaccinex Inc. **W.J. Bowers:** A. Employment/Salary (full or part-time):; Vaccinex Inc. **M. Zauderer:** A. Employment/Salary (full or part-time):; Vaccinex Inc.. **M.A. Pouladi:** None.

Poster

047. Dystonia Mechanisms and Model Systems

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 47.01/M1

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: Bachmann-Strauss Dystonia & Parkinson's Foundation

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Dystonia Coalition (NS065701)

HHMI

Kavli Institute for Brain and Mind

NSF, Temporal Dynamics of Learning Center, SBE-0542013

NSF EFRI-1137279

Title: A theoretical model for the pathogenesis of adult onset dystonia due to GNAL mutations

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Abstract: The pathogenesis of adult onset dystonia has been elusive. Recent genetic studies have implicated mutations in GNAL, which codes for the alpha subunit of the guanine nucleotide-binding protein G(olf). G(olf) is the dominantly expressed stimulatory G protein in the striatum andit mediates intracellular signaling pathways downstream from the D1-type dopamine receptors (D1Rs). Heterozygous knockouts (GNAL +/-) exhibit reduced locomotion during a 5-day period of sensitization to D-amph or cocaine. However, after a 9-day withdrawal, the locomotor behavior in response to a D-amph or cocaine challenge is normalized to that of control animals (GNAL +/+)(Corvol et al. 2007 Neuropsychopharmacology). Although this gross measure of motor function normalizes in the GNAL +/- mice, we hypothesize that other aspects of their motor functions would be pathological. Specifically, we predict that the heterozygous mice would be impaired in instrumental learning tasks, given the putative roles of the basal ganglia in learned action selection and phasic striatal dopamine release in feedback-based learning. At the mechanistic level, we hypothesize that the impaired D1R-G(olf)-AC-PKA

signaling pathway in the GNAL +/- mice leads to altered patterns of synaptic plasticity. Through a cascade of homeostatic processes downstream from PKA with increasingly longer time constants, short-time constant acute behavior and learning are relatively intact, while long time constant feedback-based learning is subtly altered. In conjunction with repeated patterns of context-specific motor selection, synaptic tagging, remodeling, and synaptogenesis and pruning lead to pathological action selection circuits in the striatum. This model sets the stage for new experimental tests of the GNAL mouse model and a rational basis for future therapeutics development in adult onset dystonias.

Disclosures: **D.A. Peterson:** None. **T.J. Sejnowski:** None.

Poster

047. Dystonia Mechanisms and Model Systems

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

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INSERM C10 02

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ANR-10-IAIHU-06

Fondation Groupama pour la Santé

AMADYS

Title: Cerebellar encoding of neck input primes cortical plasticity of hand representation: The case of cervical dystonia

Authors: *S. O. MEUNIER[1], A. KISHORE[2], C. HUBSCH[3], T. POPA[4], A. RICHARD[5], E. ROZE[3], M. VIDAILHET[3];
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Abstract: We have previously shown, in healthy subjects, that the responsiveness to plasticity induction of the cortical hand representation in the primary motor cortex can be primed by changing the excitability of the cerebellar cortex (Popa et al 2013). Such a priming effect was lost in patients with writer's cramp (Hubsch et al 2013). We reasoned that if this abnormal encoding plays a direct role in generating dystonic manifestations, patients with cervical dystonia and no hand impairment, should have a preserved cerebellar priming of hand cortical plasticity. We compared 22 patients with a cervical dystonia and 22 healthy subjects. We assessed how sham, excitatory and inhibitory stimulations of the cerebellum (obtained by means of sham, intermittent and continuous thetaburst stimulations to cerebellum respectively) influenced an externally evoked plasticity (using a 5Hz paired associative stimulation protocol) in the cortical hand area. After sham cerebellar stimulation PAS-induced plasticity was similar in healthy controls and patients. Cerebellar excitation decreased the ensuing PAS-induced plasticity and cerebellar inhibition enhanced it in the healthy controls. At contrast cerebellar inhibition decreased the PAS-induced plasticity and cerebellar excitation let it unchanged in patients (INTERVENTION*GROUP $P<0.0003$). In control experiments we explored 8 healthy subjects while they maintained a head rotation to the right. In this condition cerebellar inhibition decreased the PAS-induced plasticity and cerebellar excitation increased it. These results show that the directional changes of cerebellar priming in cervical dystonia patients were linked to the involuntary head tilt that they maintained during the recordings. Provided that head positions were matched cerebellar inhibition induced similar decrease of PAS-induced plasticity in patients and healthy controls yet cerebellar excitation was efficient only in the less affected patients and lost in the more affected (linear regression $R2 = 0.4$, $P < 0.004$). Cerebellar priming of cortical plasticity is preserved in not severely affected cervical dystonia patients stressing the role of this priming in dystonic manifestations patients. Part of this priming is most in severely affected patients suggesting a spread of the pathological process from the cortical hand representation to other body part representations receiving the abnormally encoded cerebellar input.

Disclosures: **S.O. Meunier:** A. Employment/Salary (full or part-time):; INSERM. **A. Kishore:** A. Employment/Salary (full or part-time):; SCTIMST. **C. Hubsch:** A. Employment/Salary (full or part-time):; APHP. **T. Popa:** A. Employment/Salary (full or part-time):; ANR-10-IAIHU-06. **A. Richard:** None. **E. Roze:** A. Employment/Salary (full or part-time):; APHP. **M. Vidailhet:** A. Employment/Salary (full or part-time):; APHP.

Poster

047. Dystonia Mechanisms and Model Systems

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 47.03/M3

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: T32NS007222-32

5R01NS077730-02

Title: Selective alteration of striatal cholinergic function in a symptomatic mouse model of DYT1 dystonia

Authors: *S. S. PAPPAS, W. T. DAUER;
Dept Neurol., Univ. of Michigan, Ann Arbor, MI

Abstract: Dystonia is a movement disorder characterized by prolonged involuntary twisting movements that reflect selective dysfunction of CNS motor circuits. Abnormal basal ganglia function is implicated as a key cause of dystonic movements, but the cellular basis for neuronal dysfunction remains undefined. DYT1 dystonia, the most common inherited form of primary dystonia, is caused by a dominantly inherited loss of function mutation in the TOR1A gene (encoding the protein torsinA). To model dystonia-related neuronal dysfunction in forebrain motor circuits, we deleted torsinA selectively from forebrain GABAergic and cholinergic neurons. These mice are born at normal Mendelian frequencies, are indistinguishable from littermate controls, and perform normally in neonatal motor tests. However, by two to four weeks of age they develop clear motor dysfunction, including abnormal prolonged limb clasping and truncal twisting, reduced ability to hang from a wire grid, and hyperactivity. These mice show no gross brain neuropathological changes as assessed by Nissl, hematoxylin and eosin or GFAP immunostaining. However, they exhibit a selective loss of striatal cholinergic interneurons that begins between 1 and 4 weeks postnatally. No changes in cell number are seen in GABAergic medium spiny neurons or several classes of GABAergic interneurons. Significant reduction of striatal acetylcholinesterase activity provides further evidence of cholinergic dysfunction in this model. These findings demonstrate that among striatal cell types, maturing cholinergic interneurons have a unique requirement for torsinA function. These mice model several key features of DYT1 dystonia, including a developmental onset of striatal dysfunction and temporally related motor dysfunction, paralleling the late childhood/early adolescence onset of the human illness. This behaviorally symptomatic mouse model of dystonia therefore provides a powerful platform for identifying specific circuits and cell types that drive abnormal motor function in DYT1 dystonia.

Disclosures: **S.S. Pappas:** None. **W.T. Dauer:** None.

Poster

047. Dystonia Mechanisms and Model Systems

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: MIUR GR 2009 to AP

MoH Progetto Finalizzato 2010

DMRF, Stanley Fahn Award 2011

COST project "Action BM11101"

Title: M1 muscarinic receptor subtype as a therapeutic target for the rescue of striatal synaptic plasticity alterations in basal ganglia disorders

Authors: M. MALTESE[1,2], G. MADEO[1,3], G. MARTELLA[1,2], T. SCHIRINZI[3], G. SCIAMANNA[1], G. PONTERIO[1], A. TASSONE[1], P. BONSI[1], P. J. CONN[4], *A. PISANI[3,1]; [1]Neurophysiol. and Plasticity Lab., Fondazione Santa Lucia IRCCS, Rome, Italy; [2]Systems Med., Univ. of Rome "Tor Vergata", Rome, Italy; [3]Clinica Neurologica Univ. Tor Vergata, Rome 00133, Italy; [4]Vanderbilt Ctr. for Neurosci. Drug Discovery, Nashville, TN

Abstract: Competitive antagonists of muscarinic receptors (mAChRs) are currently used in the pharmacological treatment of movement disorders such as Parkinson's disease and dystonia, although the existing drugs for clinical use are non-selective mAChR antagonists, associated with a broad array of undesirable central and peripheral side effects. Despite the clinical utility of this class of drugs, the mechanism of action still remains poorly understood. Furthermore, these anticholinergic drugs are not selective for specific mAChR subtypes and they display a wide range of negative side effects. In the present study we tested the effect of different mAChR antagonists on corticostriatal synaptic plasticity alterations recorded from medium spiny neurons (MSNs) in DYT1 dystonia heterozygous knock-in mice (Tor1a+/Δgag mice) and control mice (Tor1a+/+ mice). Tor1a+/Δgag mice exhibited a significant impairment of bidirectional corticostriatal synaptic plasticity, consisting in a loss of long term depression (LTD) and synaptic depotentiation (SD), as compared to controls. A complete rescue of physiological LTD and SD in knock-in mice was obtained by applying the M1-preferring antagonists pirenzepine (100 nM) and trihexyphenidyl (3 μM) as well as the novel selective M1 mAChR antagonist, VU0255035 (100 nM). Conversely, the non-selective mAChR antagonists orphenadrine (100 nM) produced only a partial rescue of synaptic plasticity deficits, whereas biperiden (20 μM) failed to restore synaptic plasticity. Our study demonstrate that M1 mAChR antagonism offsets synaptic plasticity deficits in the striatum of Tor1a+/Δgag mice, providing a potential mechanistic

rationale for the development of improved antimuscarinic therapies for movement disorders characterized by corticostriatal plasticity alterations, such as DYT1 dystonia and Parkinson's disease.

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Poster

047. Dystonia Mechanisms and Model Systems

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NIH Grant NS65273

Title: Electrophysiological characterization of DYT1 dystonia mouse models

Authors: *F. YOKOI[1], H. CHEN[1], C. C. CHEETHAM[2], S. L. CAMPBELL[3], J. D. SWEATT[3], Y. LI[1];
[1]Neurol., Univ. of Florida, Gainesville, FL; [2]Neurology, Neurobio., [3]Neurobio., Univ. of Alabama at Birmingham, Birmingham, AL

Abstract: DYT1 early-onset generalized torsion dystonia is an inherited movement disorder caused by mutations in one allele of *DYT1* (*TOR1A*), coding for torsinA. The most common mutation is a trinucleotide deletion (ΔGAG), which causes a deletion of a glutamic acid residue (ΔE) in the C-terminal region of torsinA (torsin$A^{\Delta E}$). Other *DYT1* mutations, such as an 18-base

pair in frame deletion and a 4-base pair frame-shift mutation, have also been reported. Electrophysiological recording in CA1 regions of hippocampal slices is one of the established experimental models to examine long-term and short-term synaptic plasticity in the brain. Hippocampal CA3 pyramidal cells project to CA1 pyramidal cells through Schaffer collaterals. We and another group previously reported that theta-burst-induced long-term potentiation (LTP) in the CA1 region is not altered in *Dyt1* ΔGAG heterozygous knock-in (KI) mice. Here, we examined short-term synaptic plasticity and synaptic transmission in the hippocampal slices. Field recordings in the hippocampal Schaffer collaterals pathway revealed significantly enhanced paired pulse ratios (PPRs) in *Dyt1* KI mice, suggesting an impaired synaptic vesicle release. Whole-cell recordings from the CA1 neurons showed that *Dyt1* KI mice exhibited normal miniature excitatory post-synaptic currents (mEPSC), suggesting that action-potential independent spontaneous pre-synaptic release was normal. On the other hand, there was a significant decrease in the frequency, but not amplitude or kinetics, of spontaneous excitatory post-synaptic currents (sEPSC) in *Dyt1* KI mice, suggesting that the action-potential dependent pre-synaptic release was impaired. Although hippocampal torsinA was reduced in *Dyt1* KI mice, it was not clear whether decreased hippocampal torsinA level itself affects the synaptic plasticity or torsin$A^{\Delta E}$ does it. To analyze effect of torsinA loss to the synaptic plasticity, *Dyt1* heterozygous knock-out (KO) mice were further examined as a model with a frame-shift mutation. *Dyt1* heterozygous KO mice showed more reduced hippocampal torsinA than *Dyt1* KI mice. Contrast to *Dyt1* KI mice, *Dyt1* heterozygous KO showed enhanced hippocampal LTP and normal PPRs in the acute brain slices. These results suggest that the extreme reduction of torsinA causes enhanced hippocampal long-term synaptic plasticity. On the other hand, the short-term plasticity deficits found in *Dyt1* KI mice may be caused by torsin$A^{\Delta E}$-specific toxic-gain of function. Torsin$A^{\Delta E}$ and decreased torsinA levels seem to affect short-term and long-term synaptic plasticity, respectively. Different mutations in *DYT1* may differently affect synaptic plasticity and symptoms.

Disclosures: **F. Yokoi:** None. **H. Chen:** None. **C.C. Cheetham:** None. **S.L. Campbell:** None. **J.D. Sweatt:** None. **Y. Li:** None.

Poster

047. Dystonia Mechanisms and Model Systems

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NIH Grant R01NS069936

NIH Grant R01NS082296

Title: Motor phenotypes associated with Ciz1 deficiency

Authors: *M. S. LEDOUX[1], K. KURUVILLA[1], H. HONDA[2], J. XIAO[1];
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Abstract: Missense mutations in Cip1-interacting zinc finger protein 1 (CIZ1) have been associated with adult-onset primary cervical dystonia. CIZ1 is a p21(Cip1/Waf1)-interacting zinc finger protein expressed in brain and involved in DNA synthesis and cell-cycle control. To date, neither nonsense nor large deletion mutations leading to haploinsufficiency have been identified in subjects with dystonia. Therefore, the relative contributions of gain- or loss-of-function to the pathogenesis of CIZ1-associated dystonia remain indefinite. To provide a platform for mechanistic studies of CIZ1 biology, we have begun to characterize *Ciz1* gene-trap knockout (KO) and *Ciz1*-floxed mice. Using relative quantitative RT-PCR, we confirmed that *Ciz1* transcripts were reduced by 50% in heterozygous gene-trap mice and absent from homozygotes. In *Ciz1*-floxed mice, there were no apparent effects of loxP sites on transcription in spinal cord, cerebellum, striatum, midbrain, thalamus or frontal cortex. In adult wild-type littermates, cerebellum showed the highest expression levels of *Ciz1*, about two fold higher than liver, whereas expression levels in cerebral cortex, midbrain, and thalamus were approximately 50% higher than liver. Adult wild-type, heterozygous and homozygous mice were weighed and subjected to a battery of motor tests including open-field activity, rotarod, vertical rope climbing, a raised-beam task, grip strength, and gait analysis (DigiGait). Initial testing showed an overall effect of genotype on weight, activity, grip strength and the raised-beam task. Ongoing experiments are assessing interactions among age, gender and genotype given that cervical dystonia is more common in females with mean age-of-onset after 45 years.

Disclosures: **M.S. LeDoux:** None. **K. Kuruvilla:** None. **H. Honda:** None. **J. Xiao:** None.

Poster

047. Dystonia Mechanisms and Model Systems

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NIH P50NS037409-13

Title: Effects of nicotinic receptors on striatal dopamine efflux in a cholinergic-specific dyt1 knock-out mouse model of dystonia

Authors: *C. THOMPSON, K. ESKOW JAUNARAJS, D. STANDAERT;
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Abstract: Early onset torsion dystonia (DYT1) is characterized by sustained muscle contractions causing twisting and repetitive movements or abnormal postures due to a mutation in the gene TOR1A, encoding the protein torsinA. Recent evidence in several mouse models of DYT1 dystonia, including cholinergic neuron-specific knockout of the DYT1 gene (ChAT-KO), shows abnormal striatal dopamine release compared to wild-type mice. This suggests that dopamine receptor signaling is particularly altered in striatal cholinergic neurons. In ChAT-KO mice, muscarinic acetylcholine receptors have reduced sensitivity to muscarinic antagonists; however, nicotinic acetylcholine receptors (nAchR) have not been examined. Because of our interest in the interplay between dopaminergic and cholinergic signaling in dystonia, we have used ChAT-KO mice as a mouse model of DYT1 dystonia in order to characterize the effects of nicotine on striatal dopamine release (DA). In a between-subject design, male ChAT-KO or control mice were implanted with a striatal microdialysis cannula and treated with either vehicle or nicotine (10mM, infused). Mice were then sacrificed and sectioned in order to verify probe placement. Results showed that ChAT-KO mice had a significantly greater DA efflux than control mice when nicotine was administered. This shows that striatal neuron nAChRs in ChAT-KO mice are more sensitive to nicotine than the control mice. Ongoing studies are looking into nicotinic involvement in other mouse models of DYT1 dystonia. Further investigation into the dopamine-acetylcholine relationship in several models of dystonia is needed to understand the mechanism between nAchRs and motor dysfunction induced by mutation in the TOR1A gene.

Disclosures: **C. Thompson:** None. **K. Eskow Jaunarajs:** None. **D. Standaert:** None.

Poster

047. Dystonia Mechanisms and Model Systems

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: R01DC011805 to KS

Title: Phenotype-specific alterations of resting-state brain activity in spasmodic dysphonia

Authors: *G. BATTISTELLA, K. SIMONYAN;
Dept. of Neurology,Mount Sinai Med. Ctr., New York, NY

Abstract: Spasmodic dysphonia (SD) is a primary task-specific focal dystonia of unknown pathophysiology, which is characterized by involuntary spasms in the laryngeal muscles leading to uncontrolled voice breaks during speaking. In this study, we investigated differences in spontaneous fluctuations of brain activity during the rest in sporadic SD patients (ADductor and ABductor types) compared to healthy volunteers (HV) and assessed SD phenotype-specific organization of resting-state networks (RSNs) in both clinical phenotypes. Resting-state fMRI images were acquired in 27 ADSD, 21 ABSD and 28 HV on a 3T Philips scanner. Independent component analysis (ICA) identified 27 RSNs across all subjects (MELODIC-ICA, FSL). We a priori selected 2 RSNs with the sensory-motor (SMC) and the language (LC) components. Dual-regression analysis was performed to identify subject-specific temporal dynamics and associated spatial maps. Statistical inference was performed using a two-sample t-test (all patients vs. HV, and ABSD vs. ADSD) at a corrected $p \leq 0.05$. Compared to HV, patients showed decreased SMC functional connectivity in the left laryngeal/orofacial primary sensorimotor cortex, inferior frontal gyrus (Broca's area), operculum, auditory cortex, and right premotor cortex. Conversely, patients showed increased LC connectivity in the left premotor cortex, which did not overlap with the region of decreased SMC connectivity. Direct comparisons between the patient groups showed that ABSD had increased SMC connectivity in the left supramarginal gyrus and superior parietal lobule, bilateral operculum, and right supplementary motor area. In addition, ABSD patients compared to ADSD showed increased LC connectivity in the left somatosensory cortex, inferior parietal lobule and right inferior/middle temporal gyri. Our findings demonstrated abnormal organization of resting-state networks related to speech sensorimotor control in SD patients, which may underlie abnormal functional connectivity during dystonic task production. Phenotype-specific differences in SMC and LC functional network organization between ADSD and ABSD may be associated with different genetic variants.

Disclosures: **G. Battistella:** None. **K. Simonyan:** None.

Poster

047. Dystonia Mechanisms and Model Systems

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: MoH, Giovani Ricercatori 2009

DMRF, Fahn Award 2011

MoH, Progetto Finalizzato 2010

Title: Alterations of the functional interplay between striatal dopamine D2 receptor and RGS9-2 in movement disorders

Authors: *P. BONSI[1], G. PONTERIO[1], G. SCIAMANNA[1,3], A. TASSONE[1], G. MANDOLESI[1,3], V. VANNI[1,3], V. ZACHARIOU[4], E. BEZARD[5], A. PISANI[2,3];
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Abstract: Striatal dopamine and acetylcholine regulate voluntary movement and their imbalance is critically involved in motor dysfunction in Parkinson's disease (PD) and dystonia. RGS9-2, striatal R7 family member regulating dopamine D2 receptor (DRD2) localization and signalling, negatively modulates levodopa-induced dyskinesia (LIDs) in experimental PD. Indeed, RGS9-2 overexpression achieved by viral vector injection into the striatum diminishes the intensity of involuntary movement caused by levodopa treatment in both rat and monkey models of PD and dyskinesia. Given previous evidence of a significant dysfunction of striatal DRD2 in DYT1 dystonia, we investigated whether viral-mediated RGS9-2 overexpression might restore DRD2 function in DYT1 mice. As previously observed, electrophysiological recordings from cholinergic interneurons (ChIs) of both hMT1 and *Tor1a*$^{+/\Delta E}$ striatal slices showed an abnormal increase of firing activity, instead of the reduction observed in wild-type neurons, in response to DRD2 activation by quinpirole. Conversely, the inhibitory effect on ChI firing activity of mu-opioid receptor, whose function is also regulated by RGS9-2, was unaltered. hMT1 mice overexpress the human protein with the DYT1 dystonia mutation, ΔE-torsinA. However, the levels of endogenous torsinA are developmentally regulated in wild-type mice, reaching a peak at P7 in the striatum. We therefore characterized the developmental expression of endogenous torsinA in knock-in *Tor1a*$^{+/\Delta E}$ striata, and found significantly reduced (~50%) torsinA levels throughout the postnatal development (P7-P60). Immunoblotting experiments demonstrated a significant reduction of DRD2 protein level at P60 in DYT1 striatal homogenates. The reduction of DRD2 level seems due to an increased rate of degradation by lysosomal proteases, since it was abolished by the protease inhibitor leupeptin. Of note, also the level of RGS9-2 was reduced at P60 in DYT1 mice. Conversely, the levels of an RGS9-2 interacting protein, Gβ5, and of the closely related RGS7 protein were unchanged in *Tor1a*$^{+/\Delta E}$ striata. *In vivo* viral-mediated delivery of RGS9-2 in DYT1 striata restored RGS9-2, but not torsinA, levels to normal values, with respect to the contralateral vehicle-injected striata. Electrophysiological recordings from ChIs in acute slices from the RGS9-2-injected striatum demonstrated a rescue of D2 receptor

inhibitory response to quinpirole. These observations demonstrate a key role of RGS9-2 in DYT1 dystonia pathophysiology, as described in LIDs in experimental PD, suggesting that the DRD2-RGS9-2 interplay may represent a common target in movement disorders.

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Poster

047. Dystonia Mechanisms and Model Systems

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Support: DMRF, Stanley-Fahn Award 2011

MoH Progetto finalizzato 2010 to AP

COST project “Action BM1101”

Title: Cross-species validation of electrophysiological abnormalities in distinct rodent models of DYT1 dystonia

Authors: *G. MADEO[1,2], G. MARTELLA[1,2], M. MALTESE[1,2], T. SCHIRINZI[1], G. SCIAMANNA[1,2], G. PONTERIO[1,2], A. TASSONE[2], G. MANDOLESI[2,1], V. VANNI[2], M. PIGNATELLI[3], R. NISTICÒ[4], P. BONSI[2], A. PISANI[1,2];
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Abstract: DYT1 dystonia is a severe movement disorder, caused by a 3-bp deletion in the C-terminal coding region of the protein torsinA (TorA). It is still unclear whether TorA mutation is responsible for the failure of cellular processes encoding motor learning and memory, and whether this impairment occurs in specific species or in selective brain regions. The present work confirms our previous findings obtained in distinct mouse models of DYT1 dystonia, specifically transgenic mouse model overexpressing human mutant torsinA (TorA) and mice with selective TorA deletion in cholinergic interneurons (ChI) (Martella et al, 2009; Sciamanna et al 2012). Electrophysiological recordings were performed from both striatal and hippocampal

acute slices in knock-in (Tor1aΔGAG+/-) mice and transgenic overexpressing human torsinA (hMT) rats and their respective wild-type littermates. The aberrant electrophysiological responses to D2 dopamine receptor (D2R) activation from ChI has been detected in the two rodent DYT1 models, Tor1aΔGAG+/- mice and hMT rats, consisting in an excitatory response to the D2R agonist quinpirole, rather than the expected inhibition occurring in wild-type animals. Moreover, alterations of corticostriatal long-term synaptic plasticity were observed in Tor1aΔGAG+/- mice. Indeed, in MSNs recorded long-term depression (LTD) could not be elicited, as compared to wild-type mice. Additionally, long-term potentiation (LTP) in mutant torsinA mice exhibited increased amplitude, and low-frequency stimulation (LFS), which induces a synaptic depotentiation (SD) in controls, failed to depotentiate corticostriatal synapses in Tor1aΔGAG+/- mice. These synaptic plasticity abnormalities exhibit a regional specificity, since a theta-burst stimulation protocol induced a LTP of normal amplitude at CA1-hippocampal synapses in Tor1aΔGAG+/- mice. These findings validate our previous results and endorse the existence of a specific electrophysiological phenotype linked to DYT1 mutation, irrespective of the species analyzed.

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Poster

047. Dystonia Mechanisms and Model Systems

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 47.11/M11

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: The Brian Jackson Dystonia Research and Discovery Fund

Title: Novel approaches for diagnosis and therapeutics for DOPA responsive dystonia

Authors: *L. JONES[1], L. GOOD[1], N. SHARMA[2], P. BHIDE[1], I. A. ARMATA[1];
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Abstract: The *GCH1* gene encodes the GTP cyclohydrolase 1, which is an enzyme involved in dopamine biosynthesis. Mutations in the *GCH1* gene are associated with dopa-responsive dystonia (DRD), a debilitating movement disorder affecting adults as well as children. Over 114 *GCH1* mutations are linked to DRD with only three single nucleotide polymorphisms (SNPs)

detected within its 5'untranslated region (5'UTR). We have previously reported that among these three 5 UTR SNPs, only the +142C>T is of biological significance because it reduces GCH1 protein levels. In support of this finding, genetic studies show that individuals with a +142T manifest DRD while individuals with a +142C are unaffected. Here we show that the presence of a +142T introduces an upstream translational codon (uAUG) leading to production of two GCH1 protein products; the normal GCH1 enzyme from the normal ATG and a second truncated abnormal protein from the uATG. The latter abnormal protein competes with the normal protein (unpublished data). We are currently developing an antibody specific for the mutant uATG-initiated peptide which can be used as a diagnostic tool for this type of DRD, as well as a tool for screening therapeutic potential of novel compounds that can tilt the balance towards the normal ATG. Accurate DRD diagnosis is critical so that the patient can benefit from L-DOPA treatment at the earliest stages of the disease. Finding novel therapy is also critical because treatment of the symptoms using L-DOPA leads to side effects such as dopa-induced dyskinesias. Our findings open new avenues for novel diagnostic and therapeutic approaches.

Disclosures: **L. Jones:** None. **L. Good:** None. **N. Sharma:** None. **P. Bhide:** None. **I.A. Armata:** None.

Poster

047. Dystonia Mechanisms and Model Systems

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Program#/Poster#: 47.12/M12

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: Allergan Education fund

Title: Kinematic motion sensors objectively characterize neck movements in cervical dystonia

Authors: *O. SAMOTUS[1], H. VAFADAR[1], F. RAHIMI[1], J. LEE[1], M. JACKMAN[2], M. JOG[2,1];
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Abstract: Cervical dystonia (CD) is characterized by varying abnormal neck posturing with superimposed hyperkinetic movement such as tremor. Visual assessments are highly subjective and often incorrectly characterize an individual's neck movements. Kinematic technology using motion sensors can quantitate the static and dynamic movements of the neck. This study aims to develop a sensor-based method for kinematic assessment of cervical dystonia. 11 CD patients

underwent a kinematic assessment in a seated position involving surface attachment of 1 electrogoniometer at spinal segments C2 and T2, an accelerometer and inclinometer on the right temple and an inclinometer on each shoulder. Neutral head position was recorded for each patient for kinematic calibration. Recordings captured CD in a seated eyes open and closed scenario. Natural position of the neck and head were marked once patient's eyes were closed for 1 minute. Additional range of motion kinematics were recorded at 90° left/right rotation of head, tilt up/down to chest, and lateral left/right tilt. Deviation from calibrated neutral position revealed the severity of a patient's fixed posturing in lateral, vertical and rotational degrees of freedom (DOF). At patient's natural position, angular tremor amplitudes for each DOF were plotted. Interpretation of these results led to grouping the 11 patients into two categories based on their prevalent neck movements. 5 patients commonly had mild to severe asymmetrical fixed posturing, accompanied by mild corrective hyperkinetic movements. 6 patients commonly had mild to severe head tremors with mild fixed posturing. As involuntary twisting of the neck and superimposed head tremors contribute to abnormal neck movements, kinematics can objectively measure the biomechanics of the neck. Kinematics can aid clinicians to better tailor focal treatments for cervical dystonia, such as botulinum neurotoxin type A injections.

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Poster

047. Dystonia Mechanisms and Model Systems

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Program#/Poster#: 47.13/N1

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Title: Focal dystonia in torsin A +/- mice after transient nerve injury

Authors: *J. VOLKMANN, I. U. ISAIAS, B. TEKIN, A. ALTTOA, D. KLEIN, T. HIGUCHI, A. REIF, C. W. IP;
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Abstract: Objective: To date no satisfying rodent model of idiopathic dystonia with typical clinical features exists. DYT1 dystonia is the most common genetic form of primary torsion dystonia in humans caused by an autosomal-dominant "loss of function" mutation in the torsin A gene. Interestingly, the penetrance of DYT1 dystonia is markedly reduced with only 30 % of carriers manifesting the typical limb-onset generalized dystonia in childhood. Here, we pursue

the hypothesis, that dystonia manifests in genetically predisposed individuals only after a "second hit" (e.g. abnormal sensory input) causing maladaptive plasticity of the basal ganglia-cortex network. To this end, we observed the motor recovery of torsin A +/- and wildtype mice after a transient nerve injury and examined dopaminergic metabolic changes in the basal ganglia in response to the trauma. Methods: Development of dystonia after a sciatic nerve crush was assessed by blinded clinical scoring of hindlimb movements and by using a gait analysis system for rodents (CatWalk XT). Immunohistochemical stainings of the peripheral nerve for myelin basic protein and neurofilament as well as electroneurographic measurements were used to exclude permanent injury of the peripheral nerve. Western blot analysis was performed for dopamine transporter (DAT), D1 and D2 receptors (striatum) as well as quantitative RT-PCR analysis for DAT (midbrain). Ex-vivo autoradiography (AR) after intravenous DAT radioligand I-123 ioflupane administration was used to assess the DAT binding potential in the striatum. Results: 8 weeks after the sciatic crush a higher dystonia score of the hindlimb and a decreased step sequence regularity index were found in torsin A +/- compared to wt mice. Histology and electroneurography excluded a different degree of peripheral nerve injury in the two groups. Instead, we found several indicators of an altered dopaminergic neurotransmission in the striatum in response to the trauma: torsin A +/- compared to wt mice showed decreased baseline mRNA and protein levels of presynaptic DAT, which increased after peripheral nerve injury in the mutant mice only. In parallel, postsynaptic D1 and D2 receptor protein levels decreased after trauma. AR exhibited a reduced DAT binding capacity in Torsin A +/- mice after peripheral nerve injury. Conclusion: Our data indicate that peripheral nerve injury triggers the clinical manifestation of focal dystonia in susceptible mice. Dopaminergic neurotransmission is altered in torsin A +/- mice and could play an important role in the central network changes leading to a dystonic phenotype after the injury.

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Poster

047. Dystonia Mechanisms and Model Systems

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: American Heart Association

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Title: Acetylcholine-induced calcium transients are sensitized in central neurons associated with DYT1 dystonia

Authors: *S. IWABUCHI, J.-Y. KOH, N. C. HARATA;
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Abstract: Enhanced activity of the cholinergic system in the brain has been associated with a movement disorder dystonia. This notion is based on the clinical findings that anti-cholinergic agents are effective in alleviating symptoms in some patients. These agents are typically antagonists of M1 muscarinic acetylcholine receptors, which are coupled to G proteins and the mobilization of intracellular calcium. However, the cellular basis for the hyper-cholinergic state is not understood well. We have tested the hypothesis that the susceptible neurons are more sensitive than wild-type neurons to acetylcholine. To this end, we have used a mouse model of DYT1 dystonia, the most common inherited form of this disorder. DYT1 dystonia is caused by the deletion of a single glutamic acid residue in torsinA (ΔE-torsinA), a protein expressed widely throughout the brain. Heterozygous ΔE-torsinA knock-in mice reproduce the genetic status of patients. We used live-cell fluorescence imaging of the intracellular calcium concentration to evaluate the responses to acetylcholine in cultured striatal and hippocampal neurons, which normally express both torsinA and M1 muscarinic receptors. A high concentration of acetylcholine (10 μM) induced calcium transients in most neurons of both heterozygous and wild-type mice. These calcium transients often oscillated in amplitude, both during and after the 20-sec application of acetylcholine. They were observed in dendrites, somata and axons, and were completely blocked by pretreatment with the broad-spectrum muscarinic antagonist atropine (0.1 μM). An intermediate concentration of acetylcholine (1 μM), however, more effectively induced calcium transients in heterozygous vs. wild-type neurons. A low concentration (0.1 μM) induced detectable calcium transients only in heterozygous neurons. Interestingly, the calcium transients in the neurons treated with low acetylcholine were observed mainly in axons. These results suggest that the mutant neurons are sensitized to acetylcholine. Since intracellular calcium is an important regulator of neuronal excitability, the observed changes in cholinergic sensitivity in different subcellular regions could underlie the hyper-cholinergic state in DYT1 dystonia.

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Poster

047. Dystonia Mechanisms and Model Systems

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: PHS NS059645

Title: Neurochemical and behavioral dysfunction in a new mouse model of dopa-responsive dystonia

Authors: *S. ROSE, X. YU, H. A. JINNAH, E. J. HESS;
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Abstract: Dystonia is characterized by involuntary muscle contractions that cause debilitating twisting movements and postures. Although the pathogenesis of dystonia is not understood, reduced dopamine neurotransmission is observed across many different forms of dystonia, including dopa-responsive dystonia (DRD). DRD is cause by mutations in genes integral to dopamine synthesis, including tyrosine hydroxylase (TH), the rate-limiting enzyme in dopamine synthesis. Therefore, to better understand the role of dopamine in dystonia, we have created a knockin mouse bearing the DRD-causing TH mutation c.1160C>A; p.381Q>K (DRD knockin mice). TH activity is reduced by ~90% *in vivo* in these mice, causing a dramatic reduction in dopamine concentration. Behaviorally, DRD knockin mice exhibit abnormal movements reminiscent of human dystonia. These movements are exacerbated by dopamine receptor antagonists, illustrating the role for reduced dopamine tone in the expression of dystonia. DRD knockin mice also exhibit abnormal circadian regulation of locomotor activity, with hyperactivity at the start of the dark cycle and hypoactivity by the end of the dark cycle. These studies establish DRD knockin mice as a useful model for examining dopaminergic mechanisms underlying dystonia.

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Poster

047. Dystonia Mechanisms and Model Systems

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

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Grant-in-Aid from the Ministry of Health, Labor and Welfare (201024171A)

Title: Paretic and paratonic dystonia alleviated by pallidal stimulation

Authors: *Y. MIYAGI[1,2], T. OHMURA[1], T. SHIRAISHI[3], K. YAMASHIRO[4];
[1]Dpt.Stereotactic and Functional Neurosurg., Kaizuka Hosp., Fukuoka, Japan; [2]Grad. Sch. of Med. Sci., Kyushu Univ., Fukuoka, Japan; [3]Dpt. Neurol., Ikeda Hosp., Kanoya, Japan; [4]Dpt. Neurosurg., Okinawa Nanbu Med. Ctr., Haebaru, Japan

Abstract: A 22-year-old woman presented with a quadriplegia after symptoms of common cold at 17 years old. Based on the tentative diagnosis as abnormal autoimmune response, she was treated by the steroid pulse therapy but the quadriplegia did not respond to steroid at all in a short term. After repeating the exacerbation and remission, the muscle weakness of her limbs resolved spontaneously and gradually over the next three years. At 20 years old, the muscle power of her limbs recovered enough so that she could work as a caregiver in the facility for the disabled. At the age of 22, she felt uneasiness in all limbs and she began to lose the muscle power of all limbs, finally presenting with a quadriplegia in the following two days. Although her limb muscles were flaccid during voluntary movement (paretic), muscle tone turned rigid immediately in response to passive movement of limb joints (paratonic). There were neither involuntary movement nor abnormal posture of the limbs. All the other neurological findings other than the muscle tone of extremities, such as cranial nerve, cognitive, autonomic and sensory systems were totally normal. The paretic/paratonic symptoms were not alleviated by any pharmacotherapy. Deep brain stimulation of the internal globus pallidus diminished both paretic and paratonic symptoms and she recovered the voluntary motor control of extremities immediately after the implantation. Dystonia is typically defined as a syndrome of involuntary movement and abnormal postures caused by abnormal co-contractions of agonist and antagonist muscles and paretic loss of voluntary motor control is very rare. Paratonia, another form of abnormally increased muscle tone with an involuntary variable resistance during passive movement, has been thought to result from frontal lobe dysfunction such as late stage of dementia. Paretic and paratonic form of dystonia has never been reported as yet. The present case suggested that some atypical features of dystonia (coexistence of paresis and paratonia) could result from pathological output of the basal ganglia.

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Poster

047. Dystonia Mechanisms and Model Systems

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NIH Grant R21DC012344

NIH Grant UL1TR000114

Title: Pathophysiology of spasmodic dysphonia: A combined transcranial magnetic stimulation and functional magnetic resonant imaging study

Authors: *T. J. KIMBERLEY[1], M. CHEN[2], R. SCHMIDT[2];
[1]Program in Physical Therapy, Univ. of MN, Minneapolis, MN; [2]Physical Med. and Rehabil., Univ. of Minnesota, Minneapolis, MN

Abstract: Introduction Adductor spasmodic dysphonia (AdSD) is a primary task specific focal dystonia affecting the laryngeal muscles during speech. The pathophysiology of AdSD is unclear. Transcranial magnetic stimulation (TMS) studies on non-affected muscles (e.g. first dorsal interosseous) have demonstrated that there was significantly greater activation in M1 cortex. However, the excitability of the laryngeal motor cortex evaluated by the affected muscle (thyroarytenoid, TA), has not been investigated. In this work we developed a method to determine cortical excitability using the TA muscle EMG responses to TMS over laryngeal motor cortex and compared the results between the AdSD and healthy individuals. Method Cortical stimulation responses were acquired by two pairs of fine-wire hooked electrodes that were inserted into the right and left TA muscles. Peripheral nerve responses to stimulation were also collected to confirm the cortical response and latency. The cortical stimulation responses were measured by cortical silent period (CSP). Peripheral response was delivered to the mastoid. Latency of response to both cortical and peripheral stimulation were quantified. The CSP values were compared between healthy (H, n=2) and AdSD (S, n=1) individuals were compared. Result Preliminary results are presented. Ipsilateral responses with no silent period were observed from mastoid stimulation; bilateral responses were observed from the cortical stimulation, verifying cortical stimulation. The average CSP values for each subject: H1: 62.97 ms (contralateral, L) and 63.28 ms (ipsilateral, R); H2: 35.90 ms (contralateral, L) and 34.50 ms (ipsilateral, R); and S1: 59.50 ms (contralateral, L) and 49.38 ms (ipsilateral, R). Discussion The differences in the response pattern between mastoid and cortical stimulation confirmed that the CSP were elicited cortically. The CSP values indicated that it was possible to assess the cortical excitability by the

proposed method. CSP values from L and R TA were similar in healthy controls, but were different in AdSD subject. This finding needs further confirmation with more data. Conclusion We have developed a valid method to evaluate the excitability of the laryngeal motor cortex. The preliminary results confirmed that the CSP responses can be cortically evoked. Future work will use the comparison of the cortical excitability between AdSD and healthy individuals. These results will help to gain a better understanding of the mechanism of the inhibition networks of the brain of AdSD.

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Poster

047. Dystonia Mechanisms and Model Systems

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: K01 AG041211-01A1

2P41-EB015897-24

Title: Diffusion tensor image analysis of a novel mouse model of dystonia

Authors: *A. BADEA[1], Y. QI[1], S. QIU[3], S. BHAGAT[4], N. CALAKOS[2];
[1]Radiology, [2]Neurol., Duke Univ. Med. Ctr., DURHAM, NC; [3]Sch. of Med., Duke Univ., Durham, NC; [4]Neurol., Duke Univ. Med. Sch., Durham, NC

Abstract: **Introduction**. Dystonia is a neurological syndrome characterized by twisting movements or sustained abnormal postures. A novel, rare *TOR1A* variant (p. F205I) identified in an individual with late-onset, focal dystonia raised the possibility that this *TOR1A* variant may contribute to the expression of dystonia (Calakos, 2010). A mouse model with a knockin mutation was created to test the functional significance of this variant. In prior studies, DTI MRI changes have been described in DYT1 patients and asymptomatic carriers as well as asymptomatic DYT1 knockin mice (Argyelan, 2009; Ulug, 2011). We examined whether similar abnormalities were present in this novel model using high field, high resolution magnetic resonance histology. **Methods.** Twelve animals (6 of each genotype; littermate controls) were perfusion-fixed and actively stained with Gadolinium prior to MR imaging at 9.4T. A diffusion tensor imaging (DTI) protocol with 6 directions was used to reconstruct tensors and derived DTI

parametric maps (Jiang et al, 2011). ANTS (Avants et al 2011) was used for registration. SurfStat (Chung et al, 2010) was used for voxel-wise statistics for log Jacobian and DTI parametric maps. A novel atlas of the mouse brain was used for a regional quantitative characterization of volume and DTI parametric changes. **Results.** Atlas-based segmentation enabled regional analysis of DTI parametric changes for 100 brain regions. FA in the inferior cerebellar peduncle was different at $p<0.05$ between the dystonia animal models and age-matched controls. Other white matter tracts showed a trend towards reduced FA at $p<=0.1$. These include the brachium of the superior colliculus (bsc), the posterior commissure (pc), and the spino-cerebellar tract (dsc+vsc). FA was reduced between 5-10 % in these white matter structures (approx. 5% reduction in FA for bsc, 8% for icp and pc, and 11% in spinocerebellar tract). Also showing a trend, towards larger FA however, was the area occupied by the gigantocellular reticular nuclei (9% larger FA than in controls). **Conclusion.** Our results confirm and extend findings in the field. We see changes in regions that have been implicated in prior studies (cerebellar tracts). In addition, there are a number of new areas implicated. Notably, unlike prior mouse models studied with this imaging modality, F205I mice have motor impairments. Efforts are underway to determine whether correlations between imaging data and behavior exist, as data from human studies have indicated.

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Poster

047. Dystonia Mechanisms and Model Systems

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: DFG Grant KFO247

Title: Distinct oscillatory pallido-cortical networks revealed by simultaneous pallidal iEEG and MEG in patients with dystonia

Authors: W.-J. NEUMANN[1], A. JHA[2], A. BOCK[1], J. HUEBL[1], G.-H. SCHNEIDER[3], T. SANDER[4], V. LITVAK[2], *A. A. KUEHN[1];
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Abstract: Question: Little is known about the organisation of oscillatory network activity in pallidocortical circuits that are being modulated by deep brain stimulation (DBS) as a treatment for dystonia. In Parkinson's Disease, simultaneous subthalamic intracranial EEG (iEEG) and magnetoencephalographic (MEG) recordings have revealed distinct sources for alpha (7-13 Hz) and beta (13-35 Hz) networks. To characterize the oscillatory pallido-cortical network in dystonia we performed simultaneous MEG and iEEG recordings from the GPi in 8 patients (age: 47.8 ± 3.7 years; 6f/2m) with dystonia. Methods: Simultaneous MEG-iEEG recordings were conducted with the patients lying at rest with eyes open. Bipolar iEEG recordings were acquired from adjacent contact pairs of the DBS electrodes. To visualize peaks of frequency specific cortico-pallidal coherence the Dynamic imaging of coherent sources (DICS) beamforming method was utilized. Standard Statistical Parametric Mapping (SPM) methods were used to identify significant clusters of coherence in 3D source space. Source activity from these clusters was extracted using the Linearly-Constrained Minimum Variance beamformer method and directionality of information flow was analysed using granger causality based methods. Results: Group level analysis of the 3D DICS images revealed significant clusters of coherence in the theta (4-8 Hz), alpha (7-13 Hz) and beta (13-30 Hz) band in distinct anatomical locations. In the theta band, a cluster spanning from subcortical areas to the temporal cortex, with the coherence maximum in the mid temporal lobe, was identified ($p < 0.01$). A cluster restricted to the cerebellum was significant in the alpha band ($p < 0.01$). Pallido-cortical coherence in the beta band (13-30 Hz) was significant in the sensorimotor areas ($p < 0.01$). Directionality analysis revealed bidirectional coupling in all networks with predominant driving of the cortical signal by the GPi in the theta and alpha band, as opposed to a predominant cortical driving of the GPi signal in the beta band. Conclusions: We have demonstrated that the human internal pallidum is interconnected with the cerebral cortex in spatially and spectrally distinct functional oscillatory networks in patients with dystonia. Pallido-cortical coherence in the beta band was localized to motor areas, supporting previous findings in patients with Parkinson's disease. More interesting, pallido-cortical coherence in the alpha band was focussed to a central cerebellar source that may hint at cerebellar involvement in patients with dystonia as has been recently discussed for results from imaging studies.

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Poster

048. Neuromuscular Diseases

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Nemours Foundation

Title: An investigation of gene expression changes in motor neurons in a mouse model of spinal muscular atrophy

Authors: *C. C. PHILLIPS[1], A. W. HARRIS[3], M. MAEDA[4], M. E. R. BUTCHBACH[4], M. A. HARRINGTON[2];
[2]Dept. of Biol. Sci., [1]Delaware State Univ., Dover, DE; [3]Ctr. for Applied Clin. Genomics,, [4]Ctr. for Applied Clin. Genomics, Nemours Biomed. Research/Alfred I. DuPont Hosp. for Children, Wilmington, DE

Abstract: Spinal Muscular Atrophy (SMA) is a genetic motor neuron disease resulting from the loss of mutation of the *survival motor neuron 1* (*SMN1*) gene. SMA is characterized by the degeneration and death of motor neurons leading to neuromuscular dysfunction and ultimately mortality in those affected. Work with mouse models for spinal muscular atrophy has shown that SMN-deficient motor neurons are impaired in central circuitry and synaptic function including loss of afferent synapses associated with proprioceptive inputs. Recent work from our lab has shown that motor neurons from mouse models are increasingly less able to fire action potentials as the disease progresses. SMN plays a crucial role in processing of mRNA, so disruption of this protein could lead to defects in expression for other proteins needed for motor neuron function. Consideration of additional gene-expression mediated dysfunction is essential to further characterize the pathophysiology of the disease. In motor neurons, the axon initial segment (AIS) functions as the spike initiation zone. We are investigating changes in the expression of genes important in the function of the AIS, including ankyrin 3 (*Ank3*), and the sodium channels NaV1.1 *(Scna1)* and NaV1.6 *(Scna8)* in motor neurons from a mouse model of SMA. In severe SMA mouse motor neurons differentiated from embryonic stem cells, using whole transcriptome sequencing, we found significant reductions in the levels of *Scna8* and *Ank3* transcripts Scna1 mRNA levels were not affected by reduced SMN protein observed in SMA motor neurons. Changes in the expression of these proteins over the course of disease progression may be a key driver of motor neuron dysfunction.

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Poster

048. Neuromuscular Diseases

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NICHD Grant R15 HD075207

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Title: Survival motor neuron protein expression in motor neurons alters their intrinsic excitability in spinal muscular atrophy

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Abstract: Spinal Muscular Atrophy (SMA) is an inherited autosomal recessive disorder caused by loss or mutation of the Survival Motor Neuron 1 (SMN1) gene, resulting in loss of lower motor neurons and atrophy of muscle. Current research with SMA mouse models has mainly focused on the synaptic properties of motor neurons, demonstrating that defects appear at the neuromuscular junction as well as at central sensorimotor synapses before motor neuron cell death. However, recent work in the SMA-Δ7 mouse model, the most commonly studied SMA model, has shown that rescuing SMN protein expression specifically in motor neurons can prevent synaptic dysfunctions at the neuromuscular junction and restore central synapses. This suggested that lack of SMN protein expression in motor neurons, and motor neurons themselves might be the origin of the main features of SMA pathology. To characterize the alterations of motor neuron properties and understand their contribution to SMA, we used the recently developed mouse model in which SMN protein expression is rescued in SMA-Δ7 motor neurons under the control of the Choline Acetyl-transferase (ChAT) gene promoter. Early alterations of motor neuron intrinsic electrophysiological properties were studied with the whole-cell patch clamp technique in spinal cord slices obtained from the lumbar enlargement of SMA-Δ7 mutant mice, SMA-Δ7 mutant mice with SMN protein re-expressed in motor neurons, and control mice. In addition, confirmation of motor neuron identity of the electrophysiologically recorded cells

was achieved through morphological characterization and ChAT expression of the recorded cells.

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Poster

048. Neuromuscular Diseases

Location: Halls A-C

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: The Health and Medical Research Fund (HMRF), Food and Health Bureau, Hong Kong Special Administrative Region Government (Ref. No: 01122026)

Title: Therapeutic potential of heat shock protein 27 on Guillain-Barré syndrome

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Abstract: Guillain-Barré syndrome (GBS) is an autoimmune disease affecting the peripheral nervous system in which a person's own immune system damages their peripheral nerves, causing muscle weakness, paralysis, and loss of sensation in lower limbs. GBS patients suffer from muscle weakness in the lower limbs progressing rapidly in the ascending fashion resulting flaccid neuromuscular paralysis. Several clinical studies indicated that antibodies against ganglioside (anti-GD1a) in the sera of GBS patients are the most commonly recognized autoimmune markers in all forms of GBS targeting the neuromuscular junction (NMJ). Clinical studies have already reported the failure of target reinnervation and nerve repair in patients. Recent studies showed that Hsp27 accelerates axonal growth in peripheral neuron and functional recovery in animal model of peripheral nerve injury. The objective of present study is to determine the protective effect of Hsp27 on GBS. Anti-gangliosides GD1a/GT1b-2b (IB7) was administered intraperitoneally after peripheral nerve crush in Hsp27 transgenic and littermate mice. Series of behaviour experiments were performed such as pinprick assay (sensory), and grip strength (motor). Animals were perfused and tissues (gastrocnemius muscle, plantar muscle, brain, sciatic nerve and dorsal root ganglion) were harvested for histological analysis. The protective effect of Hsp27 was observed in grip strength test during initial days of recovery as

compared to IB7 treated littermates. These findings were consistent with pinprick test as initial response was observed 9 days earlier in Hsp27 transgenic group. The number of innervated NMJ increase significantly in Hsp27 transgenic mice as compared to IB7 treated littermate. Further analysis on axon number will be performed. Our pilot study indicates that Hsp27 as a potential therapeutic target for GBS.

Disclosures: **P. Asthana:** None. **G. Zhang:** None. **K.A. Sheikh:** None. **C.H.E. Ma:** None.

Poster

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Program#/Poster#: 48.04/N11

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Title: Pharmacological inhibition of HDAC6 is beneficial in mutant Gars-induced CMT2 mouse model

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Abstract: Mutations in glycyl-tRNA synthetase (GARS) can lead to the axonal form of Charcot-Marie-Tooth disease (CMT) or CMT2D. CMT is the most common inherited disorder of the peripheral nervous system. Due to progressive length-dependent degeneration of both motor and sensory axons, patients suffer from distal muscle weakness and atrophy in combination with sensory deficits. An endogenous ENU-induced mutation in Gars results in the development of motor and sensory deficits in mice, reminiscent to CMT2D in patients. In these mice, the amplitudes of the compound muscle action potentials (CMAPs) and the sensory nerve action potentials (SNAPs) are severely reduced, indicating axonal degeneration of both motor and sensory axons. This leads to reduced motor performance and reduced grip strength of the paws

together with sensory loss. Moreover, the mitochondrial axonal ransport is reduced in cultured DRG neurons from mutant Gars mice hich could be restored by the pharmacological inhibition of histone deacetylase 6 (HDAC6). Therefore, we investigated if HDAC6 could serve as a therapeutic target in this mutant Gars-induced CMT2 mouse model. Pharmacological HDAC6 inhibition in these mice led to improved neuromuscular innervation increased motor and sensory nerve conduction resulting in improved motor performance. Interestingly, we discovered a direct interaction between GARS and HDAC6. The interaction is strengthened by the CMT-causing mutation, which enhances the HDAC6 activity. These results explain the beneficial effect of inhibiting HDAC6 and suggest that HDAC6 may be involved in the etiology of CMT2D. Regardless of the mechanism, our results show that HDAC6 is a promising therapeutic target for peripheral neuropathies like CMT for which currently no pharmacological therapy exists.

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Poster

048. Neuromuscular Diseases

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Program#/Poster#: 48.05/N12

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Title: Generation of experimental model of myasthenia gravis with antibodies against LRP4

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Abstract: Myasthenia gravis (MG) is an autoimmune disease characterized by ptosis, fatigue, and muscle weakness. It is caused by autoantibodies associating with postsynaptic membrane proteins at neuromuscular junctions (NMJs). Several recent studies identified autoantibodies against low-density lipoprotein receptor-related protein (LRP4), an agrin receptor that is critical for NMJ development, in patients with double-seronegative MG-that is, patients without immunoreactivity toward acetylcholine receptors (AChRs) or muscle-specific kinase (MuSK). A variety of autoantibodies have been observed in MG patients. However, generally, such antibodies have not been associated clearly with MG pathogenesis. Elucidating the potential pathogenic role of anti-LRP4 autoantibodies in MG will require the establishment of an experimental autoimmune MG (EAMG) model. In the present study, we prepared the

recombinant protein of an extracellular region of human LRP4 and injected it into female A/J mice. After two or three injections, LRP4-injected mice exhibited weight loss and reduced strength, relative to controls. Electromyography with 3-Hz repetitive stimulation showed a decremental pattern of compound muscle action potentials (CMAPs) in LRP4-injected animals that was similar to that observed in MG patients, indicating that the animals had impaired neuromuscular transmission. The LRP4-injected mice also had marked reductions in pre- and post-synaptic labeling. Furthermore, sera from LRP4-injected mice blocked agrin-induced AChR clustering while inhibiting MuSK activation in cultured myotubes. These results demonstrate that an EAMG model was established in mice via active immunization, and further provide evidence suggesting that anti-LRP4 autoantibodies may play a role in the onset of double-seronegative MG. Given that A/J mice carry mutations that result in complement (C5) deficiency, our EAMG model suggests that complement activation is dispensable in MG pathogenesis caused by anti-LRP4 antibodies.

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Poster

048. Neuromuscular Diseases

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Title: Connexin-based hemichannels in muscular dystrophies. A possible common pathological mechanism

Authors: *L. A. CEA[1], P. A. CAVIEDES[1], J. A. BEVILACQUA[2], A. M. CARDENAS[3], A. D. MARTINEZ[3], J. C. SAEZ[4];
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Abstract: Skeletal muscles fibers from *mdx* (Duchenne Muscular Dystrophy disease model) and dysferlin-null (Dysferlinopathy disease model) mice show elevated intracellular Ca^{2+} levels and sarcolemmal permeability to dyes (i.e., Evans blue, EB^{4-}), as well as extensive inflammation and cell death. However, the molecular mechanisms underlying such pathological changes remain unknown. Here, we evaluated the possible involvement of connexins-based hemichannels (Cx HC) as the mechanism involved in the increased sarcolemmal permeability to molecules observed in the aforementioned animal models. Protein levels of Cx39, Cx43 and Cx45 were elevated in *mdx Tibialis Anterior* muscles of 3 months old mice, and they were located mainly in the sarcolemma. In addition, we analyzed muscle biopsies (Quadriceps or Deltoids muscles) from 4 patients that bear dysferlin mutations, all of which exhibited Cx43 in the sarcolemmal membrane, whereas in biopsies from control individuals this protein was absent. Sarcolemma permeability, as evaluated with EB^{4-} (*in vivo*) uptake, was increased in *mdx* myofibers, and absent in myofibers from *mdx* mice treated with carbenoxolone, a hemichannels blocker. We also demonstrated in HeLa-cells that EB^{4-} passes the membrane through Cx HC. A similar mechanism might explain the EB^{-4} uptake observed by others in animal models of dysferlinopathy. Further, the annexin V (A5) reactivity was analyzed in myofibers from control and *mdx* mice, only *mdx* myofibers positive for A5 were also positive for EB^{4-}, suggesting that Cx HC are involved in apoptosis processes. Thus, inhibition of Cx HC would protect myofibers from damage induced by the absence of dystrophin (*mdx* condition), and a similar phenomenon could also be operating in dysferlinopathies. We propose that the presence of Cx HC could be a common pathway that facilitates skeletal muscle damage in muscular dystrophies.

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Poster

048. Neuromuscular Diseases

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NOMA Grant, Norway

Title: The spectrum of deletions and duplications in the Dystrophin (dmd) gene in a cohort of patients with Duchenne Muscular Dystrophy in Sri Lanka

Authors: *N. THAKUR[1,2], A. ABEYSEKERA[2], J. WANIGASINGHE[3], V. H. W. DISSANAYAKE[2];
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Abstract: Background: Duchenne muscular dystrophy (DMD), which affects 1 in 3500 newborn male, is one of the most common fatal neurodegenerative disorders in children. It is an X-Linked recessive disorder and the most severe form of dystrophopathies affecting newborn males. It is the most common muscular dystrophy in all parts of world. Deletions and duplications are thought to be the major mutation underlying this disease accounting for approximately 65% and 6% of mutations respectively. Approximately 30% of DMD patients have unidentified mutations or point mutations in the dystrophin gene. Method: 50 clinically diagnosed children with DMD were screened after taking a written informed consent with the parents. We applied Multiple Ligation Probe Amplification (MLPA) to analyze the spectrum of deletions and duplications involving the dmd gene in Sri Lankan population. Results: Detection rate of the DNA rearrangements was 88% in 50 patients including 40 (80%) deletions and 4 (8%) duplications. Among 50 patients, 40 (80%) had deletions, 4 (8%) had duplications and 6 (12%) had no deletions and duplications. Single exon involvement was seen in 8 (16%) children. Two exons involvement was seen in 3 (6%) children, three exons involvement was seen in 6 (12%), four exons involvement was seen in 1 (2%) child and more than four exons involvement was seen in 26 (52%) children. The most common deletion was the deletion spanning from exon 45 to exon 52 which was seen in 6 (12%). The next common exon deletion was single exon 45 deletion which was seen in 4 (8.0%) children. The most frequent mutant region fell within exons 45 to 55 (52%) in dmd gene, followed by exons from 21 to 44 (26%) and exons from 1 to 20 (26%) and the least common region fell within exons 56 to 79 (4%). Conclusion: This study is the first to report the deletions/duplications pattern in patients with DMD in Sri Lanka. MLPA technique can be used effectively to diagnose clinically suspected DMD patients. The deletion/duplication pattern seen in the Sri Lankan population was similar to that of the other global populations.

Disclosures: **N. Thakur:** None. **A. Abeysekera:** None. **J. Wanigasinghe:** None. **V.H.W. Dissanayake:** None.

Poster

048. Neuromuscular Diseases

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NIAAA intramural program

Title: A single mutation in the acetylcholine receptor delta-subunit causes distinct effects in two types of neuromuscular synapses

Authors: *F. ONO[1], J.-Y. PARK[1], T. WILLIAMS[1], M. MOTT[1], H. IKEDA[1], H. WEN[2], M. LINHOFF[2];
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Abstract: Mutations in acetylcholine receptor (AChR) subunits, expressed as pentamers in neuromuscular junctions (NMJ), cause various types of congenital myasthenic syndromes. In AChR pentamers, the adult ε subunit gradually replaces the embryonic γ subunit as the animal develops. Due to this switch in subunit composition, mutations in specific subunits result in synaptic phenotypes that change with developmental age. However, a mutation in any AChR subunit is considered to affect the neuromuscular junctions of all muscle fibers equally. Here, we report a zebrafish mutant of the AChR δ subunit that exhibits two distinct NMJ phenotypes specific to two muscle fiber types: slow or fast. Homozygous fish harboring a point mutation in the δ subunit form functional AChRs in slow muscles, while receptors in fast muscles are non-functional. To test the hypothesis that different subunit compositions in slow and fast muscles underlie distinct phenotypes, we stained larvae with an antibody specific for γ/ε subunits. Both wild type and the mutant larvae lacked γ/ε subunits in slow muscle synapses. These findings in zebrafish suggest that some mutations in human congenital myasthenic syndromes may affect slow and fast muscle fibers differently.

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Poster

048. Neuromuscular Diseases

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NIH/NINDS grant F31 NS77806-01

Title: Astrocytes in Amyotrophic Lateral Sclerosis (ALS): A fundamental rearrangement of vascular and synaptic organization

Authors: *I. LORENZINI[1], J. PREMINGER[2], S. NATH[3], J. D. ROTHSTEIN[2]; [2]Neurol., [1]Johns Hopkins Univ. Sch. of Med., BALTIMORE, MD; [3]Neurol., johns Hopkins Univ. Sch. of Med., BALTIMORE, MD

Abstract: Amyotrophic Lateral Sclerosis (ALS) is a neurodegenerative disease characterized by the loss of cortical and spinal motor neurons. Although motor neurons are vulnerable in ALS, other cells are also affected. Glial cells are known to be major contributors of disease onset and disease progression. Astrocytes in particular, become reactive displaying changes in structure and function. We examined in detail the astrocyte structure during normal and chronic motor neuron degeneration using highly specific inducible transgenic astroglial reporter mice. Confocal imaging analysis and three-dimensional (3D) reconstruction of protoplasmic astrocytes from motor cortex and spinal cord revealed significant differences in the surface area and volume. A major astrocyte role in the CNS is to give metabolic support to neurons through the interactions with blood vessels. In order to know if this interaction is affected during disease conditions, we created a 3D imaging assessment to show astrocytes' contact with blood vessels. Mutant astrocytes from the spinal cord lose contact with the vasculature at late stages of ALS disease and significantly extend long processes over the limit of their domain. Astrocytes also form spherical-like structures during symptomatic and end-stages of the disease suggesting a potential phagocytic role. Further studies showed that a subset of astrocytes from the ventral regions of the lumbar spinal cord have a phagocytic role during disease conditions. Overall, these changes suggest that astrocytes rearrange their structure during ALS disease affecting multiple synapses as well as the metabolic and vascular support. Results from this investigation provide a deeper understanding of the astroglial biology and pathology in the setting of neurological disease.

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Poster

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: Köhler Stiftung,Deutsche Forschungsgemeinschaft(DFG)

Title: Brain computer interface for communication in completely locked-in patient using near infrared spectroscopy

Authors: *U. CHAUDHARY[1], B. XIA[1], S. VESSER[1], G. GALLEGOS-AYALA[1], N. BIRBAUMER[1,2];
[1]Inst. of Med. Psychology and Neurobio., Univ. of Tubingen, Tubingen, Germany; [2]Irccs, Ospedale San Camillo, Venezia, Italy

Abstract: Amyotrophic lateral sclerosis (ALS) is a progressive motor disease of unknown etiology resulting eventually in a complete destruction of the peripheral and central motor system but only affecting sensory or cognitive functions to a minor degree. Almost all people with ALS experience a motor speech disorder as the disease progresses. At some point in the disease progression, 80 to 95% of patients with ALS are unable to meet their daily communication needs using natural speech. Later, most become unable to speak at all. Brain computer-interface (BCI) technology has generated considerable research interest for the "locked-in" patients such as those in the late stages of ALS. Several EEG-based BCI are currently in use namely slow cortical potential (SCP)-BCI, sensorimotor rhythm (SMR)-BCI and P300-BCI but none of them have been successful for communication in ALS patients in completely locked in state (CLIS). Hence there is a need to find an alternative neuroimaging technique to design a more effective BCI to help ALS patient in CLIS with communication. Near infrared spectroscopy (NIRS) is an emerging neuro-imaging modality which employs near-infrared light to non-invasively or invasively investigate cerebral oxygenation changes in healthy and neurologically challenged adults and children. Previous researches have shown that NIRS can be successfully used to design BCI; hence NIRS was used to design BCI to help ALS patient in CLIS with communication. Recently NIRS was used successfully to investigate the functional activations in the motor cortex of 67 year old female CLIS patient in response to auditorily presented stimuli containing correct or incorrect statements and open questions. The hemodynamic change in the motor cortex of the CLIS patient was recorded across many sessions spread over more than a year and was used to train a classifier to predict the "yes" and "no" answering pattern of the CLIS patient who was previously trained to use an EEG-BCI without success. The trained classifier was able to provide online feedback ("your answer was classified as (in) correct") to the patient with performance rate of 71.76%. This is the first carefully documented case of communication in a CLIS patient with BMI, which holds promise and raises the hope for communication in CLIS. Hence, to further validate the preliminary findings of our lab and refine the technology of NIRS-based BCI for communication in CLIS-patients extensive studies are presently carried out on CLIS patients using combined NIRS-EEG based BCIs.

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Poster

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Title: Mutation in the novel nuclear-encoded mitochondrial protein CHCHD10 in a family with autosomal dominant mitochondrial myopathy

Authors: F. FECTO[1], S. AJROUD-DRISS[1], K. AJROUD[1], I. LALANI[1], S. E. CALVO[2], V. K. MOOTHA[2], H.-X. DENG[1], N. SIDDIQUE[1], A. J. TAHMOUSH[3], T. D. HEIMAN-PATTERSON[3], *T. SIDDIQUE[1];
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Abstract: Mitochondrial myopathies belong to a larger group of systemic diseases caused by morphological or biochemical abnormalities of mitochondria. Mitochondrial disorders can be caused by mutations in either the mitochondrial or the nuclear genome. Only 5% of all mitochondrial disorders are autosomal dominant. We analyzed DNA from members of a previously reported Hispanic kindred with an autosomal dominant mitochondrial myopathy (Tahmoush et al. 1997). Linkage analysis suggested a putative locus on the pericentric region of the long arm of chromosome 22 (22q11). Using the tools of integrative genomics, we established C22orf16 (later designated as CHCHD10) as the only high scoring mitochondrial candidate gene in our minimal candidate region. Sequence analysis revealed a double missense mutation (R15S; G58R) in cis in CHCHD10 which encodes a coiled-coil helix coiled-coil helix protein of unknown function. These two mutations completely co-segregated with the disease phenotype and were absent in 1481 Caucasian and 80 Hispanic controls. Expression profiling showed that CHCHD10 is enriched in skeletal muscle. Mitochondrial localization of the CHCHD10 protein

was confirmed using immunofluorescence in cells expressing either wild-type or mutant CHCHD10. We found that expression of the G58R, but not the R15S, mutation induced mitochondrial fragmentation. Our findings identify a novel gene causing mitochondrial myopathy, thereby expanding the spectrum of mitochondrial myopathies caused by nuclear genes. Our findings also suggest a role for CHCHD10 in the morphologic remodeling of the mitochondria.

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Poster

048. Neuromuscular Diseases

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

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NS Grant 20480

Title: Morphological changes to the structure of the neuromuscular junction in a canine model of duchenne muscular dystrophy

Authors: ***S. HADDIX**[1], Y. LEE[2], I. SMITH[2,3], J. N. KORNEGAY[4,3], W. THOMPSON[2,3]; [1]Texas A&M Univ., College, TX; [2]Biol., [3]Inst. for Neurosci., [4]Col. of Vet. Med. and Biomed. Res., Texas A&M Univ., College Station, TX

Abstract: Duchenne muscular dystrophy (DMD) is an X-linked genetic disease in which mutations of the dystrophin gene lead to skeletal and cardiac muscle abnormalities. It affects 1 in 5000 boys and has no known cure or effective treatment. Previous observations have suggested that not only are muscle fibers in these patients and animal models sensitive to damage, but their neuromuscular junctions (NMJs), the cholinergic synapse between motor neurons and muscle fibers, are also dramatically affected. The similarity of the junctional alterations observed in muscles treated with myotoxic drugs suggests that a process of degeneration and regeneration in fibers produces the synaptic changes. To further assess mechanisms underlying morphological alterations, we have examined NMJs in dogs that have dystrophin mutations leading to a severe

dystrophy (Golden Retriever muscular dystrophy, GRMD), a model much more akin to human DMD than the commonly used mdx mice (to date 10 dogs analyzed, 4 in depth). Using fluorochorome-conjugated α-bungarotoxin, a snake toxin that binds specifically to muscle acetylcholine receptor (AChR), postsynaptic alterations at NMJs of GRMD extensor hallucis longus muscles were examined. We found changes to AChR aggregates that mirror those in the canonical mdx mouse. The majority of GRMD AChR aggregates were large and dispersed into fragments as compared to pretzel-shaped, continuous aggregates in controls. Moreover, there were a number of "ghost" junctions in which nerve, Schwann cells and cholinesterase were no different than other GRMD junctions, but these sites seemed, in several cases, to be dimly stained for AChR and to lack postsynaptic sarcomeric structures in the associated muscle fiber. Ghost junctions and AChR fragmentation resemble similar structures seen in normal mice after postsynaptic muscle fiber ablation and upon muscle fiber regeneration, respectively, suggesting that altered NMJs results from necrosis/regeneration of muscle fiber at the synaptic region. In addition to fragmentation of AChR aggregates, there was a distinct population of minute and compact AChR aggregates in dystrophic muscle, more prevalent in later stages of disease. There was also a change in the innervation pattern of the GRMD muscles: adjacent muscle fibers were often innervated by the same axon, apparently due to preterminal sprouting. This suggests a loss of some motor innervation to the muscles and an enlargement of motor units. Our observations suggest morphological changes at NMJs in the GRMD model accumulate with time and act as a compounding factor in the progression of the disease.

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Poster

048. Neuromuscular Diseases

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NINDS intramural research funding

Title: Astrocytes isolated from transgenic Δ7 SMA mice have altered protein secretion

Authors: ***E. FORAN**[1], T. NGUYEN[1], P. R. LEE[2], C. GRUNSEICH[1], J. NOFZIGER[1], E. S. ARNOLD[1], B. BURNETT[3], K. FISCHBECK[1];
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Abstract: Spinal muscular atrophy (SMA) is the most common inherited cause of death in infants and young children. It is caused by the deletion or mutation in the survival of motor neuron 1 (SMN1) gene, leading to a deficiency of the ubiquitously expressed SMN protein. SMA is characterized by slowly progressive weakness and atrophy of the limb muscles associated with motor neuron loss in the spinal cord. Currently, there is no disease specific effective treatment available. Loss of SMN leads to degeneration of motor neurons; however recent work has shown the importance of other cell types in CNS diseases. Astrocytes are the most numerous cell type in the CNS and are required for correct functioning of motor neurons in the spinal cord; however the characterization of the role of astrocytes in SMA is still at early stages. We present here evidence that spinal cord astrocytes from an SMA mouse model have abnormalities in the secretion of several factors that could affect motor neuron health. We used the SMN2+/+;SMNΔ7+/+;Smn-/- (SMAΔ7) mouse line to examine the effects of decreased levels of SMN on astrocytes and how they interact with motor neurons. To validate changes seen in the SMAΔ7 mouse model in a human model of SMN depletion, we have also differentiated patient and parental control-derived iPS cell lines (GM23240 and GM23241) into astrocytes and motor neurons. We found altered mRNA and protein expression, and protein secretion in astrocytes isolated from the SMAΔ7 mice in comparison to astrocytes isolated from littermate controls. In particular, the secretion profile of astrocytes isolated from the SMAΔ7 mice was abnormal. Such secretion alterations may lead to a decreased ability to support motor neurons *in vitro* and *in vivo*. We are using co-cultured motor neurons and astrocytes and motor neurons grown in astrocyte-conditioned media to determine how these alterations affect motor neuron survival, growth, and physiology. These astrocyte defects may contribute to motor neuron degeneration in SMA and thus could be a novel target for therapeutic intervention.

Disclosures: **E. Foran:** None. **T. Nguyen:** None. **P.R. Lee:** None. **C. Grunseich:** None. **J. Nofziger:** None. **E.S. Arnold:** None. **K. Fischbeck:** None. **B. Burnett:** None.

Poster

048. Neuromuscular Diseases

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 48.14/O9

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: Fapesp 2012/03498-7

Title: Effects of suramin on stretch-activated calcium channel protein TRPC1 and calsequestrin in the diaphragm of old mdx mice

Authors: *D. O. MOREIRA, H. SANTO NETO, M. MARQUES;
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Abstract: Duchenne muscular dystrophy (DMD) is a devastating inherited neuromuscular disorder with an incidence of 1 in 3500 live male births. DMD is characterized by a mutation in the X-chromosome that leads to a lack of dystrophin in skeletal and cardiac muscles. In DMD and in the mdx mice model of DMD, the absence of dystrophin leads to sarcolemma instability, increased calcium influx and consequent myonecrosis. Abnormal calcium entry and buffering seem to play important roles in dystrophy pathology and stretch activated calcium channels, such as the transient receptor potential canonical channel 1 (TRPC1), greatly contribute to calcium changes in dystrophic fibers. In this study we investigated the effects of suramin, an anti-fibrotic agent on TRPC1 levels in the diaphragm of mdx mice, at later stages of the disease (11 months). We also investigated the effects of suramin of the levels of calsequestrin, the main calcium-binding protein of the endoplasmic reticulum. Mdx mice (8 months old) received intraperitoneal injections of suramin (60 mg/kg body weight), twice a week for 3 months. Control mdx mice (8 months old) were injected with saline. Western blot analysis showed that suramin decreased the levels of TRPC1 (2.3±0.2 in control vs 1.3±0.3 in suramin-mdx, $p<0.05$) and improved the levels of calsequestrin (0.5±0.1 in control vs 0.9±0.2 in suramin-mdx, $p<0.05$). Suramin also decreased muscle fiber total calcium (29% decrease as observed with inductively coupled plasma-optical emission spectrometry). The present results suggest that suramin, in addition to its anti-fibrotic action can ameliorate dystrophy in the mdx diaphragm, at later stages of the disease, by acting on calcium entry (TRPC1) and calcium binding (calsequestrin) mechanisms.

Disclosures: **D.O. Moreira:** None. **H. Santo Neto:** None. **M. Marques:** None.

Poster

048. Neuromuscular Diseases

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Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 48.15/O10

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: FAPESP

CNPq

Title: Effects of omega-3 therapy in the cardiomyopathy of the mdx mice, at later stages of the disease

Authors: *A. F. MAURICIO, J. A. PEREIRA, H. SANTO NETO, M. J. MARQUES;
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Abstract: Duchenne muscular dystrophy is the most common and severe dystrophynopathy in childhood characterized by absence of dystrophin, with progressive muscle wasting and cardiorespiratory failure. In the absence of dystrophin there is sarcolemma instability, increased calcium influx and myonecrosis. Cardiomyopathy is one of the most frequent causes of death in DMD. In the mdx mice model of DMD, signs of cardiomyopathy are first seen around 9 months of age. We investigated the effects of omega-3 therapy in the mdx cardiomyopathy, at later stages of disease (13 months of age). Mdx mice (8 months of age) received fish oil containing eicosapentaenoic acid and docosahexanoic acid (300mg/kg via gavage, 3 days a week), for 5 months. Control mdx received nujol in an equivalent dose and period. Control mdx showed elevated (3 times) serum levels of CK-MB, an indicator of heart necrosis, compared with normal C57BL/10. Omega-3 reduced CK-MB (119±20 UI in mdx-nujol vs. 86±20 UI in mdx-omega-3). In control mdx, electrocardiogram analysis indicated alterations in the amplitudes of some waves, a decrease in the R/S ratio and a significant increase in the cardiomyopathy index. Omega-3 ameliorated some of these heart functional parameters. No changes in heart fibrosis area were seen in omega-3-mdx, with higher levels of fibrosis in the right ventricule (16±3% in mdx-nujol vs. 13±3% in mdx-omega-3). The levels of TNF-a (proinflammatory cytokine), TGF-b (profibrotic factor) and metaloproteinases(MMP)-9 and MMP-2 were all increased in the heart of control mdx in comparison to normal C57BL/10. Omega-3 significantly reduced the levels of TNF-a (1.5±0.4 in mdx-nujol vs. 1.1±0.03 in mdx-omega-3) and MMP-9 (1.3±0.1 in mdx-nujol vs. 1.1±0.09 in mdx-omega-3), with a tendency to reduce TGF-b (1.9±0.3 in mdx-nujol vs. 1.7±0.4 in mdx-omega-3; $p > 0.05$, Anova). The present results demonstrate that omega-3 is effective against cardiomyopathy in the mdx mouse, at later stages of the disease, being able to improve functional parameters and to regulate molecular markers (TNF-a, TGF-b and MMPs) of dystrophy progression, therefore deserving future investigation in DMD clinical trials.

Disclosures: **A.F. Mauricio:** None. **J.A. Pereira:** None. **H. Santo Neto:** None. **M.J. Marques:** None.

Poster

048. Neuromuscular Diseases

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NIH Grant R01 NS085207

ALSA

MDA

Judith and Jean Pape Adams

Target ALS

Brain Science Institute

Title: Generation and characterization of C9ORF72 Amyotrophic Lateral Sclerosis (ALS) astrocytes derived from patient fibroblasts

Authors: *J. T. PHAM[1,2], E. L. DALEY[1], C. J. DONNELLY[1], T. GENDRON[4], L. PETRUCELLI[4], R. G. SATTLER[1], J. D. ROTHSTEIN[1,3];
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Abstract: Amyotrophic lateral sclerosis (ALS) is one of the most common neurodegenerative diseases, characterized by progressive loss of upper and lower motor neurons as well as associated interneurons. An intronic GGGGCC hexanucleotide repeat expansion in the *C9ORF72* gene was recently identified as the most common genetic cause of ALS. Studies investigating *C9ORF72* pathogenesis have primarily focused on neurons. However, glial cells are also known to play an important role in ALS by modulating disease onset and progression. In particular, astrocytes often exhibit altered structure and functionality under disease conditions. We examined the role of astrocytes in *C9ORF72* ALS by generating astrocytes from patient fibroblasts and assessing mechanisms by which intronic repeat expansions can cause disease. First, patient fibroblasts were reprogrammed into induced pluripotent stem (iPS) cells, which were then differentiated into astrocytes (iPS-A). These iPS-A robustly immunostain for astrocyte markers, including glial fibrillary acidic protein (GFAP), CD44, S100β, and Vimentin. Co-culturing iPS-A with primary mouse cortical neurons increased mRNA expression of excitatory amino acid transporter 2 (EAAT2), an astrocyte-specific glutamate transporter tightly regulated by neurons *in vivo*. EAAT2 functionality was evaluated further with pending protein expression and functional sodium-dependent ^{3}H-glutamate transport assessments in *C9ORF72* ALS and control iPS-A. Next, we explored pathological consequences of the repeat expansion in the iPS-A. *C9ORF72* iPS astrocytes had decreased levels of total *C9ORF72* mRNA in comparison with control iPS-A as quantified by RT-PCR. Probing the iPS-A with RNA fluorescent *in situ*

hybridization showed accumulation of RNA foci containing the (GGGGCC)n repeat expansion, which were more abundant in *C9ORF72* iPS-A. We also investigated the localization of poly-glutamine-proline (poly-GP) repeat-associated non-ATG (RAN) protein product in *C9ORF72* iPS-A. Poly-GP RAN translation products were detectable by ELISA in supernatants taken from *C9ORF72* iPS-A cultures but not from control cultures. These findings show that iPS-A are a viable model, allowing us to more faithfully recapitulate processes occurring in patients and to thus gain a more thorough understanding of the disease. Results suggest that astrocytes are affected in *C9ORF72* ALS and could potentially contribute to disease pathogenesis.

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Poster

048. Neuromuscular Diseases

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 48.17/O12

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Title: Inhibition of hematopoietic prostaglandin D synthase improves symptoms of muscular dystrophy in a mouse model of Duchenne muscular dystrophy

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Abstract: Duchenne muscular dystrophy (DMD) is a genetic and lethal disease. No complete cure for the disease is still established. The fibers of skeletal muscle in DMD patients are highly susceptible to damage of the membrane and easily suffer necrotic change, because of the loss of dystrophin in the muscle. This pathological condition leads to progressive weakness of all skeletal muscles, including diaphragm. Then, the patients show weakened motor activity, such as dysbasia. In the past (SfN2013 etc.), we have reported that prostaglandin D_2 (PGD_2) synthesized by hematopoietic PGD synthase (HPGDS) may play an important role in the pathology of DMD and that the inhibition of HPGDS would be an effective therapy for DMD. Recently, a novel high-selective HPGDS inhibitor, TAS-205, was found in our laboratory. This compound is useful to validate our hypothesis. Therefore, the effect of TAS-205 was evaluated using

dystrophin-deficient (*mdx*) mice, which is an animal model of DMD. *In vitro* study, inhibitory effects of TAS-205 were evaluated on enzyme activity in cell-free assay and Ca^{2+}-ionophore stimulated PGD_2 production in a human basophilic leukemia (KU812). *In vivo* study, *mdx* mice (C57BL/6 back ground) were supplied the diet including 0.01% or 0.1% TAS-205 from 8 to 9 weeks of age in the pharmacokinetic evaluation, or from 5 to 9 weeks of age in the efficacy evaluations. To determine the plasma concentration of TAS-205, the blood was collected on day 7 after beginning of the treatment with TAS-205. At 9 weeks of age, we measured the urinary tetranor-PGDM level, a metabolite of PGD_2, and the locomotor activity during night-time. Also, the rate of necrotic fibers area was evaluated in cross sections of the diaphragm muscle. *In vitro* study, TAS-205 inhibited the enzyme activity of HPGDS (IC_{50}:56 nM) and reduced PGD_2 production in KU812 (IC_{50}:83 nM). When *mdx* mice were given the diet including 0.01% TAS-205 for 7 days, TAS-205 concentrations in plasma of *mdx* mice were 156-232 nM, which were enough to inhibit HPGDS. The urinary tetranor-PGDM concentration was significantly higher in *mdx* mice than that in wild-type mice. TAS-205 dose-dependently suppressed the urinary tetranor-PGDM amount in *mdx* mice. The locomotor activity during the night-time was significantly lower in *mdx* mice than that in wild-type mice. And, in histological study, many necrotic fibers were detected in diaphragm muscle of *mdx* mice, although it was hardly in wild-type mice. TAS-205 at high dose significantly reduced the necrotic muscle fibers and recovered the locomotor activity in *mdx* mice. These results support our hypothesis that a highly selective HPGDS inhibitor, such as TAS-205, would be an effective therapy for DMD.

Disclosures: **K. Tanaka:** A. Employment/Salary (full or part-time):; Taiho Pharmaceutical Co., Ltd.. **K. Aritake:** None. **M. Tayama:** A. Employment/Salary (full or part-time):; Taiho Pharmaceutical Co., Ltd. **K. Shigeno:** A. Employment/Salary (full or part-time):; Taiho Pharmaceutical Co., Ltd. **Y. Hayashi:** A. Employment/Salary (full or part-time):; Taiho Pharmaceutical Co., Ltd. **E. Sasaki:** A. Employment/Salary (full or part-time):; Taiho Pharmaceutical Co., Ltd. **T. Utsugi:** A. Employment/Salary (full or part-time):; Taiho Pharmaceutical Co., Ltd.. **T. Sasaoka:** None. **Y. Urade:** None.

Poster

048. Neuromuscular Diseases

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Program#/Poster#: 48.18/P1

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NIH Grant NS058901

Title: Mbnl1; Mbnl2 conditional double knockout mice as a model for the myotonic dystrophy brain

Authors: *K.-Y. LEE[1,2], F.-J. FERNANDEZ-GOMEZ[3,4], S. EDDARKAOUI[3,4], M. LI[2], J. THOMAS[2], D. FINN[2], N. HAMED[2], L. BUÉE[3,4], N. SERGEANT[3,4], M. SWANSON[2]; [1]Chang Gung Mem. Hospital, Keelung, Taipai, Taiwan; [2]Mol. Genet. and Microbiology, Ctr. for NeuroGenetics and the Genet. Inst., Univ. of Florida, Col. of Med., Gainesville, FL; [3]Inserm UMR837-1 and Univ. Lille Nord de France, Jean-Pierre Aubert Res. Center, Alzheimer & Tauopathies, F-59045, Lille, France; [4]Regional Univ. Hosp. of Lille, Lille, France

Abstract: Myotonic dystrophy (DM), the most common form of adult-onset muscular dystrophy, is a multi-systemic disorder that has severe effects on brain function. Neuropathological studies reveal neurofibrillary tangles in DM and mis-splicing of microtubule-associated protein Tau (MAPT). Muscleblind-like (MBNL) proteins are developmentally regulated alternative splicing factors and the MBNL gene family consists of three paralogs (MBNL1, MBNL2, MBNL3) with distinct spatial and temporal expression patterns. In the mouse, Mbnl3 is primarily expressed during embryogenesis while Mbnl1 and Mbnl2 promote adult isoform splicing patterns for specific target genes. Although Mbnl1 is predominantly expressed in adult skeletal muscle, Mbnl2 is highly expressed in the adult brain. In DM, abnormal expansions of CTG and CCTG microsatellites in the DMPK and CNBP genes, respectively, results in the expression of C(C)UGexp mutant RNAs that bind, and subsequently sequester, the MBNL proteins resulting in MBNL loss-of-function. This disease model is supported by Mbnl1 and Mbnl2 single knockout (KO) mouse models that recapitulate the major manifestations of DM in skeletal muscle (myotonia) and the brain (learning/memory deficits, REM sleep mis-regulation). To test the hypothesis that compound loss of MBNL activity would recapitulate additional CNS features of DM disease, we have generated neuron-specific Mbnl1; Mbnl2 double KO (DKO) mice (Mbnl1ΔE3/ΔE3; Mbnl2cond/cond; Nestin-Cre+/- (Nestin-Cre DKO). Mbnl DKOs are small, show impaired motor functions and are characterized by a nearly complete reversal to fetal brain splicing patterns, including Mapt exons 2, 3 and 10. Specific anti-Tau antibodies and two-dimensional electrophoresis (2DE) immunoblotting revealed that Tau protein expression was profoundly altered in the Mbnl1; Mbnl2 DKO brain with equivalent expression of the 0N4R (Tau isoform lacking exons 2 and 3 but including exon 10) and 0N3R (lacking exon 10) Tau isoforms. We conclude that Mbnl1; Mbnl2 DKO mice provide a novel animal model to characterize the molecular events leading to DM CNS disease.

Disclosures: **K. Lee:** None. **F. Fernandez-Gomez:** None. **S. Eddarkaoui:** None. **M. Li:** None. **J. Thomas:** None. **D. Finn:** None. **N. Hamed:** None. **L. Buée:** None. **N. Sergeant:** None. **M. Swanson:** None.

Poster

049. Epilepsy: Synapses and Ion Channels

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 49.01/P2

Topic: C.07. Epilepsy

Title: Evaluations of seizures and behavior in Slack-/- mice suggest separate pathways for epilepsy and developmental delay in patients with Slack-associated epileptic encephalopathies

Authors: *I. H. QURAISHI[1], R. L. COUTURE[2], M. L. SCHWARTZ[2], L. K. KACZMAREK[3]; [1]Dept. of Neurol., [2]Neurobio., [3]Pharmacol. and Cell. and Mol. Physiol., Yale Univ., New Haven, CT

Abstract: Slack (Kcnt1) is a Na^+-activated K^+ channel that has been implicated in several epilepsy syndromes including Malignant Migrating Partial Seizures of Infancy (MMPSI). Patients with mutations in Slack develop intractable epilepsy with neurodevelopmental or psychiatric comorbidities. We previously demonstrated that epilepsy-associated Slack mutations are paradoxically associated with gain of function in the channel conductance, and that Slack interacts with proteins necessary for normal neuronal development, particularly Fragile X Mental Retardation Protein (FMRP). To determine whether the epilepsy and behavioral phenotypes in patients with these mutations are functions of the increased current or of loss of FMRP binding, we performed tests of seizure thresholds and behavioral screening in adult male Slack-/- mice. Slack-/- mice did not have spontaneous seizures. Acute seizure thresholds were measured with maximum electroshock and pentylenetetrazole. Maximum electroshock seizure thresholds were slightly lower in Slack-/- than Slack+/+ mice. Measurement of severity of fixed high current electroshock seizures was confounded by decreased mortality in the Slack-/- mice. With pentylenetetrazole there was no difference in latency to Racine 5 seizures between groups. Behavioral screening was done with open field and rotorod tasks. In an open field test Slack-/- mice had less exploratory behavior than wild type animals. Rotorod testing suggests impaired motor learning. These results suggest that loss of Slack interactions, in the absence of increased the current seen in the human epilepsy mutations, may be sufficient to produce neurodevelopmental changes without spontaneous seizures or large changes in seizure thresholds.

Disclosures: I.H. Quraishi: None. **R.L. Couture:** None. **M.L. Schwartz:** None. **L.K. Kaczmarek:** None.

Poster

049. Epilepsy: Synapses and Ion Channels

Location: Halls A-C

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Program#/Poster#: 49.02/P3

Topic: C.07. Epilepsy

Support: FAPESP

PROEX-CAPES

FAPESP-Cinapce

CNPq

FAEPA

Title: Chronic high intensity sound stimulation inhibits Schaffer-CA1 hippocampal long-term potentiation in Wistar rats but not in those of the Wistar Audiogenic Rat (WAR) strain

Authors: *A. O. CUNHA[1], J. A. C. OLIVEIRA[2], S. S. ALMEIDA[3], N. GARCIA-CAIRASCO[2], R. M. X. LEAO[2];
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Abstract: High intensity sound stimulation can cause convulsive seizures in susceptible mice and rats. The repetition of these stimuli, in turn, will evoke a change in seizure behavioural repertoire together with the appearance of epileptic EEG activity in limbic structures. In the hippocampus, seizures lead to indiscriminate and widespread long-term potentiation (LTP) due to structural and biochemical alterations. Here, we investigated if LTP of hippocampal Schaffer-CA1 synapses is altered by chronic high intensity sound stimulation (HISS) in normal Wistar rats and in a strain of audiogenic seizure-prone rats (Wistar Audiogenic Rats - WAR). HISS consisted of two daily acoustic stimulations (120 dB) for ten days. Animals were observed before, during and after HISS and their seizures were analysed and scored for severity. Then, the rats were trained during 4 days (6 sessions) in a circular pool, divided in quadrants with a hidden platform and then tested for spatial navigation. One week after the end of the experiments, animals were killed and their brains were removed and sliced in order to perform hippocampal electrophysiology. Field potentials (fEPSPs) in the stratum radiatum of CA1, were elicited by stimulation of Schaffer collaterals. LTP was induced by three trains of stimulation at 50 or 100 Hz for 1 second. After chronic HISS all WARs exhibited mesencephalic seizures and 50% of these animals developed limbic recruitment, while only 26% of Wistar rats presented mesencephalic seizures. HISS did not alter spatial navigation in both strains, although WARs

presented impaired navigation in comparison to Wistar rats. Surprisingly, HISS strongly supressed LTP in slices of Wistar-naïve animals, and inhibited LTP in slices from Wistar rats which presented mesencephalic seizures, but had no effect in LTP of WARs. We also observed that LTP in slices from control WARs rats had slower onsets than in control Wistar rats. These findings indicate that homeostatic processes seem to act on the Schaffer-CA1 synapses, preventing their potentiation probably to compensate for hyperactive afferent circuits in response to HISS, preventing seizures and not affecting spatial navigation. In audiogenic seizure-prone WARs these mechanisms seem to be disrupted and could account for their seizure susceptibility.

Disclosures: **A.O. Cunha:** None. **J.A.C. Oliveira:** None. **S.S. Almeida:** None. **N. Garcia-Cairasco:** None. **R.M.X. Leao:** None.

Poster

049. Epilepsy: Synapses and Ion Channels

Location: Halls A-C

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Program#/Poster#: 49.03/P4

Topic: C.07. Epilepsy

Support: UC President's Postdoctoral Fellowship

Title: Impulse response of local field potential to spikes relates activity in distant regions of the human brain

Authors: *B. A. LOPOUR[1], I. FRIED[2], D. L. RINGACH[3];
[1]Biomed. Engin., Univ. of California, Irvine, CA; [2]Neurosurg., [3]Neurobio. and Psychology, Univ. of California, Los Angeles, CA

Abstract: Simultaneous analysis of single neuron spiking and the local field potential (LFP) can be used to study both local processing of groups of neurons as well as network-level properties of neuronal circuits (Einevoll 2013). The simplest example of this is the spike-triggered average of the LFP, which has been applied to thalamocortical connectivity (Swadlow 2002, Jin 2011) and the cortex-basal ganglia network (Goldberg 2004). An alternative approach is to calculate the impulse response, which accounts for spatiotemporal correlations within spike trains that may make the spike-triggered LFP difficult to interpret (Einevoll 2013). The impulse response has been used to analyze the accuracy of LFP estimates based on multi-unit activity (MUA) (Rasch 2009) and functional connectivity within cortical circuits (Nauhaus 2012). In both studies, data were recorded from a grid of electrodes implanted in the cortex of nonhuman

primates. Here we apply the impulse response technique to MUA and LFP measurements recorded simultaneously from multiple regions of the human brain, and we show that it can be used to uncover correlated activity between distant brain regions and even across hemispheres. Recordings were taken from bilateral depth electrodes implanted in patients undergoing clinical evaluation for epilepsy surgery. Electrode locations were based solely on clinical criteria and typically included both temporal and frontal lobe regions. Eight to twelve electrodes were implanted in each patient, and the data were recorded from eight microwires extending from the tip of each one. Using simultaneous measurements from each brain region, we calculated the impulse response of the LFP to MUA between all possible pairs of recordings. We applied this technique to both the raw LFP and the amplitude spectrum of the LFP at frequencies between 1-100Hz, as a means of testing whether or not the LFP activity was phase-locked to the timing of the MUA. In each patient, we found significant LFP impulse responses to MUA recorded in a different brain region. Further, analysis of the amplitude revealed that in some cases the LFP had frequency components that were correlated (but not phase-locked) to multi-unit activity. As expected, these significant impulse responses were not observed between all pairs of brain regions. However, preliminary data suggest that strong correlations between LFP and MUA may be related to each patient's individual epileptic network, rather than functioning as a general measure of anatomical connectivity. This may have implications for the identification of the epileptogenic zone and the surgical treatment of epilepsy.

Disclosures: **B.A. Lopour:** None. **I. Fried:** None. **D.L. Ringach:** None.

Poster

049. Epilepsy: Synapses and Ion Channels

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Topic: C.07. Epilepsy

Support: Swedish Research Council

Epitarget

Title: Collagen VI modulates synaptic transmission in the hippocampus

Authors: ***T. RAMOS-MORENO**[1], A. CIFRA[2], L. NIKITIDOU[3], M. AHL[2], S. H. CHRISTIANSEN[4], C. R. GØTZSCHE[4], D. P. WOLDBYE[4], M. KOKAIA[2];
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Abstract: Epilepsy is a common neurological disorder that affects 1% of the general population worldwide. Drug resistance to available therapies occurs in the 30-40% of the cases and no biomarkers for the disease progression are currently available. Therefore, a deeper understanding of the cellular and molecular mechanisms underlying epilepsy and epileptogenesis is required. We have recently found that the mRNA and protein levels of collagen VI (CVI), a component of the extracellular matrix, are increased in the rat hippocampus 4 weeks after status epilepticus, a well-characterized model of epilepsy. In the present study, we explored basic mechanisms of CVI action in the naïve hippocampus as a prerequisite for elucidating its implications in epilepsy. Hippocampal slices from naïve rats were incubated in CVI for 2 or 6 h. Field recordings were performed in the CA1 region of the hippocampus, in order to determine how CVI could affect basal synaptic transmission and paired-pulse facilitation, a form of short-term plasticity dependent on presynaptic mechanisms. Our results suggest that CVI has diverse effects depending on for how long the tissue is exposed to its increased levels. In the short term (2h), CVI may decrease inhibitory synaptic transmission and increase excitatory synaptic transmission, effects that may worsen the condition of hyperexcitability generally associated with epilepsy. Conversely, in the long term (6h), CVI may reduce transmitter release probability in excitatory synapses, thus counteracting hyperexcitability. Next step will be to explore CVI effects on the single-cell level in order to better clarify its mechanisms of action.

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Poster

049. Epilepsy: Synapses and Ion Channels

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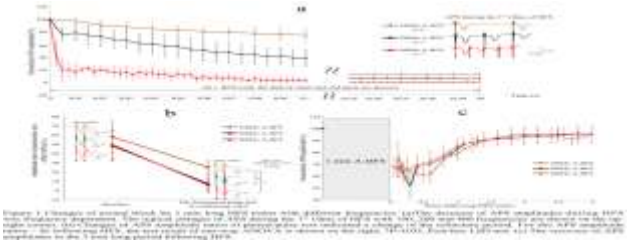
Topic: C.07. Epilepsy

Support: Major State Basic Research Development Program of China (No. 2011CB504400)

Title: Frequency dependent axonal block by high frequency stimulation in hippocampal CA1 region of rats

Authors: *Y. YU, Z. FENG, J. CAO, N. HU, Z. WANG;
Zhejiang Univ., Zhe Jiang, China

Abstract: Recent studies have shown that the axonal block may be one of the important underlying mechanisms for the therapeutic effects of high frequency stimulation (HFS) on brain disorders. In addition, refractory periods could be the mechanism of axonal block. But if HFS with higher frequencies over 100 Hz could generate axonal block more rapidly is unknown yet. To address this issue, we applied 1 minute long pulse trains to alveus fiber tract of hippocampus in anesthetized rats, and recorded antidromically-evoked population spike (APS) in the pyramidal layer of CA1 region. The APS amplitude of the first 100 ms and the last 50 ms periods of the HFS trains were measured to determine the generation speed of axonal block and the suppression degree of axons respectively. Paired-pulse test stimuli with an inter-pulse interval of 2.5 ms were delivered to evoke two APSs (APS1 and APS2) before (i.e., baseline) and ~8 s following HFS. The results showed that even within the very first 100 ms period, the APS amplitude decreased to 4.27±4.43% by the 400 Hz HFS, much more suppression than 38.69±14.16% of 200 Hz HFS and 68.97±11.66% of 100 Hz HFS. The suppression differences among the three frequencies remained till the end of HFS with all of the APS amplitude decreasing to less than 11.86±4.56% (Figure 1a). Paired-pulse tests applied at ~8 s following HFS showed that the ratio of APS2/APS1 decreased to 6.03±6.89% by the 400 Hz HFS, indicating a change of the refractory period by HFS. In addition, the decrease of the ratios were also frequency dependent (Figure 1b). The evoked APS by the test pulses following HFS trains showed that the entire recovery periods were not significantly different among the HFS with different frequencies (Figure 1c). The present results indicated that the axonal blocks caused by HFS were frequency-dependent up to 400 Hz, a much higher frequency than the common used ~130 Hz frequency in clinical application. It is potential that the HFS could modulate the excitability or activity of the neurons in the downstream and upstream areas by affecting axonal fibers in a much larger frequency ranges.



Disclosures: **Y. Yu:** 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.; Zhejiang University. **Z. Feng:** None. **J. Cao:** None. **N. Hu:** None. **Z. Wang:** None.

Poster

049. Epilepsy: Synapses and Ion Channels

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 49.06/P7

Topic: C.07. Epilepsy

Title: Approaches to understanding human ion channel genetic variation and disease - an example with a KCNT1 variant and infantile epilepsy disorder

Authors: K. M. PADILLA[1], B. M. ANTONIO[1], S. C. SANTOS[1], Z. LIN[1], J. W. THEILE[1], M. L. CHAPMAN[1], S. PETROU[2], D. B. GOLDSTEIN[3], *D. S. KRAFTE[4];
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Abstract: Understanding the functional significance of ion channel genetic variation is essential to realize the potential of precision medicine and targeted therapies for human disease. We have approached this challenge by analyzing a *de novo* variant in the KCNT1 protein, P924L, which is linked to epilepsy of infancy with migrating focal seizures (EIMFS). A recent publication demonstrated gain-of-function for P924L when expressed in Xenopus oocytes (Milligan et al 2014 Annals of Neurology doi: 10.1002/ana.24128). We designed a strategy to analyze KCNT1 P924L gene expression in recombinant cell systems and in iPSC-derived cortical neuron engineering using TALENs. The latter approach was unsuccessful in 2 iPSC-derived cell lines. In the 1st case, KCNT1 wt mRNA expression was absent in differentiated neurons while in the 2nd case 28 nucleofections and analysis of >1300 clones failed to yield appropriate targeting. Heterologous recombinant expression in HEK-293 cells was more successful leading to robust expression of wt KCNT1 with 77% of clones expressing functional KCNT1 channels. KCNT1 P924L was more difficult to constitutively express with only 12% of clones resulting in stable expression. This suggests possible negative selection when expressing P924L which was unexpected. To investigate the underlying cause we created inducible expression systems for wt and P924L KCNT1 and analyzed function and cell viability from 0-24 hrs post-induction using a ^{86}Rb flux assay. Compared to wt, P924L demonstrated lower signals at 24 hrs than at earlier time points. Cell viability, however, was similar between wt and P924L indicating the flux differences are likely not due to a toxic effect of the variant. Blocking KCNT1 channels by using an inhibitor during the experiment for all steps except the assessment of function restored the KCNT1 P924L ^{86}Rb signal to levels similar to wt. This result is consistent with published data demonstrating dysregulation of P924L leading to gain-of-function. Our results were confirmed by

electrophysiological analysis with current densities of 211 ± 33 pA/pF for P924L vs 9 ± 3 pA/pF for wt (both at 0 mV, following establishment of whole cell recording mode). In summary, our results with inducible expression in mammalian cells are consistent with those reported for gain-of-function with P924L in the Xenopus oocyte system. Chronic upregulation of KCNT1 P924L may lead to indirect effects on cell properties and viability which could explain why constitutive expression systems are poorly suited for detailed variant analysis for this ion channel.

Disclosures: **K.M. Padilla:** None. **B.M. Antonio:** None. **S.C. Santos:** None. **Z. Lin:** None. **J.W. Theile:** None. **M.L. Chapman:** None. **S. Petrou:** None. **D.B. Goldstein:** None. **D.S. Krafte:** None.

Poster

049. Epilepsy: Synapses and Ion Channels

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 49.07/P8

Topic: C.07. Epilepsy

Support: CURE Taking Flight Award

Title: IGF-1 promotes epileptogenesis after injury through activation of Akt-mTOR, but not MAPK(ERK), signaling

Authors: Y. SONG[1], K. J. STALEY[2], *Y. BERDICHEVSKY[1];
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Abstract: Insulin-Like Growth Factor-1 (IGF-1) levels are elevated in brain tissues following head injury, and there is a significant elevation in IGF-1 receptor (IGF-1R) phosphorylation after traumatic brain injury (TBI). Phosphorylation of IGF-1R may lead to activation of MAPK (ERK) and PI3K-Akt signaling cascades, and IGF-1 was found to promote growth cone motility and neurite outgrowth. IGF-1 is neuroprotective in animal models of hypoxic-ischemic and traumatic brain injury; however, its role in neural circuit re-organization and signaling pathway activation suggests that it may contribute to epileptogenesis after injury. We investigated the role of IGF-1 in epileptogenesis in organotypic hippocampal culture model of posttraumatic epilepsy. We applied IGF-1 during the latent period between trauma and appearance of spontaneous seizures, and measured lactate production (biomarker of increased seizure activity), lactate dehydrogenase (LDH, biomarker of increased cell death), electrical activity, and phosphorylation of Akt, MAPK, and S6 (marker of mTOR activation) proteins. We then applied IGF-1 together with

inhibitors of Akt and MAPK and measured lactate and LDH levels. We found that IGF-1 application increases lactate and LDH release and phosphorylation of Akt and S6, but not MAPK. We also found that chronic IGF-1 application during the latent period causes an increase in electrographic ictal activity. Proepileptogenic effect of IGF-1 was significantly reduced by inhibitors of Akt, but not MAPK. We conclude that IGF-1 is neuroprotective immediately after injury, but proepileptogenic during the latent period and beyond through activation of Akt-mTOR signaling.

Disclosures: **Y. Song:** None. **K.J. Staley:** None. **Y. Berdichevsky:** None.

Poster

049. Epilepsy: Synapses and Ion Channels

Location: Halls A-C

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Program#/Poster#: 49.08/P9

Topic: C.07. Epilepsy

Support: NIH NINDS NS29709

NIH NINDS NS076916

Title: Increased risk of hypoxic depolarization of the brainstem autonomic circuit in a mouse SUDEP model

Authors: *I. AIBA, J. L. NOEBELS;
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Abstract: A subpopulation of epilepsy patients experience a sudden reduction in cardiorespiratory function and die in the absence of resuscitation. The phenomenon is termed sudden unexpected death in epilepsy (SUDEP) and is the leading cause of epilepsy-related mortality, however the mechanisms are not well understood. Here we investigated a potential contribution of hypoxic depolarization of brainstem autonomic nuclei in an established Kv1.1 knockout (KO) mouse model. Susceptibility of the juvenile Kv1.1 KO mouse brainstem to hypoxic insult was first evaluated in an acute transverse slices. Acute exposure to an artificial CSF solution lacking oxygen and glucose (0% O2, 0 mM glucose) resulted in the generation of a slow propagating wave of depolarization (i.e. Anoxic Depolarization: AD) detected by intrinsic optical signal and extracellular DC potential shift. The spontaneous wave of depolarization invaded the dorsal vagal nucleus (X), the hypoglossal nucleus (XII) and the nucleus of the tractus

solitarius (NTS). Kv1.1 KO brainstem slices showed a higher susceptibility to AD as determined by a significantly faster AD onset than wild-type (WT). Increasing the glucose concentration of the hypoxic solution (i.e. 0% O2, 5 mM glucose) did not affect AD onset in Kv1.1 KO slices whereas AD was largely delayed or prevented in WT slices. These *in vitro* data indicate that Kv1.1 KO mice have an increased risk of deleterious brainstem depolarization under severe hypoxia. We next examined whether such brainstem depolarization could occur during a seizure *in vivo*. Recurrent seizures were evoked by topical application of 4AP (100 mM, ~10 μl) in anesthetized mice and DC field potentials in the dorsal medulla were recorded with a glass microelectrode (~0.3 mm depth, 0.5 mm rostral to the obex). Cortical activity, heart and breathing rates were simultaneously monitored. WT mice showed relatively stable cardiorespiratory tone during seizures and none died. In contrast, the Kv1.1 KO mice showed severe apnea following seizure onset, and 83% (5/6) of mice died following a period of bradycardia and intermittent asystoles. In the dying Kv1.1 KO mice, a slow negative DC potential shift was detected in the brainstem coincident with the onset of the terminal cardiac arrhythmias. Together these data demonstrate that terminal autonomic instability is accompanied by deleterious brainstem depolarization in this model. The lower brainstem AD threshold may increase the risk of death during otherwise recoverable central autonomic dysregulation, and suggests a novel and distinct therapeutic target for the prevention of seizure-related morality.
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Disclosures: **I. Aiba:** None. **J.L. Noebels:** None.

Poster

049. Epilepsy: Synapses and Ion Channels

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Topic: C.07. Epilepsy

Support: NRF Korea 2009-0081468

NRF Korea 2013-070465

NRF Korea 2013R1A2A2A01014688

NRF Korea 2013M3C7A1044016

Title: Melatonin inhibits voltage sensitive Calcium channel mediated neurotransmitter release

Authors: J. KWON[1], T.-Y. CHOI[1], E. S. DURRANCE[1], S.-H. JO[2], K.-T. KIM[3], *S.-Y. CHOI[1]; [1]Sch. of Dent., Seoul Natl. Univ., Seoul, Korea, Republic of; [2]Kangwon Natl. Univ. Sch. of Med., Chuncheon, Korea, Republic of; [3]Pohang Univ. of Sci. and Technol., Pohang, Korea, Republic of

Abstract: Melatonin is involved in various neuronal functions such as circadian rhythmicity and thermoregulation. Melatonin has a wide range of pharmacologically effective concentration levels from the nanomolar to millimolar levels. Recently, the antiepileptic effect of high dose melatonin has been the focus of clinical studies; however, its detailed mechanism especially in relation to neurotransmitter release and synaptic transmission remains unclear. We studied the effect of melatonin at high concentrations on the neurotransmitter release by monitoring norepinephrine release in PC12 cells, and excitatory postsynaptic potential in rat hippocampal slices. Melatonin inhibits the 70 mM K+-induced Ca2+ increase at millimolar levels without effect on bradykinin-triggered Ca2+ increase in PC12 cells. Melatonin (1 mM) did not affect A2A adenosine receptor-evoked cAMP production, and classical melatonin receptor antagonists did not reverse the melatonin-induced inhibitory effect, suggesting G-protein coupled receptor independency. Melatonin inhibits the 70 mM K+-induced norepinephrine release at a similar effective concentration range in PC12 cells. We confirmed that melatonin (100 μM) inhibits excitatory synaptic transmission of the hippocampal Schaffer collateral pathway with the decrease in basal synaptic transmission and the increase in paired pulse ratio. These results show that melatonin inhibits neurotransmitter release through the blocking of voltage-sensitive Ca2+ channels and suggest a possible mechanism for the antiepileptic effect of melatonin.

Disclosures: **J. Kwon:** None. **T. Choi:** None. **S. Choi:** None. **E.S. Durrance:** None. **S. Jo:** None. **K. Kim:** None.

Poster

049. Epilepsy: Synapses and Ion Channels

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 49.10/P11

Topic: C.07. Epilepsy

Support: 1RO1NSO78331-01

1R01DC013048-01

Title: Ultrastructural changes in astrocyte enwrapping and excitatory synapses in the hippocampal CA1 stratum radiatum following kainic acid induced status epilepticus

Authors: C. CLARKSON[1], M. GIBBONS[2], J. A. WHITE[3], K. S. WILCOX[2], *M. E. RUBIO[1]; [1]Otolaryngology, Univ. of Pittsburgh Med. Sch., Pittsburgh, PA; [2]Dept. of Pharmacol. & Toxicology, [3]Dept. of Bioengineering, Univ. of Utah, Salt Lake City, UT

Abstract: Reactive astrogliosis is a common pathology in both animal models and human epilepsy. However, very little is known about ultrastructural changes that occur in reactive astrocytes at the tripartite synapse. We used electron microscopy to determine the ultrastructural response of astrocytes and synapses to kainic acid (KA) induced-status epilepticus (SE) in the hippocampal stratum radiatum. Rats were implanted with cortical EEG electrodes, administered KA to induce SE, and chronically recorded with video-EEG for 7 days to insure that animals were still within the latent period. Animals were sacrificed and perfused at day 7 following KA. Using 3D reconstructions from images after serial ultrathin sections we determined the percentage of astrocyte enwrapping around CA1 excitatory synapses and investigated ultrastructural changes at presynaptic endings and postsynaptic spines that might indicate changes in normal excitatory transmission. Consistent with previous findings, we qualitatively observed that KA treatment leads to neuronal cell death and consequently to a decrease in spines within CA1 stratum radiatum. Additional analysis of reactive astrocytes revealed thicker processes and increased microtubule content. In addition, presynaptic endings making more than one synaptic contact on different spines were more frequently observed in the KA treatment than in non-treated tissue. Quantitative analysis of astrocyte enwrapping showed an increase in astrocyte plasma membrane apposition around presynaptic endings, but not around CA1 spines. We also observed that KA treatment tended to increase the number of docked synaptic vesicles as well as the length and thickness of the postsynaptic densities. Taken together, our results show ultrastructural remodeling of astrocytes and CA1 excitatory synapses in response to KA induced SE and also imply that understanding excitatory transmission during epileptogenesis should take into account distinct and specific changes in astrocyte ensheathment of synapses.

Disclosures: **C. Clarkson:** None. **M. Gibbons:** None. **J.A. White:** None. **K.S. Wilcox:** None. **M.E. Rubio:** None.

Poster

049. Epilepsy: Synapses and Ion Channels

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 49.11/P12

Topic: C.07. Epilepsy

Support: Jiangsu Specially-appointed professor grant

Title: Overactive Slack channel (KCNT1) mutants lead to epilepsy by two different mechanisms

Authors: *Z. ZHANG, Q.-Y. TANG, F.-F. ZHANG, J. XU;
Physiol. and Biophysics, Xuzhou Med. Col., Xuzhou, China

Abstract: Qiong-Yao Tang, Fei-Fei Zhang, Jie Xu, Zhe Zhang [1]Jiangsu Province Key Laboratory of Anesthesiology, Xuzhou Medical College, Xuzhou, Jiangsu Province, CHINA [2]Jiangsu Province Key Laboratory of Anesthesia and Analgesia Application Technology, Xuzhou Medical College, Xuzhou, Jiangsu Province, CHINA, 221004 So far, 12 sodium-activated potassium channel mutants (KCNT1, Slack) had been linked in severe early-onset epilepsy by whole genome sequence. However, how the biophysical property of Slack channel had been changed by these mutants and the underlying mechanism remain unknown. In this abstract, open probability and sodium affinity of Slack channel mutants that had been found in family epilepsy patients were examined by using inside-out patch recording in Xenopus oocytes expressing system. By measuring the Kd value sodium sensitivity of each mutant and Po, we found some mutants on the RCK2 domain increased the channel open probability without changing sodium binding affinity, but one mutant (Y775H) on the RCK2 domain and 2 mutants on the RCK1 domain do increased channel acitvity and decreased the Kd value of sodium sensitivity.In addition, molecular simulation results suggested that mutant Y775H increased sodium binding affinity by changing the local structure of sodium binding site on the RCK2 domain. Thus, we set up a two-step activation kinetic model to explain the gating mechanism change underlying overactivity of these mutant channels. Also, our data supported that Slack channel possesses two sodium binding sites located on RCK1 and RCK2 domain respectively.

Disclosures: **Z. Zhang:** None. **Q. Tang:** None. **F. Zhang:** None. **J. Xu:** None.

Poster

049. Epilepsy: Synapses and Ion Channels

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 49.12/Q1

Topic: C.07. Epilepsy

Title: Neurons in the amygdala of enriched rats with acquired reflex epilepsy are activated by a sound-induced seizure

Authors: E. M. O'NEIL, M. G. RODEN, S. E. NYSTROM, H. A. HOLDEN, H. K. ANDERSON, *M. C. ZRULL;
Psychol, Appalachian State Univ., BOONE, NC

Abstract: In reflex epilepsy, an environmental stimulus evokes seizure activity. This disorder is modeled in rat with the neural network for sound-induced or audiogenic seizures (AGS) and its physiology researched primarily in genetically epilepsy prone rats (GEPRs). For AGS in GEPRs, abnormal neural activity begins in the midbrain and spreads to the forebrain via the lateral amygdala (LA). Long-Evans rats are resistant but can be primed for AGS, and it is assumed that the same neural network supporting AGS in GEPRs mediates seizures in rats with acquired reflex epilepsy. Environmental enrichment (EE) can promote change in injured neural systems, which in turn, may promote recovery from behavioral deficits. In this study, seizure-induced neural activity in the LA and basolateral amygdala (BLA) of rats with acquired AGS and experiencing EE or not was examined using the activity marker c-fos. On postnatal day (pnd) 18, Long-Evans rats (n=8) were primed for AGS (120 dB, 10 kHz tone pips, 8 min). Age-matched rats (n=8) served as controls. The primed rats were tested for AGS susceptibility (120 dB noise) 12 times between pnd 32 and 62. During this period, half of the AGS and control rats experienced 18 EE sessions in a cage with ramps, objects, and familiar and novel conspecifics. On pnd 62, after AGS induction for primed rats, all rats spent 60 min in the quiet dark to allow expression of c-fos protein. After sacrifice, brain tissue was Nissl stained or processed for c-fos immunohistochemistry. Sections with LA and BLA were drawn from Nissl material using a projection microscope, and c-fos positive neurons activated by AGS were plotted on to the drawings of LA and BLA using digital microscopy. Counts of plotted neurons were made, AGS brains were compared to controls, and AGS brains with EE were compared to EE controls. LA and BLA from rats with AGS history showed 617% and 139% more c-fos positive neurons, respectively, than LA and BLA from control brains ($p<.05$). Interestingly, LA and BLA from rats with AGS and EE history showed 1,361% and 277% more c-fos positive neurons, respectively, than in EE control brains ($p<.05$). While enhanced amygdala activation in enriched AGS rats is interesting, both groups with acquired AGS susceptibility did exhibit more neural activation in the LA than BLA suggesting the pathway for forebrain recruitment during seizures is similar to that in rats with inherited epilepsy (i.e., GEPRs). Neural pathways underlying epilepsy in other species differ; however, the results indicate that networks for acquired and inherited epilepsy in rat function similarly implying knowledge of one form of epilepsy may advance knowledge and treatment of other forms of seizure disorders.

Disclosures: **E.M. O'Neil:** None. **M.G. Roden:** None. **S.E. Nystrom:** None. **H.A. Holden:** None. **M.C. Zrull:** None. **H.K. Anderson:** None.

Poster

049. Epilepsy: Synapses and Ion Channels

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 49.13/Q2

Topic: C.07. Epilepsy

Title: L-arginine restores synaptic plasticity in the visual cortex of an animal model of developmental epilepsy and dyslexia

Authors: *H. MENDONÇA[1,2], P. CAMPELLO-COSTA[1], K. M. JACOBS[3];
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Abstract: The rat freeze lesion (FL) model of microgyria replicates pathological features of human 4-layered polymicrogyria associated with epilepsy and dylslexia, including focal loss of deep layers and delayed onset to epileptiform activity. Synaptic plasticity is the main process related to learning and memory, and it has been shown to be modulated after epileptiform activity and to play a role in seizure generation. The expression of layer V synaptic plasticity relies on nitric oxide (NO) and group 1 glutamate metabotropic receptor (mGluRI) signaling. Since we have previously shown that mGluRI activation reverts hiperexcitability, reducing LTP and restoring LTD in microgyral cotex (Mendonça et al - SfN 2013), we aimed to investigate the effects of NO production modulation on visual cortex layer V synaptic plasticity of normal and freeze-lesioned rats. FL were made in the visual cortex of Sprague-Dawley rats on postnatal day (P) 1. From P9 on, animals received daily intraperitoneal injections of l-name (30 mg/kg), l-arginine (750 mg/kg) or vehicle to modulate NO levels. Slices containing the lesion or equivalent regions of control rats ranging from P13 to P17 were used for field potentials recordings. A bipolar stimulating electrode was placed within layer V and the recording one was placed within the same layer at a lateral distance of 0.5 mm, and 0.5 mm from the microgyrus. Synaptic plasticity induction experiments were performed as follows: 50% of maximal amplitude intensity stimuli were applied every 15 s during 10 min of baseline recordings. Long term potentiation (LTP) induction consisted of 5 theta burst trains (1 min intervals), with each train = 4 pulses at 100 Hz, repeated 10 times at 5 Hz. Long term depression (LTD) induction consisted of 900 pulses at 1 Hz. Post test recordings consisted of the same conditions of baseline recording but lasting 30 minutes. Our results show that normal cortex synaptic plasticity depends on NO synthesis, since L-name treatment inhibits LTP and reduces LTD. On the other hand, l-arginine bath application on control slices promoted LTP, whereas l-arginine treatment of control animals occluded high frequency stimulation-induced LTP. In addition, l-arginine treatment promoted

LTD reduction on control cortices. Freeze-lesion reduces the amplitude of LTP and blocks LTD induction in the visual cortex. Although l-name treatment didn't modulated synaptic plasticity in microgyral cortex, l-arginine restored both LTP and LTD, suggesting l-arginine treatment as a possibility to reestablish cognitive, learning and memory functions that are impaired in microgyral animals.

Disclosures: **H. Mendonça:** None. **K.M. Jacobs:** None. **P. Campello-Costa:** None.

Poster

049. Epilepsy: Synapses and Ion Channels

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Program#/Poster#: 49.14/Q3

Topic: C.07. Epilepsy

Support: Inserm

Axa Research Fund

Title: Control of GABAergic synapses by A2A receptors during development

Authors: ***S. ZAPPETTINI**[1,2], S. GILISSEN[3], F. GOMEZ CASTRO[3], M. ESCLAPEZ[1,2], S. LÉVI[3], C. BERNARD[1,2];
[1]Inst. De Neurosciences Des Systèmesinserm UMR11, Marseille, France; [2]Aix Marseille Université, INS, Marseille, France; [3]INSERM UMR-839, Inst. du Fer à Moulin, Paris, France

Abstract: Consumption of drugs of abuse during pregnancy negatively impact upon brain development. The most widely used psychoactive drug in the world, including during pregnancy, is caffeine. At non-toxic doses, caffeine antagonizes adenosine receptors. In the adult brain, Adenosine (ADO), a degradation product of ATP, controls neurotransmitter release mainly through inhibitory A1 and facilitatory A2A receptors. These receptors appear also important during early phases of development. A2A receptors are present on some types of migrating GABA neurons, and control their migration speed. Here, we present evidence that A2A receptors also control GABAergic synapses. We used hippocampal slices from male GIN mice pups and we recorded CA3 pyramidal cells in voltage clamp whole cell mode in the presence of TTX (1 μM). Our results show that blockade of A2A receptors with SCH58261 (100 nM), a specific antagonist of A2A receptors led to an irreversible decrease of miniature IPSC frequency and amplitude from post natal day 4 to post natal day 12. This decrease was time-dependent and

maximal at postnatal day 6 (-50%). The loss of mIPSCs was clathrin-dependent, suggesting that blockade of A2A receptors triggered the internalization of GABAa receptors. A2A receptors are positively coupled to adenylyl cyclase and protein kinase A signaling cascade. Using a pharmacological approach, we demonstrated A2AR stabilize inhibitory synapses through activation of the AC/cAMP/PKA cascade. We thus hypothesize that a continuous A2A receptors activation stabilizes postsynaptic GABAa receptors during development.

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Poster

049. Epilepsy: Synapses and Ion Channels

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Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 49.15/Q4

Topic: C.07. Epilepsy

Support: NIH NS048336

NIH NS065187

Title: The reduction of Nav1.6 current suppresses high potassium induced seizure-like activity in hippocampal slices

Authors: *B. S. TANAKA[1], A. L. GOLDIN[2];
[1]Microbiology & Mol. Genet., UC Irvine, Irvine, CA; [2]Microbiology & Mol. Genetics; Anat. & Neurobio., Univ. of California, Irvine, Irvine, CA

Abstract: *SCN1A* mutations are the main cause of genetic epilepsy with febrile seizures plus (GEFS+), a disorder characterized by myoclonic seizures, ataxia, absences and atonic seizures. Mutations in the mouse *Scn8a* gene, which encodes the $Na_v1.6$ voltage-gated sodium channel, have shown reduced seizure susceptibility in chemically induced seizure models. To determine the general neuronal population in the hippocampus, excitatory or inhibitory neurons, that contributes to reduced seizure susceptibility, we induced synchronous seizure-like burst activity using elevated extracellular potassium in hippocampal slices from heterozygous $Scn8a^{flox/+}$ mice with restricted deletions of *Scn8a*. Restricted deletions of *Scn8a* in discrete cell types were generated by crossing *Scn8a*-floxed mice with either EMX-Cre (excitatory) or DLX-Cre (inhibitory) mice. Increased latency to the initial epileptiform burst discharge and reduced

frequency of burst discharges was observed in Scn8a$^{flox/+}$;EMX-Cre mice but not Scn8a$^{flox/+}$;DLX-Cre mice. Reduced action potential firing and depolarized spike threshold in CA1 pyramidal neurons suggests that decrease $Na_v1.6$ activity in hippocampal excitatory neurons contribute to reduced susceptibility to seizure-like burst activity by shifting the spike threshold in the depolarized direction. To determine whether the susceptibility to high potassium induced seizure-like activity could be reduced pharmacologically, we selectively targeted $Na_v1.6$ channels with an isoform-specific toxin to block $Na_v1.6$ activity in hippocampal slices. The tetrodotoxin (TTX) metabolite 4,9 anhydrous TTX (ah-TTX) shows potency and a 5-fold selectivity for $Na_v1.6$ channels over $Na_v1.1$ and $Na_v1.2$ channels when expressed in Xenopus oocytes. At a concentration of 100 nM TTX the population spike amplitude in CA3 layer was reduced ~40-50%, however 100 nM ah-TTX did not affect population spike amplitude. Although ah-TTX is not as potent as previously reported, seizure-like activity in hippocampal slices was suppressed by 100 nM ah-TTX, and did not affect sodium channel dependent activity in the DG-CA3 pathway. These results suggest that mutant $Na_v1.6$ channels in hippocampal excitatory neurons are responsible for modulating susceptibility of seizure-like burst activity to prevent hyperexcitability, and that selective targeting of $Na_v1.6$ channels may suppress neuronal hyperexcitability in the hippocampus.

Disclosures: **B.S. Tanaka:** None. **A.L. Goldin:** None.

Poster

049. Epilepsy: Synapses and Ion Channels

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Topic: C.07. Epilepsy

Support: NIH grant HD 067517

Title: Human gain-of-function Slack (KCNT1) potassium channel mutations increase positive cooperativity between individual Slack channels

Authors: ***J. KRONENGOLD**[1], G. E. KIM[2], I. QURAISHI[3], H. C. MARTIN[4], G. BARCIA[5], J. C. TAYLOR[4], L. COLLEAUX[6], R. NABBOUT[5], L. K. KACZMAREK[1];
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Abstract: The Slack (Slo 2.2, KCNT1) gene encodes a large unitary conductance potassium channel that is widely expressed in neurons. Slack channels are weakly voltage dependent but are activated by elevations in intracellular sodium which contribute to a slow afterhyperpolarization (sAHP) and regulation of spike frequency. We expressed the rat Slack-B channel in Xenopus oocytes and found different patterns of channel activity in single channel patch clamp recordings. Specifically, we observe a low channel open probability pattern when few (1-3) channels are present in the patch and a high channel open probability when 4-5 or more channels are present. On-cell recordings show coupled gating of the fully open state while all points amplitude histograms show a marked deviation from a binomial distribution that is expected for independently gating channels. The different patterns of Slack channel activity likely reflect an allosteric interaction that only occurs when the channels assemble into clusters, as is the case *in vivo*. Recent studies have identified de novo KCNT1 point mutations in Epilepsy of infancy with migrating focal seizures (EIMFS), Autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE), and Ohtahara syndrome. In addition to exhibiting early onset epileptic seizures there are devastating effects on development and intellectual function. The mutations are found in RCK1 and RCK2 of the large 900aa cytoplasmic C-terminal domain of Slack with one located in the S5 pore forming domain of the channel. Voltage clamp studies of the ADNFLE (G288S, R398Q, R474H, Y796H, M896I, R928C) EIFMS (G288S, R398Q, R428Q,A934T) and Ohtahara syndrome (A966T) mutations showed 3-12 fold increases in macroscopic currents with no change in levels of channel expression. Single channel studies show no significant changes in unitary conductance except for G288S which shows a 50% decrease. We have found that positive cooperative gating is substantially enhanced in the human gain-of-function Slack point mutations. Mutations show a significant increase in NPo in multichannel patches relative to the wild type multichannel patches Our findings show that the increases in current result not from an intrinsic increase in the open probability of individual channels but from strongly enhanced cooperativity in the opening of individual channel subunits in a cluster. To our knowledge this is the first report of channelopathies that results from altered channel-channel interactions.

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Poster

049. Epilepsy: Synapses and Ion Channels

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Title: Activation of M1 muscarinic acetylcholine receptors on parvalbumin-positive neurons contributes to pilocarpine-induced seizures

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Abstract: A common animal model of temporal lobe epilepsy employs the proconvulsant muscarinic acetylcholine receptor (mAChR) agonist pilocarpine, yet the mechanisms underlying pilocarpine-induced epileptogenesis are poorly understood. Global M1 mAChRs knockout mice are resistant to pilocarpine-induced seizures (Hamilton et al. 1997). M1 mAChRs are present on both glutamatergic and GABAergic microcircuits, yet it is unclear how pilocarpine activation of M1 mAChRs disrupts excitation/inhibition balance. Gamma oscillations, generated by fast-spiking parvalbumin-positive (PV) inhibitory interneurons, are associated with seizure onset and are susceptible to depolarization block during epileptiform discharges *in vitro* (Cammarota et al. 2013). Here, we test the hypothesis that M1 mAChR activation of PV interneurons contributes to pilocarpine-induced seizures. PV+ cells were visualized in acute mouse hippocampal slices by stereotaxic injection of a floxed YFP adeno-associated virus (AAV) or by crossing PV-CRE mice with the RosaYFP reporter line (PV-Rosa mice). Action potential (AP) frequency was monitored in loose patch mode. In PV-Rosa mice under loose patch conditions, AP frequency increased in CA1 PV cells after bath application of 200 μM pilocarpine (from 2.3 ± 1.4 Hz to 13.7 ± 4.5 Hz; p=0.044, n=5). In the presence of ionotropic glutamate and GABAA receptor antagonists (DNQX, APV, and gabazine), pilocarpine increased AP frequency (from 3.3 ± 2.8 Hz to 13.8 ± 5.6 Hz, p=0.003, n=9). . In the continued presence of pilocarpine, a subset (6/9) of PV cells progressed to depolarization block (DB). Pilocarpine-induced increases in AP frequency and induction of DB were prevented in mice in which M1 mAChRs were genetically deleted from PV cells (PV-m1KO mice, from 2.1 ± 1.2 Hz to 3.7 ± 1.9 Hz, p=0.68, n=6), consistent with

a direct postsynaptic effect on M1 mAChRs. In whole-cell mode, pilocarpine depolarized PV cells in PV-Rosa mice (from -55.0 ± 1.5 MV to -49.8 ± 1.4 mV, p=0.0015, n=11) but not PV-m1KO mice (from -51.7 ± 2.2 to -49.4 ± 1.4, p=0.21, n=6). In behavioral experiments, WT or PV-m1KO mice were injected with atropine (1 mg/kg i.p.) 30 minutes prior to pilocarpine injection (155 mg/kg i.p.). Pilocarpine-induced seizures were scored blind on a modified Racine scale (0-6). Seizures were less severe in PV-M1KO (3.9 ± 0.5, n=10) than WT (5.4 ± 0.3, n=10, p=0.027) mice at 30 min, which remained significant at 45 min (p=0.004). Therefore, we propose that pilocarpine activation of M1 mAChRs can lead to overexcitation of a subset of PV cells, weakening PV-mediated GABAergic inhibition, dysregulating excitation/inhibition balance, and increasing the susceptibility to seizures.

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Poster

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Topic: C.07. Epilepsy

Title: SCN2A mutations and epilepsy: Is there a meaning in the subthreshold range?

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Abstract: Different *SCN2A* mutations have been shown to cause epilepsy-syndromes, such as benign familial neonatal-infantile seizures (BFNIS), a mild epileptic syndrome presenting within the first days to months of life and subsequent spontaneous resolution of seizures. Functional studies of several $Na_V1.2$ mutations revealed a clear gain of the channel's function including changes in activation and inactivation, accelerated recovery from fast inactivation and an increase in the persistent Na^+ current. For one mutation found in a BFNIS familiy originating from Madagascar we found an increase in the subthreshold Na^+ current elicited by using a single action potential as a voltage stimulus. Here, we analyzed already described as well as novel identified *SCN2A* mutations concerning their subtreshold behaviour to assess the functional consequences of this phenomenon. The studied mutations were engineered using site-directed mutagenesis into adult pCDNA3.1-$hNa_V1.2$. Both wildtype (WT) $hNa_V1.2$ and mutant $hNa_V1.2$ respectively, were coexpressed with pGFP-IRES-beta1 and pCD8-IRES-beta2 in tsA201 cells.

Currents of transfected cells were recorded after 24-48h using the whole-cell patch clamp technique. Single action potentials as well as different voltage ramps were used to study subthreshold sodium currents. Although most of the studied *SCN2A* mutations showed a clear gain of function effect with an increase in window and persistent current, for one of the studied mutations we could not detect any changes in the channel's function compared to the WT. Using a single action potential as physiological voltage stimulus as well as different voltage ramps mimicking the AP stimulus we found an increase in the subthreshold Na^+ currents in some of the studied mutant channels. Even the mutation which did not show any differences in channel gating exhibited an increase in the elicited subthreshold sodium current. We suggest that the observed increase in the subthreshold sodium current found in different *SCN2A* mutations might be adequate to provoke hyperexcitability of neurons and may explain the occurrence of seizures.

Disclosures: **U.B. Hedrich:** None. **S. Müller:** None. **S. Lauxmann:** None. **H. Lerche:** None.

Poster

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Topic: C.07. Epilepsy

Support: PRTLI Cycle 5 2010

Title: Hippocampal synaptic alterations in the nonsense-mediated decay pathway after status epilepticus

Authors: *C. MOONEY, E. M. JIMENEZ-MATEOS, D. C. HENSHALL;
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Abstract: Epilepsy is a common neurological disorder characterised by recurring seizures due to abnormal excessive neuronal firing. The most common form of epilepsy in adults is temporal lobe epilepsy (TLE) in which seizures originate in the temporal lobe. Despite advances in TLE treatment, ~30% of TLE patients remain drug refractory, hence new therapeutic approaches targeting the underlying cause of epileptogenesis and not the symptoms of the disorder are required. The Nonsense-mediated decay (NMD) pathway recognises aberrant mRNA transcripts containing premature termination codons which if allowed to be translated would result in synthesis of truncated proteins with a deleterious effect on the cell. Malfunctioning of this system has been implicated in genetic epilepsies, affecting mainly GABA receptor protein levels

to increase neuronal excitability. However, it is now apparent that NMD also regulates the levels of normal physiological mRNA transcripts, particularly at synapses where it may provide a means of tightly controlled translational regulation. Since modifications in synaptic transmission and GABAergic malfunctions are strongly implicated in epilepsy and little is currently known on its role in sporadic epilepsy, we examined the mechanism of NMD in an experimental model of kainate-induced status epilepticus (SE). Immunoblot analysis determined that protein levels of NMD components Upf1 and Upf3b were bi-directionally altered in a time-dependent manner after SE. Moreover, the transcript levels of the NMD targeted mRNA PSD-95Δexon18 showed an inverse expression pattern to these proteins. Next, immunohistochemical analysis of brain slices showed changes in Upf1 in the dendrites of the hippocampus after SE. To investigate further into changes in the levels of synaptically-located Upf1 we prepared hippocampal synaptoneurosomes from control and SE mice using density centrifugation separation and this also confirmed altered synaptic Upf1 after SE. Subsequently, mass spectrometry analysis of immunoprecipitated Upf1 from synaptoneurosomes revealed known and novel Upf1 interacting proteins after SE, suggesting a change in NMD activity after SE in the hippocampus. Together, these experiments indicate synaptic alterations in NMD processing of mRNA after SE. Further investigation into the role of NMD in SE and its target transcripts could identify new therapeutics in the treatment of epilepsy.

Disclosures: **C. Mooney:** None. **E.M. Jimenez-Mateos:** None. **D.C. Henshall:** None.

Poster

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Topic: C.07. Epilepsy

Support: NIH Grant R01 NS033300

Title: De novo mutations identified in the β subunit of GABAA receptor in patients with infantile spasms (IS) and Lennox-Gastaut syndrome (LGS) by the Epi4K consortium alter GABAA receptor function

Authors: ***V. C. SATPUTE**[1], K. M. VERDIER[2], C. C. HERNANDEZ[2], N. HU[2], T. EPI4K INVESTIGATORS[3], R. L. MACDONALD[2];
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Abstract: Exome sequencing of genomes of patients with epilepsy has revealed multiple rare nonsynonymous single nucleotide polymorphisms (nsSNPs). However, determining the precise role of these nsSNPs remains a daunting task, especially for epilepsy with considerable genetic and phenotypic variability. Furthermore, the functional consequences of the amino acid change produced by the nsSNP remains crucial question which needs to be answered empirically since the software predictions are still far from perfect. Thus, we examined the effects of novel de novo mutations identified in GABAA receptor β subunit genes (GABRB1 and GABRB3) by the Epi4K consortium (Allen A et al., 2013). The mutations were present in children suffering from the severe epileptic encephalopathies - IS and LGS, with unaffected parents. Three patients with LGS had unique single nucleotide missense mutations in GABRB3 and two IS patients had unique mutations GABRB1 and GABRB3. GABAA receptors are the primary mediators of synaptic inhibition in the brain and mutations in the α1, β3 and γ2 subunits have been associated with human epilepsy. The β3 subunits are critical, especially during neurodevelopment, and have been implicated in childhood absence epilepsy, while the β1 subunit has not been associated with epilepsy yet. To understand the functional consequences of the β subunit mutations on GABAA receptor function, we used α1β1/3γ2 subunit transfected HEK293T cells, which do not express endogenous GABAA receptors. Using whole cell voltage clamp recordings we found that the all the β subunit mutations altered GABA evoked currents. We used flow cytometry to evaluate whether reduced surface GABAA subunits could contribute to altered currents, and found no reduction in the surface expression levels of the β1/3 or α, γ subunits. In conclusion the β subunit mutations identified in children suffering from epileptic encephalopathies have different effects on the function of GABAA receptors and could result in reduced GABAergic inhibition in patients. Further investigation is needed to understand the mechanisms by which the GABAA receptor β subunit mutations might contribute to epileptogenesis.

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Poster

049. Epilepsy: Synapses and Ion Channels

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Topic: C.07. Epilepsy

Title: The antiepileptic drug levetiracetam suppresses non-convulsive seizure activity and reduces ischemic brain damage in rats subjected to permanent middle cerebral artery occlusion

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Abstract: The antiepileptic drug Levetiracetam (Lev) has neuroprotective properties in experimental stroke, cerebral hemorrhage and neurotrauma. In these conditions, non-convulsive seizures (NCSs) propagate from the core of the focal lesion into perilesional tissue, enlarging the damaged area and promoting epileptogenesis. Here, we explore whether Lev neuroprotective effect is accompanied by changes in NCS generation or propagation. In particular, we performed continuous EEG recordings before and after the permanent occlusion of the middle cerebral artery (pMCAO) in rats that received Lev (100 mg/kg) or its vehicle immediately before surgery. Both in Lev-treated and in control rats, EEG activity was suppressed after pMCAO. In control but not in Lev-treated rats, EEG activity reappeared approximately 30-45 min after pMCAO. It initially consisted in single spikes and, then, evolved into spike-and-wave and polyspike-and-wave discharges. In Lev-treated rats, only rare spike events were observed and the EEG power was significantly smaller than in controls. Approximately 24 hours after pMCAO, EEG activity increased in Lev-treated rats because of the appearance of polyspike events whose power was, however, significantly smaller than in controls. In rats sacrificed 24 hours after pMCAO, the ischemic lesion was approximately 50% smaller in Lev-treated than in control rats. A similar neuroprotection was observed in rats sacrificed 72 hours after pMCAO. In conclusion, in rats subjected to pMCAO, a single Lev injection suppresses NCS occurrence for at least 24 hours. This electrophysiological effect could explain the long lasting reduction of ischemic brain damage caused by this drug.

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Poster

049. Epilepsy: Synapses and Ion Channels

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Topic: C.07. Epilepsy

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Title: Epileptic Scn1a mutation reduces action potential firing of pyramidal cells

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Abstract: Most of the sodium channel mutations that cause epilepsy are in the SCN1A gene. We previously showed that Scn1a-D1866Y, a mutation associated with Genetic Epilepsy with Febrile Seizure Plus (GEFS+), results in gain-of-function of sodium currents in dissociated parvalbumin-expressing (PV) interneurons. Although Scn1a is more abundant in PV interneurons, a mutation in Scn1a can also alter the biophysiological properties of the excitatory neurons. Based on the expression pattern of Scn1a and the gain-of-function in PV interneurons by Scn1a-D1866Y, it is likely that Scn1a-D1866Y would alter the firing properties of excitatory neurons. We predict that this effect will not be present in juvenile mice but in older mice after Scn1a expression reaches adult levels. The overall goal of this study is to determine the mechanism by which the Scn1a-D1866Y leads to the development of seizures. To examine changes in the excitatory neurons resulting from the D1866Y mutation, we recorded action potential firing of pyramidal neurons in the CA1 region of hippocampal slices. We also measured seizure-like activity in the slices by local field recordings. Current clamp recordings from pyramidal neurons in CA1 of P16-P17 mice demonstrated no differences in action potential firing with or without Scn1a-D1866Y. However pyramidal neurons in CA1 from Scn1aD1866Y/+ P21-P24 mice demonstrated reduced action potential firing compared to wild-type littermates. Despite the reduced action potential firing in pyramidal neurons, the hippocampal network demonstrated a lower latency and higher frequency of seizure-like events. Our results indicate that an increase in excitatory neuron firing is not necessary for the induction of seizure-like events in hippocampal slices. The D1866Y mutation reduces excitability of pyramidal neurons, which could be a direct effect on the intrinsic properties of those neurons or a result of increased interneuron inhibition. Since decreased pyramidal cell activity is unlikely to directly cause seizure activity, it is likely that the D1866Y mutation has a significant effect on interneurons that in conjunction with the effect on pyramidal neurons results in an overall increase in synchrony and seizure susceptibility. These results along with the previous analysis of other mutations in the Scn1a demonstrates that different mutations in Scn1a can result in a similar epileptic phenotype by altering different aspects of sodium channel function and neuronal excitability. These differences have important implications, because interventions that alleviate seizures resulting from some mutations may exacerbate seizure activity resulting from other mutations.

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Poster

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ANR PRTS, Vibration Project

Title: Measurement of neuronal population excitability based on selective activation of interneurons

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Abstract: Regarding the major importance of GABAergic interneuron function in brain functions, a dysregulation of this cell types naturally lead to many pathological states, including fragile X syndrome, autism spectrum, Down syndrome, schizophrenia, affective disorders, and epilepsy. In the epileptic brain, a functional impairment of interneurons contributes to both interictal, ictal activity. When the inhibitory barrage collapse the focal ictal discharges propagates further across the cortex. The impairment of GABAergic inhibition could result from a decrease of GABAA receptor density, a shift of the chloride reversal potential toward more positive value, a decrease of excitability, a decrease of synapse number or even cell death. Therefore a simple method to selectively probe the functionality of interneuron synapses within a healthy or a pathologic brain tissue would be of a major importance. We have defined a new method of direct bipolar supra-threshold low-frequency neurostimulation, optimally designed, by using combined translational approaches including computational model, patch clamp and field recording in brain slices, field recordings *in vivo*. We show that direct bipolar stimulation is able to selectively activate GABAergic IPSP on pyramidal cells if the stimulation intensity is tuned just above the excitability threshold. Optimum stimulation frequency is around 8Hz. This protocol leads to the selective emergence of the responses of interneurons in healthy and pathological brain tissue. Our first results show that this protocol allows for a quantification of

the degree of excitability of a neuro-glial network. Results and predictions of lumped network computational model, were validated with patch-clamp recordings made on pyramidal cells and putative interneurons in organotypic hippocampal slices, *in vivo* in a mouse model of epilepsy (kaïnate injected in the hippocampus). Moreover, a clinical study is being conducted in this direction in patients undergoing pre-surgical evaluation at the University Hospital of Rennes. Such neurostimulation revealed distinct electrophysiological responses between healthy and epileptogenic brain areas in humans, but could also be useful in other diseases.

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Poster

049. Epilepsy: Synapses and Ion Channels

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Title: Propylparaben reduces the neuronal hippocampal damage as results of pilocarpine-induced status epilepticus in rat: Correlation with the release of GABA and glutamate

Authors: *C. E. SANTANA, SR[1], G. VALLE-DORADO[1], S. OROZCO-SUAREZ[2], L. BRUNO-BLANCH[3], A. TALEVI[3], L. ROCHA-ARRIETA[1];
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Abstract: Previous studies indicate that the administration of propylparaben (PPB) (an antimicrobial agent with low toxicity and widely used), reduces the seizure activity induced by pentylenetetrazole and inhibits voltage-dependent sodium channels in isolated adult rat cardiomyocytes. The aim the present study was to determine if PPB is able to induce neuroprotective effects in hippocampus when applied 3 h after status epilepticus (SE). Male Wistar rats previously implanted with a bipolar electrode coupled to a guide cannula into the

right ventral hippocampus, were subjected to microdialysis experiments during which SE was induce by pilocarpine administration (300 mg/kg, i.p., SE group, n=6). Diazepam (DZP 2.5 mg/kg, i.m.) was applied 2 h after the SE establishment. The SE+PPB group (n=6) was manipulated as described above, except that animals received PPB (178 mg/kg, i.p.) administration 1 h after DZP. One day after the SE, the animals were sacrificed and the brain used to evaluate the site of electrode/cannula implantation (Nissl staining) and neuronal damage (FLUORO-JADE B). SE and SE+PPB groups showed similar basal levels of GABA and glutamate of (0.34±0.06 μM and 1.20±0.29 μM, respectively). The SE was established at 43.2±2.5 min after pilocarpine injection. Increased GABA and glutamate release (135% and 140% respectively) was evident the perfusate collected 47 min after SE establishment, a situation that was progressive until the end of the experiment (6.5 h after pilocarpine). DZP administration reduced the behavioral and electrographic epileptic activity, but not GABA and glutamate release (270% and 330% respectively; $p<0.05$, when compared with basal conditions). In contrast, the SE+PPB group showed a progressive decrease of the GABA and glutamate levels after PPB administration (155% and 177%, $p<0.001$; respectively and at the end of the experiment), an effect associated with a significant decrease in the epileptiform electrographic activity. When compared with SE group, SE+PPB group demonstrated decreased neuronal damage in dorsal hippocampus as follows: dentate gyrus (81%, $p<0.05$), CA3 (86%, $p<0.001$) and CA1 (98%, $p<0.001$). PPB reduces the GABA and glutamate release, effect associated with decreased of neuronal damage.

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Poster

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Title: Inhibition of Nav1.6 channels reduces hyper-excitability of Subiculum neurons in epilepsy

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Abstract: Temporal lobe epilepsy (TLE) is a common form of adult epilepsy. Seizures in patients with TLE can be difficult to suppress resulting in approximately 30% of patients being classed as therapy resistant. Subiculum neurons serve as the primary output center for the hippocampus, receiving information directly from the CA1 and projecting out to cortex and subcortical regions. Subiculum neurons are spared in TLE and become hyper-excitable. Since sodium (Na) channels play a critical role in the generation of action potentials, alterations in Na channel function would facilitate this hyper-excitability. The tetrodotoxin metabolite, 4,9-anhydro tetrodotoxin (4,9-ah-TTX), has been shown to have greater potency against the Nav1.6 isoform than other neuronally expressed Na channel isoforms. At a concentration of 100 nM, 4,9-ah-TTX inhibited Nav1.6 channel currents by approximately 50%, while having no effect on Nav1.2 channel currents. In this study, we determined the effects of 4,9-ah-TTX on membrane excitability and resurgent (INaR) Na currents in both control and TLE Subiculum neurons. Action potentials (AP) were evoked by a series of depolarizing current injection steps under whole cell current clamp conditions. Subiculum neurons from TLE brain slice preparations had higher AP firing frequencies than control. At a current injection step of 470 pA, frequencies were increased from 36.6 ± 0.4 Hz (n = 16) in control to 44.4 ± 0.4 Hz (n = 7) in TLE. Bath application 4,9-ah-TTX (100 nM) significantly reduced firing frequencies in both control (23.3 ± 0.4 Hz ; n = 7) and TLE neurons (22.8 ± 0.3 Hz; n = 7). Stimulation of the pyramidal cell layer of the CA1 region evoked a burst of APs in control neurons. In TLE neurons, stimulation evoked a greater number of APs and longer durations of depolarizing events. Application of 100 nM 4,9-ah-TTX significantly reduced the number of APs evoked in both control (from 2.1 ± 0.1 APs ; n = 7 to 1.5 ± 0.3 APs ; n = 7) and TLE neurons (from 3.3 ± 0.1 APs ; n = 6 to 1.4 ± 0.3 APs; n = 6). Duration of the depolarizing events were also reduced. INaR currents were also recorded from Subiculum neurons and were increased in TLE. Control amplitudes were increased from -783.4 ± 16.1 pA (n = 5) to -1209 ± 149.7 pA (n = 5) in TLE. Bath application of 100 nM 4,9-ah-TTX reduced the amplitude of INaR currents by 45.7 % in control and 46.8 % in TLE neurons. These data suggest that the Nav1.6 isoform plays an important role in controlling neuronal membrane excitability of Subiculum neurons. Alterations in the activity of Nav1.6 could be important in facilitating neuronal hyperexcitability in TLE.

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Poster

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Program#/Poster#: 49.26/R6

Topic: C.07. Epilepsy

Support: Research grants from National Natural Science Foundation of China No. 81371427

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The Scientific Research Foundation for the Returned Overseas Chinese Scholars, State Education Ministry No. 43

Title: Newly born neurons in dentate gyrus are functionally integrated into neuronal circuitry in mice exhibiting chronic temporal lobe epilepsy

Authors: *J. LIU, M. HU, X. CHEN, J. ZHANG, P. YANG, Y. LIU;
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Abstract: Neurogenesis in subgranular zone-granule cell layer (SGZ-GCL) of dentate gyrus (DG) declines substantially in chronic temporal lobe epilepsy (TLE). Some studies have linked this decline to altered basal proliferation rate or neuronal differentiation. However, whether or not altered recruitment of newly-born cells into local circuits also attributes to the decline is still unexplored. To address this issue, we visualized the newly-born cells of DG that extended axons to CA3 area or integrated into memory circuits in mice exhibiting chronic TLE. At chronic stage of pilocarpine induced TLE mouse model (2 months after status epilepticus), newly-born cells in DG were labeled by intraperitoneal injection of proliferation marker 5-bromo-2'-deoxyuridine (BrdU) in chronically epileptic and age-matched mice. Retrograde tracer cholera toxin B subunit (CTB) was injected iontophoretically into CA3b area of hippocampus and the newly-born cells extending axons to CA3 area along the mossy fiber tract were then quantified via dual immunofluorescence of BrdU and CTB. c-Fos, an immediate early gene product, is highly correlated with neuronal firing and can be induced by the recall of spatial memory. To identify the newly-born cells incorporating into dentate gyrus circuits supporting spatial memory, mice were trained in Morris Water Maze either 4 or 6 weeks after BrdU labeling and spatial memory was tested 10 days after the training. Mice were then killed 90 min after the test, and c-Fos and BrdU expression were quantified using immunofluorescence approaches. 23.7±4.1% or 33.1±3.9% of total BrdU+ cells in SGZ-GCL were co-localized with CTB in chronically epileptic hippocampus at 6 weeks or 8 weeks post-BrdU labeling, while the percentage is 25.7±3.0% ($P>0.05$) or 35.1±4.4% ($P>0.05$) respectively in the age-matched intact hippocampus. In the spatial memory test, chronically epileptic animals exhibited spatial cognitive deficits when compared to the age-matched normal animals. After spatial memory test, 20.9±4.4% or 30.1±5.0% of total BrdU+ cells in SGZ-GCL were c-fos positive in chronically epileptic hippocampus at 6 or 8 weeks post-BrdU labeling, in comparison to 21.3±3.9% ($P>0.05$) or 31.1±4.0% ($P>0.05$) such newly-born cells expressing c-fos in the age-matched intact

hippocampus. These results demonstrate that the newly-born cells in chronically epileptic hippocampus are still able to recruit into the existing hippocampal circuitry supporting spatial memory, and largely diminished hippocampal neurogenesis in chronic TLE is not associated with decreased integration ability of surviving newly-born cells.

Disclosures: **J. Liu:** None. **M. Hu:** None. **X. Chen:** None. **J. Zhang:** None. **P. Yang:** None. **Y. Liu:** None.

Poster

049. Epilepsy: Synapses and Ion Channels

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Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 49.27/R7

Topic: C.07. Epilepsy

Support: NIH CounterACT Grant 5U01NS058162-07

Defense Threat Reduction Agency – Joint Science and Technology Office, Medical S & T Division Grants CBM.NEURO,01.10.US.18 and CBM.NEURO.01.10.US.15

Title: Reduced GABAergic inhibition in the basolateral amygdala after soman exposure: pathophysiological mechanisms underlying increased anxiety

Authors: ***J. P. APLAND**[1], E. M. PRAGER[2], V. I. PIDOPLICHKO[2], V. ARONIADOU-ANDERJASKA[2], M. F. M. BRAGA[2];
[1]Neurobehavioral Toxicol, USAMRICD, Gunpowder, MD; [2]Anatomy, Physiology, and Genet., Uniformed Services Univ. of the Hlth. Sci., Bethesda, MD

Abstract: The recent sarin attack in Syria killed 1,429 people, including 426 children, and left countless more to deal with the health consequences of the exposure. Prior to the Syrian chemical warfare agent assault, the nerve agent attacks in Japan left many victims suffering from neuropsychiatric illnesses, particularly anxiety disorders, more than a decade later. Uncovering the neuro-pathophysiologic mechanisms underlying the development of anxiety after nerve agent exposure is necessary for successful treatment. Anxiety is associated with hyperexcitability of the basolateral amygdala (BLA). The present study sought to determine the nature of the nerve agent-induced alterations in the BLA, which could explain the development of anxiety. At 24 hours and 14 days after exposure of rats to soman, at a dose that induced prolonged status epilepticus, spontaneous inhibitory postsynaptic currents (sIPSCs) in the BLA were reduced,

along with reduction in the frequency but not amplitude of miniature IPSCs. In addition, activation of $_7$-nicotinic acetylcholine receptors, cholinergic receptors that participate in the regulation of BLA excitability and are involved in anxiety, increased spontaneous excitatory postsynaptic currents (sEPSCs) in both the soman-exposed rats and the controls, but was less effective in increasing sIPSCs in the soman-exposed rats. Despite the loss of both interneurons and principal cells after soman-induced status epilepticus, the frequency of sEPSCs was increased in the soman-exposed rats. Impaired function and cholinergic modulation of GABAergic inhibition in the BLA may underlie anxiety disorders that develop after nerve agent exposure. The experimental protocol was approved by the Animal Care and Use Committee at the United States Army Medical Research Institute of Chemical Defense and all procedures were conducted in accordance with the principles stated in the Guide for the Care and Use of Laboratory Animals and the Animal Welfare Act of 1966 (P.L. 89-544), as amended. The views expressed in this abstract are those of the authors and do not reflect the official policy of the Department of Army, Department of Defense, or the U.S. Government.

Disclosures: **J.P. Apland:** None. **E.M. Prager:** None. **V.I. Pidoplichko:** None. **V. Aroniadou-Anderjaska:** None. **M.F.M. Braga:** None.

Poster

049. Epilepsy: Synapses and Ion Channels

Location: Halls A-C

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Topic: C.07. Epilepsy

Support: NIH Grant NS031718-20A1

NIH Grant DP1 OD003347

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CIHR Grant MFE115462

Title: Early-life seizures induced synaptic metaplasticity in hippocampal CA1 neurons

Authors: *H. SUN, F. E. JENSEN;
Dept. of Neurol., Univ. of Pennsylvania, Philadelphia, PA

Abstract: Neonatal seizures, often refractory to current drugs, can result in chronic epilepsy and long-term cognitive deficits. Using an established neonatal hypoxic seizure (HS) model, we have previously shown that LTP is occluded in hippocampal slices removed from rats at 48-72h following early life seizures, in part due to seizure-induced decreases in silent synapses. However, our results also show an unexpected occlusion of NMDA-dependent LTD. The threshold for both LTP and LTD can be modulated by priming neuronal network activities, displaying "metaplasticity" - the plasticity of long-term synaptic plasticity. Given HS-induced changes in both LTP and LTD, we hypothesize that HS-induced neuronal hyperactivity evokes a rightward shift of metaplastic modulation of LTP and LTD. HS were induced by graded global hypoxia at postnatal day 10. *Ex vivo* hippocampal slices were prepared from rats at 48-72h following HS as well as littermate controls. Extracellular field potential (fEPSP) recordings were obtained from the apical dendritic layer (stratum radiatum) of the CA1 region by stimulating the Schaffer collateral pathway. fEPSP slopes were measured before and after a train of stimulation at 0.1, 1Hz (1200pulses), 5, 10, 20 Hz (900pulses) and two trains of 100Hz (100pulses, 20s interval). Consistent with previous findings, the potentiation of fEPSP slopes in response to 100Hz stimulation was significantly attenuated in post-HS slices (potentiated by 10.6 ± 7.2%, n=6, $p<0.05$) compared to those from P12-13 controls (potentiated by 58.3 ± 18.7%, n=5). The depression of fEPSP slopes in response to 1Hz stimulation was also significantly attenuated in post-HS slices (reduced by 19.4 ± 7.6%, n=7, $p<0.05$) compared to those from P12-13 controls (reduced by 59.2 ± 11.9%, n=5). In addition, HS induced a rightward shift of the LTP/LTD induction threshold (5Hz: -25.8±5.6%, n=5; 10Hz: -11.2±6.6%, n=6; 20Hz: -12.7±7.6%, n=7) compared to littermate controls (5Hz: -20.5±7.2%, n=4; 10Hz: 3.6±9.2%, n=4; 20Hz: 20.6±5.8%, n=5). Overall, our data reveal a metaplastic modulation of LTP/LTD induction in hippocampus following HS, which indicates a homeostatic modulation of synaptic plasticity following early life seizures. In addition, these data suggest that the early life seizure-induced LTP/LTD impairment might be modifiable and treatable not only by pharmacologic agents but activity or stimulation such as TMS.

Disclosures: **H. Sun:** None. **F.E. Jensen:** None.

Poster

049. Epilepsy: Synapses and Ion Channels

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Topic: C.07. Epilepsy

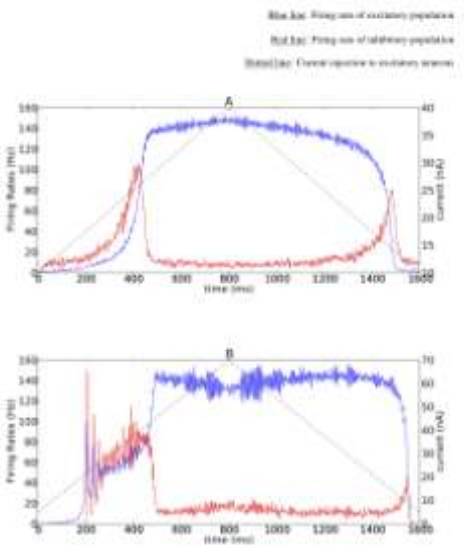
Support: NSF Grant DMS 0847749

Title: A computational model of the influence of depolarization block on initiation of seizure-like activity

Authors: C. KIM[1], *D. Q. NYKAMP[2];
[1]Sch. of Mathematics, Univ. of Minnesota, MInneapolis, MN; [2]Sch. Mathematics, Univ. Minnesota, Minneapolis, MN

Abstract: Under pathological conditions the interaction of excitatory and inhibitory neurons can become unbalanced, leading to seizure-like activity in the network. The interplay between excitatory and inhibitory neurons has been studied in rat hippocampal CA1, where spontaneous seizure-like activity was produced by blocking potassium ion channels and decreasing magnesium. The main finding from the study is that the excitatory neurons exhibit runaway excitation as the inhibitory neurons enter long-lasting depolarization block. In this study, we employ a modified Wilson-Cowan model to examine the dynamics of an EI network under similar pathological conditions. To model depolarization block, we modified the inhibitory population's activation function so that large input silences the population. Phase plane analysis shows that the network can be in as many as three different states, rest state, active state, and seizure state, where the inhibitory population enters depolarization block in the seizure state. The network can be bistable, where the rest and seizure state coexist, or if the external input to the inhibitory population is reduced, the network can become tristable, where all three state coexist for the same parameters. In addition, convergent connectivity structure from excitatory neurons onto inhibitory neurons is found to facilitate the transition to seizure state by reducing the strength of inhibitory drive when the network is near seizure state. The predictions of Wilson-Cowan model is tested with a network of Morris-Lecar (ML) neurons. We find that both bistable (Figure A) and tristable (Figure B) states are present in the ML network. Hysteresis is observed at the transitions between different states, providing evidence that the emergence of three states is a network phenomenon. It is also observed that the network enters seizure state at a low

current input when the convergent connectivity structure is



promoted.

Disclosures: **C. Kim:** None. **D.Q. Nykamp:** None.

Poster

050. Neurological Disease: Cellular Mechanisms and Oxidative Stress

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 50.01/R10

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant NS073670

Title: GMF- deficient mice are protected against MPTP-induced dopaminergic neurotoxicity

Authors: S. ZAHEER[1], M. M. KHAN[1], R. THANGAVEL[1], J. NEHMAN[1], D. KEMPURAJ[1], *A. ZAHEER[1,2];
[1]Neurol., Univ. of Iowa, Iowa City, IA; [2]VAHCS, Iowa City, IA

Abstract: Parkinson's disease (PD), one of the most prevalent and progressive neurodegenerative diseases, is characterized by the loss of dopaminergic neurons in the substantia nigra pars compacta and associated with movement disorders. Recent studies have indicated that glia maturation factor (GMF), a proinflammatory protein, is involved in the pathogenesis of neurodegenerative diseases. However, the exact association and underlying mechanism of GMF in the loss of dopaminergic (DA) neurons in PD is not clear. In the present study, we demonstrate that deficiency of GMF suppresses dopaminergic (DA) neuron loss, glial activation, and expression of proinflammatory mediators in the substantia nigra pars compacta (SN) and striatum (STR) of 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP) treated mice. Dopaminergic neuron numbers in the SN and fiber densities in the STR were significantly reduced in wild type (Wt) mice after MPTP treatment compared with GMF-deficient (GMF-KO) mice. We also compared the motor abnormalities caused by MPTP treatment in Wt and GMF-KO mice as measured by Rota rod and grip strength test. Results show that the deficits in motor coordination and decrease in dopamine and its metabolite content were protected significantly in GMF-KO mice after MPTP treatment when compared with control Wt mice under identical experimental conditions. Current data provide the first evidence that deficiency of GMF rescues the DA neuron loss following MPTP administration in mice. Thus depletion of endogenous GMF represents an effective and selective strategy to slow down the MPTP-induced neurodegeneration.

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Poster

050. Neurological Disease: Cellular Mechanisms and Oxidative Stress

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 50.02/R11

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: Grants-in-Aid for Scientific Research (S) 23228004

Grants-in-Aid for Scientific Research (C) 24580147

Enryo memorial foundation for promoting science to one of the author (K.M.)

Title: Comparison of glutamate- and hydrogen peroxide-induced HT22 cell death ~ Role of rapid and persistent Erk1/2 activation by oxidative stress ~

Authors: *K. SATO, Y. YAMANAKA, Y. ASAKURA, T. NEDACHI;
Toyo Univ., Gunma, Japan

Abstract: During ischemia reperfusion, excessive glutamate release induces neuronal death in central nervous system. This glutamate toxicity is mediated by at least two distinct mechanisms, induction of oxidative stress caused by the inhibition of cystine/glutamate transporter and glutamate receptor mediated excitotoxicity. HT22 mouse hippocampus neural cells have been often used as a model for studying glutamate induced oxidative stress, because this cell line does not have ionotropic glutamate receptor. It has been demonstrated that the persistent Erk1/2 activation, regulated by oxidative inhibition of Erk phosphatase, plays a central role on this glutamate-induced cell death. However, in the present study, we found hydrogen peroxide (H2O2), the other oxidative stressor, indeed promoted cell death but were independent of persistent Erk1/2 activation in HT22 cells. We herein report the detail comparative analysis how H2O2 and glutamate controls HT22 cell death. Initially, we treated HT22 cells with various concentration of H2O2 or glutamate and examined several cellular responses. We found similar changes of cell fate in response to H2O2 and glutamate (i.e. cell death and the release of growth factors was similarly regulated by H2O2 and glutamate). On the contrary, different time-dependent patterns of ERK1/2 activation were observed. HT22 cells were treated with various concentration of H2O2 and glutamate for different time and Erk1/2 phosphorylation was monitored. Low concentration of H2O2 or glutamate treatments similarly induced acute and transient Erk1/2 phosphorylation within 1 hour; on the other hand, when we elevated glutamate concentration, persistent Erk1/2 phosphorylation was observed 16-24 hours after glutamate treatment. This persistent phosphorylation of Erk1/2 was not stimulated by high concentration of H2O2. Moreover, U0126 inhibited glutamate induced cell death, but was not effective against H2O2 induced cell death. Overall, these results strongly suggest that the persistent ERK1/2 activation in response to high concentration of glutamate plays an important role on glutamate induced cell death; however, H2O2-dependent cell death was controlled by the other mechanisms. mGluRs-dependent signals may be involved in this process since antagonists for mGluRs attenuated U0126-dependent cell protective effect. In conclusion, two oxidative stressors, H2O2 and glutamate, similarly induced HT22 cell death; however, underlying mechanisms were completely distinct. Differential Erk1/2 regulation, glutamate-induced persistent Erk1/2 activation could be a strong candidate to consist the distinct mechanism.

Disclosures: **K. Sato:** None. **Y. Asakura:** None. **T. Nedachi:** None. **Y. Yamanaka:** None.

Poster

050. Neurological Disease: Cellular Mechanisms and Oxidative Stress

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 50.03/R12

Topic: C.08. Ischemia

Support: Grant-in Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan

Title: Involvement of endoplasmic reticulum stress and calcium dyshomeostasis in zinc-induced neurotoxicity

Authors: *M. KAWAHARA[1], D. MIZUNO[2];
[2]Bio-analytical Chem., [1]Musashino Univ., Nishitokyo, Japan

Abstract: Zinc (Zn) is abundantly present in brain and secreted from synaptic vesicles into synaptic clefts along with glutamate during neuronal excitation. It is widely accepted that the secreted Zn ion plays crucial roles in the information processing processes and the formation of memory. However, excess Zn secreted after transient global ischemia is toxic and plays central roles in the pathogenesis of vascular type dementia (VD). We have investigated the molecular mechanism of Zn-induced neurotoxicity using GT1-7 cells (immortalized hypothalamic neurons), which are more susceptible to Zn compared to other neuronal cells. The exposure to Zn induced the elevation of intracellular calcium levels. The calcium channel blockers attenuated Zn-induced neuronal death. Our analysis using the DNA microarray revealed that expressions of several genes, such as metal-related genes (metallothionein, zinc transporter 1), endoplasmic reticulum (ER)-stress related genes (GADD34, GADD45, p8), calcium-related genes (activity-related cytoskeleton protein (Arc)) were affected after Zn exposure. We have demonstrated that substances which attenuate Zn-induced neurotoxicity such as carnosine (β-alanyl histidine) or histidine inhibited the expressions of GADD34, p8, and Arc, meanwhile they did not influence the expression of metal-related genes. Furthermore, dantrolene, which prevents calcium release from ER, attenuated Zn-induced neurotoxicity, and thapsigargin, which increases intracellular calcium by blocking influx into ER, enhanced Zn-induced neurotoxicity. Furthermore, the addition of calcium into culture media prevented from Zn-induced neurotoxicity. Our results suggest that ER stress and calcium dyshomeostasis may underlie the molecular mechanism of Zn-induced neurotoxicity, and finally the pathogenesis of VD. Furthermore, we propose carnosine as a possible candidate for drugs of VD considering its beneficial characteristics and published a patent of carnosine (patent No. JP5382633). We also discuss the molecular mechanism of carnosine in preventing VD and other neurodegenerative diseases.

Disclosures: **M. Kawahara:** None. **D. Mizuno:** None.

Poster

050. Neurological Disease: Cellular Mechanisms and Oxidative Stress

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 50.04/S1

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant RO1AG037320

Title: The effects of partial PPARγ agonist on neuroinflammation- induced memory deficits

Authors: *H. D'ANGELO, A. CROCKETT, S. HOPP, S. ROYER, L. ADZOVIC, G. WENK; Neurosci., The Ohio State Univ., Columbus, OH

Abstract: There are numerous neurodegenerative diseases characterized by cognitive impairment, neuroinflammation and metabolic dysfunction. Recent studies have linked neuroinflammation as a possible contributor to the exacerbation of glucose metabolic dysfunction and the related cognitive impairments. Diabetes mellitus is perhaps the most widely known metabolic disease in the periphery. The epidemiological, clinical, and pathological links between type two diabetes mellitus (T2DM) and Alzheimer's disease suggest similar or overlapping mechanisms in neuroinflammation- induced glucose dysregulation. A class of drugs that have been used to treat T2DM, thiazolidinediones, are peroxisome proliferator- activated receptor gamma (PPARγ) agonists and have severe adverse side effects. Our aim was to find a partial agonist of this receptor in order to reduce the severity of the side effects while maintaining a therapeutic effect. We investigated the drug honokiol, a partial PPARγ agonist, on a rat model of neuroinflammation. Three-month-old rats were infused intraventricularly with lipopolysaccharide or artificial CSF and then injected with honokiol or vehicle. After four weeks of the drug treatment, the rats' spatial memory was assessed using the Morris water maze task. Preliminary results indicate promising anti- inflammatory effects of honokiol by the attenuation of the LPS- induced behavioral deficit.

Disclosures: H. D'Angelo: None. **A. Crockett:** None. **S. Hopp:** None. **S. Royer:** None. **L. Adzovic:** None. **G. Wenk:** None.

Poster

050. Neurological Disease: Cellular Mechanisms and Oxidative Stress

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 50.05/S2

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: CIHR-VIH-105439

Title: The phosphodiesterase inhibitor ibudilast attenuates glial cell reactivity, production of proinflammatory cytokines and neuronal loss in experimental glaucoma

Authors: *J. CUEVA VARGAS, N. BELFORTE, A. DI POLO;
Dept. of Neurosci., Univ. of Montreal Hosp. Res. Ctr., Montreal, QC, Canada

Abstract: Purpose: Ibudilast, a phosphodiesterase inhibitor with glial cell modulation, anti-inflammatory and vasodilator properties, has been used for over 20 years for the treatment of asthma and stroke. Here, we characterized the role of ibudilast on the response of glia and retinal ganglion cells (RGCs) to ocular hypertension (OHT) glaucoma. **Methods:** OHT was induced by injection of hypertonic saline solution into an episcleral vein in Brown Norway rats. Ibudilast or vehicle was administered by intravitreal injection. Animals were euthanized at 3 weeks after OHT induction, and the retinas and optic nerves were collected for histological and biochemical analyses. Tumor necrosis factor α, (TNFα), interleukin 1 (IL-1β), interleukin 6 (IL-6), macrophage migration inhibitory factor (MIF), glial fibrillary acidic protein (GFAP) and ionized calcium binding adaptor molecule 1 (Iba1) expression were evaluated by immunohistochemistry and western blots. RGC soma or axon density was assessed on Brn3a-stained flat-mounted retinas or toluidine blue-stained optic nerve cross sections, respectively. **Results:** Our data demonstrate a striking decrease in the number of GFAP-positive astrocytes and Iba1-labeled microglia in ibudilast-treated glaucomatous retinas and optic nerves compared to vehicle-treated controls. Ibudilast treatment also led to a marked reduction in the levels of the pro-inflammatory cytokines TNFα, IL-1β, IL-6, and MIF in ocular hypertensive eyes compared to vehicle treatment. Ibudilast promoted robust RGC soma (91%, 1884±43 RGCs/mm^2, n=5) and axonal protection (90%, 90639±2657 axons, n=5) with respect to vehicle (68% soma, 1475±95 RGCs/mm^2, n=6; 61% axons, 61318±8112 axons, n=6). Ibudilast did not alter intraocular pressure (IOP) in after hypertonic saline injection; therefore these responses could not be attributed to changes in IOP. **Conclusions:** Our data demonstrate that Ibudilast attenuates glial cell reactivity, reduces production of pro-inflammatory cytokines, and promotes RGC soma and axon protection in experimental glaucoma.

Disclosures: **J. Cueva Vargas:** None. **N. Belforte:** None. **A. Di Polo:** None.

Poster

050. Neurological Disease: Cellular Mechanisms and Oxidative Stress

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 50.06/S3

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: SafeMinds Grant

Title: Influence of a postnatal peripheral immune challenge on neuroimmune response in the developing rat brain

Authors: *K. L. JONES[1], K. STREIFEL[2], L. HEUER[1], C. BOOSALIS[2], P. LEIN[2], J. VAN DE WATER[1];
[1]Div. of Rheumatology/Allergy and Clinical Immunology, [2]Univ. of California - Davis, Davis, CA

Abstract: Research suggests that peripheral immune challenge during the perinatal period may modulate neurodevelopment. While the vast majority of this research has focused on infection or inflammation during gestation, there is emerging evidence that immune challenge during early postnatal development may also influence the developing brain. Interestingly, the studies performed to date have focused on viral or bacterial infection as the source of immune challenge. Here we addressed whether a non-pathogenic immune stimulus alters biomarkers of neuroinflammation in the developing brain. We tested Lewis rats, which are genetically predisposed to a Th1 pro-inflammatory response, and Brown Norway rats, which are skewed towards an allergy-promoting Th2 response. Male and female offspring of timed-pregnant Lewis and BN dams were immunized on postnatal day (PD) 10 with one of the following treatments: immune challenge (immune challenge and adjuvant), adjuvant-control (mixture of adjuvant and saline), or saline-control (saline only). The immune challenge treatment consisted of a mixture of rotavirus, diphtheria, tetanus, pertussis, *Haemophilus influenza* type b, pneumococcal conjugate, and inactivated polio vaccines in conjunction with an adjuvant, representing the immunization schedule typically administered to humans at 2 months of age. Serum, spleen and brain samples were collected at 2 or 5 days post-immunization. Cytokine profiles were determined using Luminex multiplex technologies, and microglial and astroglial activation states were assessed using immunohistochemistry. Preliminary results suggest that treatment effects are observed, but may vary due to factors such as sex, strain, sample type, and age. These results indicate that while a non-pathogenic immune challenge during the early postnatal period stimulates cytokine/chemokine production in the developing brain, the effects are complex and influenced by various host factors.

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Poster

050. Neurological Disease: Cellular Mechanisms and Oxidative Stress

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 50.07/S4

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Title: Neuroinflammation and neuronal cell death in the retina and cerebral vision structures in an ocular hypertensive rat model

Authors: *A. SAPIENZA, C. ROUBEIX, C. BOUCHER, D. GODEFROY, J. DEGARDIN, F. BRIGNOLE-BAUDOUIN, W. ROSTÈNE, C. BAUDOUIN, S. MELIK-PARSADANIANTZ; Vision Inst., Paris, France

Abstract: Glaucoma is the second leading cause of irreversible blindness worldwide. It is characterized by a progressive retinal neuropathy generally combined with an ocular hypertension due to trabecular meshwork degeneration. Accumulating evidence demonstrates that glaucoma not only affects the retina, but also damages the central visual pathways. The aim of this study was to analyze, in a rat model of ocular hypertension, neuroinflammation and neuronal cell death emerging from pathological retina propagating to the cerebral visual areas. Ocular hypertension model was performed by cauterization of 3 episcleral veins of right eyes in Long-Evans male rats to induce stable intra-ocular pressure increase (19.4$\pm$1.4 to 33.1$\pm$2.4 mmHg). To observe whether neurodegenerative processes occur, we counted retinal ganglion cells ((RGC) Brn3a positive cells)) in whole flat mounted retinas and parvalbumin immunoreactive (PV-IR) neurons in lateral geniculate nucleus (LGN). Retinas and cerebral vision structures (optic tract (OT), LGN, superior colliculus (SC) and visual cortex (Ctx)) were collected after microdissection by punch technique from 6- and 12-weeks ocular hypertensive and control animals. In retinas and microdissected areas, neuroinflammation was evaluated using mRNA expression levels analyze of proinflammatory cytokines, reactive glial cells, chemokines and oxidative stress markers. Gene expression analysis in 6- and 12-weeks retinas of hypertensive eyes revealed a significant increase in CCL2, IL-1β and in NADPH oxidase 2 mRNA expression compared to contralateral and naive eyes. Furthermore, microglial cells and RGC counting also showed a peripheral RGC loss associated with microglial activation and proliferation at all time point examined. We also demonstrated an up-regulation of proinflammatory markers such as IL-6, IL-1β and CCL2 in OT, LGN, SC and Ctx at 6-and 12-weeks in left-brains compared to right and naive brains. Microglial cells and PV-IR neurons counting in LGN revealed no difference at 6 weeks in left-brain compared to controls. However,

in 12-week ocular hypertensive animals, microglial cell activation was closely correlated to LGN neuron death. Ocular hypertension therefore induces neuroinflammation in retina and in the main brain structures involved in vision. It appears that neuroinflammation spreads from sick neurons to healthy neurons through synaptic connections along anatomic neuronal pathway. These findings open a new avenue in our understanding of how glaucoma develops and how it is able to spread along the cerebral visual structures and may offer new therapeutic approaches.

Disclosures: **A. Sapienza:** None. **C. Roubeix:** None. **C. Boucher:** None. **D. Godefroy:** None. **J. Degardin:** None. **F. Brignole-Baudouin:** None. **W. Rostène:** None. **C. Baudouin:** None. **S. Melik-Parsadaniantz:** None.

Poster

050. Neurological Disease: Cellular Mechanisms and Oxidative Stress

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 50.08/S5

Topic: C.08. Ischemia

Support: AHA 13SDG13970009

Title: Acid-sensing ion channels contribute to acidosis-induced neuronal injury in cerebellar slices

Authors: ***X. ZHA**[1], N. JIANG[1,2], Y. JI[2];
[1]Cell Biol. and Neurosci., Univ. of South Alabama, Mobile, AL; [2]Sch. of Life Sci., Shanghai Univ., Shanghai, China

Abstract: Acid-sensing ion channels (ASICs), a family of proton-gated cation channels, are the main proton receptor in brain neurons. Previous studies have shown that ASICs contribute to neuronal injury in cortical, hippocampal and striatal neurons. Whether ASICs mediate acidosis-induced neuronal injury in other brain regions remains unclear. We used here organotypic cerebellar slices to study neuronal injury in cerebellar neurons. We prepared cerebellar slices from postnatal 8-10 day old mice, and cultured them for 10-15 days. After 2 weeks in culture, the overall architecture of the slices was intact. Immunofluorescence labeling showed that Purkinje neurons and cerebellar granule cells were maintained, and PSD-95 clusters formed along the dendrites of Purkinje neurons. We then induced neuronal injury, either by incubating the slices with a pH 6 medium or with oxygen-glucose deprivation (OGD). As expected, pH 6 and OGD treatment induced delayed neuronal injury in these slices. Deleting the ASIC1a gene attenuated

pH 6- and OGD-induced neuronal injury. These data show that organotypic cerebellar slices are a good *ex vivo* system to study mechanisms underlying acidosis-induced neuronal injury.

Disclosures: **X. Zha:** None. **N. Jiang:** None. **Y. Ji:** None.

Poster

050. Neurological Disease: Cellular Mechanisms and Oxidative Stress

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 50.09/S6

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Title: Early life arsenic exposure causes dopaminergic dysfunction and its reversibility in developing brain of rats

Authors: *L. P. CHANDRAVANSHI[1], R. SHUKLA[1], R. GUPTA[2], Y. DHURIYA[2], V. KHANNA[2];
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Abstract: Continuing exposures to arsenic continue to pose a significant threat to public health throughout the world including India due to drinking of arsenic contaminated drinking water. Although a lot of information available on arsenic induced neurotoxicity in adults, knowledge of such effect on children is very little. Studies have been carried out to investigate the impact of early life following arsenic exposure (2 mg/kg and 4 mg/kg body weight, p.o.) to rats from PD22 to PD59 results in various neurobehavioural and neurochemical alterations observed on PD60. In spontaneous locomotor activity test, a significant increased in the total distance (28%, 66%) arsenic exposed groups remarkably compared to control group, and there was a dose-related increase observed. An increase in the binding of DA-D2 receptors in corpus striatum (38%, 56%) and the expression of tyrosine hydroxilase (TH) in corpus striatum (1.93, 2.73 -fold) furthermore associated with a significant increase in the mRNA expression of DAR-D2 (68%, 97%) as compared to controls. Impairment in mitochondrial functional parameters, status of antioxidant enzymes, accumulation of oxidative damage markers, ROS generation, mitochondrial membrane potential and assay of mitochondrial complex I, II and IV with the altered expression of pro-apoptotic, anti-apoptotic proteins were observed in the corpus striatum of rats exposed to arsenic. Increased the content of arsenic (2.65-fold, 4-fold) in the corpus striatum of rats exposed to arsenic in a dose dependent manner was observed as compared to controls. In conclusion, our findings suggest that the postnatal period of brain is more vulnerable to arsenic and most of these changes were persisted 30 days after withdrawal of exposure of arsenic on PD90. This study also

has suggested a correlation between low dose of arsenic exposure and potential dopaminergic neurotoxicity in the corpus striatum of the brain.

Disclosures: **L.P. Chandravanshi:** None. **R. Shukla:** None. **R. Gupta:** None. **Y. Dhuriya:** None. **V. Khanna:** None.

Poster

050. Neurological Disease: Cellular Mechanisms and Oxidative Stress

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 50.10/S7

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: National Science Council of the Republic of China (Grant number: NSC 102-2628-B 002-014-MY3

Title: Prenatal infection affects the neuronal architecture and cognitive function in adult mice

Authors: ***W. LI**[1], Y.-C. CHANG[1], L.-H. LEE[4,5], L.-J. LEE[1,2,3];
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Abstract: Environmental factors such as prenatal infection are involved in the pathogenic processes of neurodevelopmental psychiatric disorders. In the present study, we administered a viral mimic, polyriboinosinic-polyribocytidilic acid (poly I:C, 20 mg/Kg, i.p.), to pregnant B6 mice at gestational day 9.5. Neonates born to these poly I:C-treated dams showed an increase of microglia in the hippocampus, indicating an activation of the immune system in the brains. Moreover, a significant increase in the number of dopamine-producing neurons in the ventral tegmental area (VTA) was observed in adult male poly I:C-offspring compared with age-matched saline-offspring. Poly I:C-offspring also exhibited hypolocomotor activity in a novel open-field arena but did not display signs of anxiety or depression in the elevated plus maze or the forced swim test, respectively. However, the short-term memory of the poly I:C-offspring was impaired in a novel object recognition task. Therefore, the dendritic architecture of granule cells in the dentate gyrus (DG) and pyramidal neurons in the medial prefrontal cortex (mPFC) were examined. The dendritic complexity was reduced in the DG granule cells of poly I:C-offspring and exhibited shorter dendritic length compared with saline-offspring. The density of

dendritic spines in the DG granule cells was also decreased in poly I:C-offspring. Furthermore, the dendritic complexity and spine density was reduced in the layer II/III mPFC pyramidal neurons of the poly I:C-offspring. Together, these data demonstrate impaired short-term memory and altered dendritic architecture in adult poly I:C-offspring, which validates the prenatal infection paradigm as a model for neurodevelopmental psychiatric disorders.

Disclosures: **W. Li:** None. **Y. Chang:** None. **L. Lee:** None. **L. Lee:** None.

Poster

050. Neurological Disease: Cellular Mechanisms and Oxidative Stress

Location: Halls A-C

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Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: Swedish Medical Research Council Grant Nr 2710

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Society for Study on Neuroprotection & Neuroplasticity (SSNN) Cluj-Napoca, Romania

SAIOTEK, and IT/491/10 (Basque Government.

Swedish Strategic Research Foundation

Title: Nicotine administration induces blood-brain barrier breakdown, brain edema formation and neuronal injuries. Exacerbation by cold environment

Authors: *S. SHARMA[1], J. V. LAFUENTE[2], A. NOZARI[3], A. SHARMA[4], Z. TIAN[5], D. F. MURESANU[6];
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Abstract: Recent studies show that prenatal exposure of nicotine in small amounts leads to neuronal damages in several brain areas. However, exposure of nicotine to young adults causing brain dysfunction is still not well known. In this investigation we examined a low dose of

nicotine in young adult rats on brain dysfunction e.g., breakdown of the blood-brain barrier (BBB) permeability, brain edema formation and neuronal injuries. Since environmental factors i.e., heat or cold could also influence nicotine neurotoxicity, we examined effects of cold exposure on nicotine neurotoxicity using standard procedures. Young adults male Wistar rats (20 to 25 weeks of age) were exposed to Nicotine hydrochloride (9 mg/kg, s.c.) once daily for 1 week either at room temperature (21±1°C) or at cold environment (+8±1°C). On the 8th day, BBB permeability to Evans blue albumin and brain water content was examined. The neuronal damages were evaluated using Nissl or Haematoxylin & Eosin methods using standard histopathological procedures. Rats subjected to nicotine exposure at room temperature showed 64 % increase in Evans blue albumin extravasation as compared to saline treated rats under identical conditions. These nicotine treated rats also exhibited increase in brain water content by 0.6 to 0.9 % from the saline group. A significant increase in number of neuronal distortion and damages were seen in in the brain stem, cerebral cortex and cerebellum following nicotine exposure in rats as compared to saline treated group at room temperature. Interestingly, nicotine exospore in rats at cold environment further exacerbated the brain pathology in wide areas of the brain. Thus, the neuronal damages were intensively seen in hippocampus, striatum and thalamus apart from brain stem, cerebral cortex and cerebellum. The breakdown of the BBB to Evans blue was further increased by 80 to 120 % from the identically exposed animals to nicotine at room temperature. The brain edema formation as measured using brain water content was enhanced by 1.5 to 2 % in cod environment following nicotine exposure as compared those treated at room temperature. Taken together these results demonstrate that small doses of nicotine is able to induce profound neurotoxicity in young adult rats and this neurotoxicity is further exacerbated when identical nicotine exposure is made under cold environments. Further studies are in progress to find out the role of oxidative stress in causing nicotine induced neurotoxicity.

Disclosures: **S. Sharma:** None. **J.V. Lafuente:** None. **A. Nozari:** None. **D.F. Muresanu:** None. **A. Sharma:** None. **Z. Tian:** None.

Poster

050. Neurological Disease: Cellular Mechanisms and Oxidative Stress

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 50.12/S9

Topic: C.11. Neurotoxicity, Inflammation, and Neuroprotection

Support: Core Research for Evolutional Science and Technology (CREST), Japan Science and Technology Agency (JST)

Title: Repulsive guidance molecule-a is involved in neuronal damage induced by Th17 cells in experimental autoimmune encephalomyelitis

Authors: *S. TANABE, T. YAMASHITA;
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Abstract: Multiple sclerosis is a chronic autoimmune disease, characterized by various symptoms such as motor dysfunction and visual problem. In multiple sclerosis, immune cells such as T cells and monocytes infiltrate to central nervous system (CNS) and induce inflammation, resulting in demyelination and neurodegeneration. It has been reported that the pathogenesis of multiple sclerosis is attributed to acquirements of autoimmunity to CNS component such as myelin sheath. Moreover, earlier studies revealed that CD4+ helper T cells are known to play crucial roles for inducing inflammation in CNS. In particular, T helper type 17 cells (Th17 cell), CD4+ T cells characterized by producing interleukin 17 (IL-17), are recently identified as critical drivers of autoimmune diseases including multiple sclerosis. Repulsive guidance molecule-a (RGMa) is a glycosylphosphatidylinositol (GPI)-anchored membrane protein known to play a role in axonal growth repulsion. We previously reported RGMa is expressed in dendritic cells and involved in activation of T cells in experimental autoimmune encephalomyelitis (EAE), which is mouse model of multiple sclerosis. We injected RGMa neutralizing antibody to active EAE immunized with myelin oligodendrocyte glycoprotein (MOG35-55) and observed attenuation of EAE severity. However, we did not assess the possible role of RGMa in other immune cells than dendritic cells. Here, we demonstrate RGMa is involved in neurodegeneration in EAE. We found the high expression of RGMa in Th17 cells and RGMa depletion attenuated the severity of EAE induced by adoptive transfer of MOG specific Th17 cells. Moreover, RGMa depletion reduced axonal damage in inflammatory lesions without altering immune responses. In *in vitro* co-culture system, Th17 cells induced death of cortical neurons by a mechanism dependent on RGMa. Thus, RGMa is involved in Th17 cells-mediated neuronal damage.

Disclosures: **S. Tanabe:** None. **T. Yamashita:** None.

Poster

051. Schizophrenia and Bipolar Disorder

Location: Halls A-C

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Program#/Poster#: 51.01/S10

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: RO1MH91130-01-A1 (A.G.)

Title: CB_1-dependent lack of social novelty preference in the sub-chronic PCP rat model of schizophrenia

Authors: *A. SEILLIER, A. GIUFFRIDA;
Pharmacol., UTHSCSA, SAN ANTONIO, TX

Abstract: We previously showed that social withdrawal in the PCP rat model of schizophrenia resulted from deficient endocannabinoid-induced activation of CB_1 receptors. To understand the underlying cognitive mechanisms of the social deficit in PCP-treated rats, we developed a behavioral test adapted from "the three-chamber social approach task" to assess sociability (i.e. social approach) and social novelty preference (which relies on social recognition). In contrast to the mouse experimental protocol, our rats were tested in an open arena virtually divided in quadrants. In the sociability phase, rats were allowed to explore the arena with two wire mesh cages at the opposite corners, one of which contained an unfamiliar rat, whereas the other one was left empty. As expected, control rats spent more time exploring the cage containing the unfamiliar rat, showing a clear preference for a "social" versus a "neutral" object. In the social novelty phase, a new rat was placed in the empty wire mesh cage, whereas the previously unfamiliar rat, now familiar, remained in the cage at the opposite corner. During this phase, rats spent more time exploring the "novel" rather than the "familiar" cage. In contrast, PCP-treated rats showed intact sociability, but lacked social novelty preference. To test whether the deficit in novelty discrimination was due to impaired social recognition or lack of novelty preference, we assessed the ability PCP-treated rats to discriminate between social (bedding from their home or a different cage) and non-social (new bedding or no bedding) olfactory cues contained in Petri dishes. Both saline- and PCP-treated animals were able to discriminate the social versus non-social cues. However, control animals showed a slight preference for the bedding from a different cage, whereas PCP-treated rats preferred the bedding from their home cage. Preliminary data indicate that the lack of social novelty preference in PCP animals is reversed by the CB_1 antagonist AM251, suggesting that increased activity at CB_1 receptors contributed to this behavioral deficit. In agreement with this hypothesis, the cannabinoid agonist CP55,940, which had no effect on sociability, dose-dependently suppressed the preference for social novelty in control animals. Taken together, these data suggest that PCP-treated rats have a deficit in social cognition resulting from a diminished interest for social novelty, possibly induced by increased stimulation of CB_1 receptors. This deficit, however, does not contribute to the social withdrawal observed in PCP-treated animals, as the latter is due to deficient, rather than increased, CB_1 stimulation.

Disclosures: **A. Seillier:** None. **A. Giuffrida:** None.

Poster

051. Schizophrenia and Bipolar Disorder

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 51.02/S11

Topic: C.15. Schizophrenia and Bi-polar Disorder

Title: Assessment of chronic perampanel treatment on hyperactivity and hippocampal reactivity of GluA1 lacking mice

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Abstract: Neurotransmitter glutamate mediates most of excitatory transmission and is involved in essential functions of the brain, but also plays a role in pathophysiological conditions. Glutamatergic signalling is therefore important therapeutic target, like in neuropsychiatric disorders. Gria1−/− mouse line with knocked-out Gria1 gene encoding for GluA1 subunit of ionotropic AMPA glutamate receptor display some behavioural features of schizoaffective disorder, for which they have been suggested as a putative model. The hyperactivity provoked by novelty in the Gria1−/− is the dominant behavioural feature of this mouse line. Here we have tested a novel antiepileptic drug, a non-competitive antagonist of AMPA receptors, perampanel, for its efficacy in attenuating hyperactivity of Gria1−/− mice. Four-week treatment with the perampanel at the dose of 60 mg/kg, supplemented in the diet, blocked novelty-induced locomotor hyperactivity of the Gria1−/− mice, while doses of 6 and 30 mg/kg were ineffective. We also sought to see if the blockade of AMPA receptors could reverse the strong induction of neuronal activity in the dorsal hippocampus of Gria1−/− mice, by monitoring the immediate early gene c-Fos protein expression as a marker of neuronal activity. Perampanel treatment (60 mg/kg) reduced the higher c-Fos expression of Gria1−/− mice in the dentate gyrus and normalized excessive c-Fos levels of Gria1−/− mice in the CA1 subfield to the levels of WT mice. In summary, abnormal glutamatergic transmission underlies at least partly the hyperactive phenotype of the Gria1−/− mouse model, which could be particularly exploited for *in vivo* screening of novel drugs that target glutamatergic transmission.

Disclosures: **M. Maksimovic:** None. **E.R. Korpi:** None.

Poster

051. Schizophrenia and Bipolar Disorder

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 51.03/S12

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: Neuropôle de Recherche Francilien (NeRF), "Région Ile-de France"

Title: Serotonin receptor 2B knockout mice present an antipsychotic-sensitive schizophrenic-like phenotype

Authors: *P. M. PITYCHOUTIS[1,2], J. ADRIEN[3], L. MAROTEAUX[2];
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Abstract: Impulsivity, very broadly defined as action without foresight, novelty-seeking and hyperlocomotion, shares common playground with numerous mental disorders, such as schizophrenia. In a recent study conducted in a Finish cohort, we reported that a population-specific serotonin 2B receptor (5-HT2BR) receptor stop-codon (i.e. HTR2B Q20*) predisposes to severe impulsivity. Moreover, the genetic ablation of the 5-HT2BR gene in mice (5-HT2B−/−) yielded a hyperlocomotor and novelty-seeker phenotype, characterized by enhanced impulsivity. In the same cohort, psychosis was numerically more prevalent in HTR2B Q*20 carriers, with early-onset schizophrenia being observed in this group. However, the extent to which 5-HT2BR is implicated in the neurobiology of schizophrenia has never been studied. Therefore, in the present study we investigated the effects of the genetic ablation of the 5-HT2BR, across a battery of schizophrenia-relevant behavioral paradigms and demonstrate herein that loss of function of 5-HT2BR confers a wide spectrum of schizophrenic-like behavioral and psychopharmacological phenotypes in mice. Adult male 5-HT2B-/- mice and their respective wild-type (WT) controls were used in the present study. Domains related to the positive, negative and cognitive symptom-clusters of schizophrenia were affected in 5-HT2B-/- mice that closely resemble the behavioral alterations observed in schizophrenic patients. In particular, genetic ablation of the 5-HT2BR induced deficits in sensorimotor gating, selective attention, and learning and memory processes. Moreover, 5-HT2B-/- mice presented with enhanced locomotor response to the psychostimulant properties of dizocilpine and amphetamine, as well as robust alterations in sleep architecture. At the neurochemical level, ablation of the 5-HT2BR induced a region-dependent decrease of dopamine (DA) concentrations in the dorsal striatum (dSTR) and glutamate content in the nucleus accumbens (NAC) accompanied by respective alterations in dopaminergic and glutamatergic receptors' expression. Importantly, most of these schizophrenic-like phenotypes and endophenotypes were rescued by chronic treatment with the typical antipsychotic haloperidol. In conclusion, the present study reveals that 5-HT2BR deficiency may induce a

panel of schizophrenic-like behavioral phenotypes accompanied by robust region-distinctive dopaminergic and glutamatergic neurochemical alterations. The latter implies an important role for 5-HT2BR in the harnessing of dopaminergic and glutamatergic activity.

Disclosures: **P.M. Pitychoutis:** None. **J. Adrien:** None. **L. Maroteaux:** None.

Poster

051. Schizophrenia and Bipolar Disorder

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 51.04/T1

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: NIH Grant RO1MH61887

Title: Cortex-specific disruption of p90 Ribosomal S6 kinase 2 augments 5HT2A signaling *in vivo*

Authors: ***H. ZHU**[1], R. T. STRACHAN[2], D. J. URBAN[2], M. S. FARRELL[3], A. HANAUER[4], B. L. ROTH[3];
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Abstract: Coffin-Lowry syndrome (CLS) is an X-linked syndromic form of mental retardation characterized by various skeletal anomalies. It is caused by mutations of the p90 ribosomal s6 kinase 2 (RPS6KA3) gene that encodes for the growth factor-regulated serine/threonine kinase, ribosomal S6 kinase 2 (RSK2). In our previous study, we found that RSK2 protein interacted with the 5-HT2A serotonin receptor intracellular loop 3 and phosphorylated Ser-314 within that loop, which led to a tonic inhibition of 5HT2A receptor signaling *in vitro*. To test whether RSK2 inhibits 5-HT2A receptor signaling *in vivo* and whether the disruption of RSK2 leads to schizophrenia-like psychosis in CLS patients, we crossed a floxed RSK2 mouse with Emx1-Cre mice to selectively disrupt the RSK2 gene in cortical pyramidal neurons. Cortex-selective disruption of RSK2 gene augmented 5-HT2A receptor signaling, which was demonstrated by the significant increase of DOI-induced c-fos activation and head-twitching behavior. 5-HT2A receptor antagonists did not normalize the PCP-induced hyperlocomotion and disruption of prepulse inhibition, which indicated the decreased sensitivity of receptor to its antagonist. The augmentation of 5-HT2A receptor signaling was not due to the alteration of receptor expression,

which was normal in the cortex. In conclusion, we found that cortex-specific disruption of RSK2 augments 5-HT2A receptor signaling and decreases the antagonist 5-HT2A sensitivity without altering receptor expression. Thus, RSK2 modulates both agonist and antagonist properties of 5HT2A receptor *in vivo* and disruption of RSK2 leads to schizophrenia-like behaviors in animals.

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Poster

051. Schizophrenia and Bipolar Disorder

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 51.05/T2

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: Supported by USPHS grant P50 MH103222

Title: Prenatal kynurenine treatment in mice: Effects on placental and fetal brain kynurenines

Authors: *S. BEGGIATO[1], K. V. SATHYASAYKUMAR[1], F. NOTARANGELO[1], F. GIORGINI[2], P. J. MUCHOWSKI[3], R. SCHWARCZ[1];
[1]Psychiatry, Maryland Psychiatric Research, Univ. of Maryl, Baltimore, MD; [2]Dept. of Genet., Univ. of Leicester,, Leicester, United Kingdom; [3]Gladstone Inst. Neurol. Dis., San Francisco, CA

Abstract: Studies in animals and humans suggest a pathophysiologically significant association between elevated brain kynurenic acid (KYNA) levels and cognitive dysfunction in schizophrenia (SZ) (Schwarcz et al., Nat. Rev. Neurosci., 2012). The increase in KYNA in the disease may be secondary to a genetic disruption of kynurenine 3-monooxygenase (KMO), a pivotal kynurenine pathway (KP) enzyme with links to SZ endophenotypes (Wonodi et al., Arch. Gen. Psychiat., 2011). Prenatal exposure to kynurenine (the direct bioprecursor of KYNA) induces cognitive impairments reminiscent of SZ in adult rats (Pocivavsek et al., Psychopharmacology, 2014), suggesting a developmental dimension to the link between KYNA and SZ. To begin exploring the possible role of KMO in this scenario, we now exposed pregnant wild-type (WT= Kmo+/+), heterozygous (HET = Kmo+/-) (Giorgini et al., J. Biol. Chem., 2013) C57BL/6 mice to kynurenine (10 mg/day, given with chow) during the last week of gestation, i.e. from embryonic days (ED) 10/11 to ED17/18. The dams were euthanized on ED17/18, and

the levels of KYNA and 3-hydroxykynurenine (3-HK), the product of KMO, were determined in placenta and fetal brain of HET and KMO knockout (KO = Kmo-/-) mice (from HET parents). Compared to WT animals (from WT parents), basal levels of KYNA in both placenta and fetal brain tended to be higher in these HET and KO mice. In contrast, basal 3-HK levels in placenta and fetal brain were lower in both groups of mutant animals, reaching a significant reduction in the fetal KO brain ($p<0.05$ vs. WT). Preliminary data indicated that prenatal treatment with kynurenine caused a significantly larger increase in KYNA levels in the placenta and in the fetal brain of KO as compared to WT mice; a trend toward higher KYNA levels compared to WT mice was also observed in both tissues of HET animals. Conversely, 3-HK levels in placenta and fetal brain of kynurenine-treated HET and KO mice were lower than in the corresponding tissues of the WT animals (all $p<0.01$). Jointly, these results revealed that the ratio between KYNA and 3-HK, which are believed to have opposing functions in the brain, is greatly skewed towards KYNA in the fetal brain when KMO is compromised or eliminated. As qualitatively similar phenomena were observed in the placentas of the mutant mice, it remains unclear if the effects seen in the fetal brain of the mutants are tissue-autonomous or secondary to placental biochemistry. The present data therefore raise the possibility that KP activity in the placenta exerts an important influence on fetal brain development (Hsiao and Patterson, Dev. Neurobiol., 2012) and may thus contribute to the development of adult mental disorders including SZ.

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Poster

051. Schizophrenia and Bipolar Disorder

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Program#/Poster#: 51.06/T3

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: NSC 102-2410-H-431-005-MY3

NSC 101-2410-H-431-007

NSC 100-2410-H-431-003

Title: Examinations of conditioned taste aversion and episodic memory for schizophrenia-like rats

Authors: *A. C.-W. HUANG, F.-Y. WU, C.-W. WU;
Psychol, Fo Guang Univ., Yilan County, Taiwan

Abstract: The second author has an equal contribution to the first author. 1 **Abstract** Many cognitive dysfunctions occurred at schizophrenia patients. Of particular, deficits of explicit and implicit memory may be important symptoms for schizophrenia. However, the emerged issue remains unclear in the animal model. At the beginning of the experiment, methamphetamine (MAMPH) or its vehicle normal saline was intraperitoneally administrated once every other day to develop behavioral sensitization reflecting in hyperactivity served as the animal model of schizophrenia. Thus, all of rats were assigned into Saline and MAMPH groups. Later, all of rats were trained to freely drink a 0.1 % saccharin solution (conditioned stimulus, CS) to associate with 0.15 M lithium chloride (unconditioned stimulus, US) to form conditioned taste aversion learning (CTA); indicating one of implicit memory. Moreover, all rats encountered the procedure of E-maze task to acquire episodic memory; indicating one of explicit memory. The results showed that rats with MAMPH administrations induced hyperactivity to develop an animal model of schizophrenia. MAMPH-induced schizophrenia-like rats would impair implicit memory-conditioned taste aversion as well as explicit memory-episodic memory. The findings of the present study may provide a good animal model for treating schizophrenia. Further, how schizophrenia affects the explicit and implicit memory. The emerged issues should be discussed further. *Keywords:* Methamphetamine, schizophrenia, implicit memory, explicit memory, conditioned taste aversion, episodic memory 2

Disclosures: **A.C. Huang:** None. **F. Wu:** None. **C. Wu:** None.

Poster

051. Schizophrenia and Bipolar Disorder

Location: Halls A-C

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Program#/Poster#: 51.07/T4

Topic: C.15. Schizophrenia and Bi-polar Disorder

Title: Reverse translation animal preparations of hippocampal glutamatergic dysfunction in schizophrenia: GluN1, plasticity changes and genetic manipulations

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Abstract: The molecular, cellular and circuit mechanisms of schizophrenia (SZ) are unknown, hugely disadvantaging animal model research. Current research in this area comes largely from preparations based on risk genes and psychosis-relevant pharmacology, without reference to molecular targets. Nonetheless, animal models are necessary because of their dynamic and experimental characteristics. As tissue findings of SZ neurobiology advance, there is a greater opportunity for validating animal preparations. Neurobiological studies in serious brain diseases assess *in vivo* imaging outcomes and postmortem tissue pathology, among other biomarkers. Considerable clinical data suggest hippocampal involvement in schizophrenic psychosis, a body of knowledge to which our laboratory has contributed. We have verified the reported increase in *in vivo* hippocampal perfusion in the illness and demonstrated GluN1 reductions in dentate gyrus (DG) and increased synaptic plasticity markers in CA3 which could underlie hippocampal neuronal hyperactivity, including increased GluN2B, PSD95, and BDNF as well as increased spines on CA3 pyramidal apical dendrites, suggesting synaptic strengthening (Tamminga, et al., 2010; Stan et al., 2014: Li et al., 2014). This has allowed us to target these molecular changes, and also neuronal activity markers in CA3 in animals as molecular and cellular characteristics of SZ psychosis. We are developing animal preparations of SZ psychosis through reverse translation, directly mimicking the biology of the human condition in the mouse. To proceed, we crossed a POMC-Cre mouse line with a floxed P-GluN1 mouse to create a DG-specific knock down of GluN1 protein. These animals have reduced levels of GluN1 restricted to DG ($p<0.0001$). Behaviorally, these mice show decreased pre-pulse inhibition, reduced learning in the Morris Water Maze (MWM), increased freezing in a fear conditioning (FC) paradigm and an increased latency to respond in the passive avoidance (PA) paradigm. Although these animals generated a true behavioral phenotype, the tissue phenotype did not obtain; therefore, we drove the DG KO with PCP sub-chronically. Here, both the appropriate behavioral phenotype described above and the molecular outcomes derived from patient tissue including both the DG GluN1 reduction and the CA3 increased GluN2B ($p=0.035$). We have additional mouse lines on which to test animal preparations for psychosis-related findings, including the Disc1 transgenic mouse (Hikida, et al, 2007) and early environmental stressors. We base our assessment of psychosis animal model validity on the closeness of its fit with the biology of the human SZ psychosis condition.

Disclosures: **S. Southcott:** None. **M. Yanagi:** None. **J. Lister:** None. **C. Tamminga:** None.

Poster

051. Schizophrenia and Bipolar Disorder

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 51.08/T5

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: R01MH084894

Title: Structural basis of heteromeric comunication between 5-HT2A and mGlu2 receptors and its role in schizophrenia

Authors: *J. L. MORENO[1], P. MIRANDA-AZPIAZU[2], A. GARCIA-BEA[2], R. DIEZ-ALARCIA[2], A. M. GABILONDO[2], J. LOPEZ-GIMENEZ[3], G. MILLIGAN[4], J. MEANA[2], J. GONZALEZ-MAESO[1];
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Abstract: 5-Hydroxytryptamine 2A (5-HT2A) and metabotropic glutamate 2 (mGlu2) receptors are G protein-coupled receptors (GPCRs) that have been linked to the pathophysiology of schizophrenia, as well as to the mechanism of action of atypical antipsychotic drugs (e.g., clozapine and risperidone), and the new class of potential antipsychotic drugs acting as agonists of mGlu2/3 receptors (e.g., LY379268 and LY404039). GPCRs have been thought to function as monomers. Nevertheless, extensive evidences corroborates the existence of GPCR homo- and hetero-dimers/oligomers that differentially alter receptor-G protein coupling preferences and consequently G protein-dependent signaling. We previously demonstrated that the Gq/11-coupled 5-HT2A receptor and the Gi/o-coupled mGlu2 receptor form a GPCR heteromeric complex through which serotonin and glutamate ligands modulate the pattern of G protein coupling in tissue culture and mammalian brain frontal cortex. Using flow cytometry-based FRET, our previous findings show that any two of the three residues (A6774.40, A6814.44, A6854,48) located at the intracellular end of the transmembrane domain 4 are necessary for the mGlu2 to be assembled as a GPCR heteromeric complex with the 5-HT2A receptor. Here we focused our attention on the ability of the mGlu2 receptor to crosstalk and activate Gq/11 protein-dependent signaling through the 5-HT2A-mGlu2 heteromeric receptor complex. By measuring elevations of intracelullar [Ca2+], we show that expression of 5-HT2A and mGlu2 as a GPCR heteromer is necessary to induce Gq/11 protein-dependent signaling by the mGlu2/3 receptor agonist LY379268. We also demonstrate that although co-expression of mGlu2 receptor together with a mutant 5-HT2A receptor defective in G protein activation abolishes LY379268-induced Gq/11 protein-dependent signaling, an mGlu2 receptor that couples to and activates G proteins is also required to encode this particular signaling outcome. In addition, we show that the signaling crosstalk between the components of the 5-HT2A-mGlu2 heteromeric receptor is dysregulated in postmortem frontal cortex of schizophrenic subjects. These findigns may help in the development of more specific and effective therapeutic antipsychotic drugs for the treatment of schizophrenia. Supported by R01MH084894

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Poster

051. Schizophrenia and Bipolar Disorder

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Program#/Poster#: 51.09/T6

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: PHS Grant MH-083729 to JPB and RS

Title: Prenatal kynurenine exposure alters maturation of the conditioned fear response: Normalization with an α7nAChR partial agonist

Authors: *M. L. PERSHING[1], D. H. LINDQUIST[1], A. POCIVAVSEK[2], K. Y. TSENG[3], R. SCHWARCZ[2], J. P. BRUNO[1];
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Abstract: Schizophrenia (SZ) is a neuropsychiatric disorder involving maturational alterations in brain circuitry. Symptoms emerge post-puberty and include core cognitive deficits that are ineffectively treated with current pharmacotherapies. Elevations in kynurenic acid (KYNA), an endogenous α7 nicotinic acetylcholine receptor (nAChR) and NMDAR antagonist, may underlie cognitive deficits in SZ. We have reported that elevating KYNA in developing rats alters cortical maturation and yields deficits in prefrontal cortex (PFC)-mediated tasks. Trace fear conditioning (TFC), in which a tone must be associated with a later occurring footshock, requires intact cholinergic/glutamatergic signaling and sustained firing of layer 2/3 PFC neurons. We predicted that rats sustaining elevated KYNA levels during fetal development [embryonic days (ED) 15-22] would exhibit altered cholinergic/glutamatergic gene expression and impaired TFC when tested as adults but not as juveniles; furthermore, that administration of SSR180711, an α7nAChR partial agonist, would ameliorate TFC deficits, given its ability to increase α7nAChR expression and glutamate release. Pregnant Wistar rats were fed a mash diet (ECON) or a diet containing L-kynurenine (the bioprecursor of KYNA; 100 mg/dam/day; EKYN) during the last prenatal week (ED15-22). Brain mRNA levels (α7nAChR and NMDAR subunits) and TFC - 10 pairings of a 15 sec tone and 1 sec footshock, separated by a 10 sec trace interval - were assessed on postnatal days (PD) 31-33 (juvenile rats) and PD65-80 (adult rats). A separate group of adult

EKYNs was tested on TFC following SSR180711 administration (1 mg/kg, i.p.). Brain KYNA was increased in EKYN fetuses (470%, forebrain) and adults (80%, PFC), relative to age-matched ECONs. Freezing behavior was assessed after TFC. Adult EKYNs, relative to ECONs, froze significantly less during the tone (58 ± 3% vs 72 ± 5%) and trace interval (57 ± 4% vs 69 ± 4%). In contrast, juvenile EKYNs demonstrated increased freezing during the tone (68 ± 6% vs 55 ± 5%) and trace interval (71 ± 7% vs 56 ± 6%) compared to ECONs. Preliminary data suggest that NR2B mRNA was increased in EKYNs at PD32 (+48%) and PD65 (+13%). Expression of α7nAChR mRNA was reduced (-11%) on PD65. SSR180711 administration restored conditioned freezing in PD65 EKYNs, to ECON levels, across the tone and trace interval. Taken together, these results, obtained in an animal model with face and construct validity, suggest that a sustained impairment of α7nAChR/NMDAR function may contribute to deficits in executive function in SZ, and support the proposition that α7nAChRs constitute promising targets for therapeutic intervention.

Disclosures: **M.L. Pershing:** None. **D.H. Lindquist:** None. **A. Pocivavsek:** None. **K.Y. Tseng:** None. **R. Schwarcz:** None. **J.P. Bruno:** None.

Poster

051. Schizophrenia and Bipolar Disorder

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Program#/Poster#: 51.10/T7

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: PHS Grant MH-083729

Title: Acute elevations of brain kynurenic acid induce working memory deficits: Relative contributions of α7 nicotinic and NMDA receptor activity

Authors: *D. PHENIS[1], S. A. VUNCK[1], R. SCHWARCZ[2], J. P. BRUNO[1];
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Abstract: Core cognitive deficits in attention and working memory are present in schizophrenia (SZ) but remain largely unresolved by current drug interventions. Patients with SZ have elevated kynurenic acid (KYNA) levels in the brain which may contribute to cognitive impairments through either negative allosteric modulation (NAM) of α7 nicotinic acetylcholine receptors (α7nAChR) or antagonism of N-methyl-D-aspartate receptors (NMDAR) at the glycineB site. Acute elevations of brain KYNA in animals also lead to cognitive deficits (e.g. contextual

learning, spatial working memory, attentional set-shifting). The present studies were designed to determine whether a) acute increases in KYNA in rats induce performance deficits in the operant delayed non-match-to-position (DNMTP) working memory (WM) task and b) using drugs selective for the two receptor populations, whether these deficits reflect antagonism at α7nAChRs or NMDARs. The DNMTP task is divided into three components sample, retention, and choice phases. The daily sessions consisted of 120 trials delays were 5, 10 & 15 sec. The test compounds administered to separate groups of rats were kynurenine (KYN the bioprecursor of KYNA), galantamine [GAL a positive allosteric modulator of α7nAChR], 4-chloro-kynurenine [4-Cl-KYN bioprecursor of 7-chlorokynurenic acid (7-Cl-KYNA), an antagonist of NMDAR/glycineB], and D-cycloserine (DSC an agonist at NMDAR/glycineB). Dose-response curves were generated for KYNA and 7-Cl-KYNA, and alleviation of deficits was attempted using co-administration of GAL or DSC. Acute elevation of KYNA via a KYN injection (100 mg/kg) produced delay-dependent deficits in DNMTP % choice accuracy (5 sec VEH 96% vs KYN 84% 10 sec VEH 85% vs KYN 72% 15 sec VEH 78% vs KYN 50%). Deficits resulting from elevated KYNA were fully reversed by GAL (3 mg/kg) (15 sec KYN 50% vs KYN+GAL 78%). Acute elevation of 7-Cl-KYNA, via its precursor 4-Cl-KYN (100 mg/kg), reduced DNMTP performance but independent of delay length (5 sec VEH 95% vs 4-Cl-KYN 48% 10 sec VEH 88% vs 4-Cl-KYN 41% 15 sec VEH 79% vs 4-Cl-KYN 36%). Deficits following an elevation in 7-Cl-KYNA were reversed by DSC (30 mg/kg) but were unaffected by GAL (15 sec GAL 36% vs DSC 78%). Our results support antagonism of α7nAChRs as opposed to NMDAR/glycineB inhibition as the mechanism underlying KYN-induced reductions in WM. The reversal of KYN-induced, delay-dependent deficits in performance of the WM task with GAL support continued focus on allosteric modulation of α7nAChRs as a target for cognition enhancement in SZ. Performance deficits following antagonism of the glycineB site do not resemble those seen following KYN in either profile or reversibility.

Disclosures: **D. Phenis:** None. **S.A. Vunck:** None. **R. Schwarcz:** None. **J.P. Bruno:** None.

Poster

051. Schizophrenia and Bipolar Disorder

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Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: PHS Grant MH-083729

Title: Rats exposed to elevated brain levels of kynurenic acid during the prenatal period exhibit an enhanced vulnerability to working memory deficits as adults

Authors: *S. A. VUNCK[1], D. PHENIS[1], K. Y. TSENG[2], R. SCHWARCZ[3], J. P. BRUNO[1]; [1]The Ohio State Univ., Columbus, OH; [2]The Chicago Med. Sch. at Rosalind Franklin Univ. of Med. and Sci., Chicago, IL; [3]Maryland Psychiatric Res. Center, Univ. Maryland Sch. of Med., Baltimore, MD

Abstract: Schizophrenia (SZ) is a debilitating neuropsychiatric disorder arising from neurodevelopmental alterations in brain circuitry. These changes underlie core deficits in cognitive control that are predictive of outcome yet are poorly addressed with current pharmacotherapies. Kynurenic acid (KYNA) is a negative allosteric modulator at α7 nicotinic acetylcholine receptors (α7nAChR) and an antagonist at NMDA/ glycineB receptors. KYNA levels are elevated in the brains of patients with SZ, and increased KYNA levels produce cognitive deficits in animals. We previously reported that chronic perinatal (ED15-PD21) exposure to KYNA's bioprecursor kynurenine (KYN) elevates KYNA throughout development and adulthood, and leads to deficits in hippocampus- and prefrontal cortex-mediated cognitive tasks. The current study used the operant delayed non-match to position (DNMTP) task to determine if adult rats exposed prenatally to elevated KYN exhibit overt working memory (WM) deficits or a vulnerability that can be exposed through pharmacological challenges. Pregnant Wistar dams received KYN (100 mg/day in mash) during the last prenatal week (ED15-22) (EKYNs) or regular mash (ECONs). KYNA levels were determined on ED21 and PD56. Cognition was assessed at PD56-80, using the DNMTP task (delays 5, 10 & 15 sec). Animals were challenged acutely with KYN or 4-chlorokynurenine (4-Cl-KYN; the bioprecursor of the selective NMDAR antagonist 7-Cl-KYNA) and tested for alleviation of deficits following co-administration of the positive α7nAChR modulator galantamine (GAL 3 mg/kg; i.p.). EKYN rats had elevated brain KYNA levels compared to ECONs [ED21 (+470%) and PD56 (+80%)]. Adult EKYNs and ECONs acquired the task at similar rates. However, EKYNs, but not ECONS, exhibited enhanced vulnerability to WM deficits following acute challenge with drugs/doses that further compromised choice accuracy (%) by antagonizing either the α7nAChR (KYN 25 mg/kg; ECON 77% vs EKYN 43% at 15 sec delay) or the NMDAR (4-Cl-KYN 25 mg/kg; ECON 65% vs EKYN 39% at 15 sec delay). Deficits from acute drug challenges of KYN, but not 4-Cl-KYN, were fully reversed by co-administration of GAL (ECON 90% vs EKYN 89% at 15 sec delay). Our results demonstrate that acquisition of an operant WM task in adulthood is not impaired by embryonic exposure to elevated KYNA. However, this prenatal treatment appears to cause maturational changes in circuits underlying WM, resulting in an enhanced vulnerability to pharmacological challenges that antagonize glutamatergic or cholinergic activity. This work supports further research using prenatal KYNA elevation to study cognitive deficits and vulnerabilities related to SZ.

Disclosures: **S.A. Vunck:** None. **D. Phenis:** None. **K.Y. Tseng:** None. **R. Schwarcz:** None. **J.P. Bruno:** None.

Poster

051. Schizophrenia and Bipolar Disorder

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 51.12/T9

Topic: C.15. Schizophrenia and Bi-polar Disorder

Title: D-cycloserine affects pyridoxal-5-phosphate complex causing a lowering of kynurenic acid formation

Authors: *H. BARAN[1,2], B. KEPPLINGER[1,3];
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Abstract: The anti-mycobacterial drug, D-cycloserine has the capacity to lower significantly the kynurenic acid (KYNA) synthesis in mammalian and human brains in an *in vitro* study (H. Baran and B. Kepplinger, Eur. Neuropsychopharmacol., 2014). D-Cycloserine, a partial agonist at the glycine modulatory site of the glutamatergic NMDA receptor improves cognitive function and exerts anticonvulsive activities. The present study further evaluated the inhibitory action of D-cycloserine on kynurenic acid synthesis. The activity of enzymes synthesizing KYNA, kynurenine aminotransferases I, II and III (KAT I, KAT II and KAT III) in human brain in the presence of D-cycloserine or in the presence of drug with nootropic/neurotropic effects Citicholine (Startonyl) in an *in vitro* study was measured. Parallel to KYNA measurement we searched the alteration of pyridoxal-5-phosphate complex content in the incubation medium. Human post mortem samples of frontal cortex from the Neurological Institute of Medical University Vienna (N = 5) were used. The study was carried out according to the ethical regulations of Austria. Determining KATs activities as previously described we found that D-cycloserine but not Startonyl lowered dose-dependently and significantly KATs activities, respectively KYNA level, of human brain homogenate. Furthermore, D-cycloserine but not Startonyl lowered dose-dependently and significantly the content of pyridoxal-5-phosphate complex in the incubation medium. Interestingly, lowering of pyridoxal-5-phosphate complex was accompanied by formation of new peak probably pyridoxal or its derivate. No formation of such peak could be seen in the presence of Startonyl. Higher doses of D-cycloserine (>ca 800 μM) blocked KATs activities and lowered the levels of pyridoxal-5-phosphate complex most completely. Revealed data demonstrate that D-Cycloserine but not Startonyl is able to affect KYNA synthesis and this due to lowering of pyridoxal-5-phosphate complex which is required for transamination. Lowering of KYNA content due to D-cycloserine action might be involved in

the improvement of symptoms like dementia, cognition and/or delusion in Schizophrenia, Alzheimer's, HIV-1 infected patients or Parkinson's patients. Notable, metabolites formed due to degradation of pyridoxal-5-phosphate complex could be toxic and in part be responsible for the side effects of D-cycloserine. The nootropic/neurotropic effects of Startonyl are not involve in alteration of KYNA formation in the brain, at least *in vitro* study. Startonyl was provided by Chiesi Farmaceutici S.p.A., Italy. Supported by NFB - Life Science, Austria, LS 10-032. *former facility

Disclosures: **H. Baran:** None. **B. Kepplinger:** None.

Poster

051. Schizophrenia and Bipolar Disorder

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Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: Dainippon Sumitomo

Title: Role of 5-HT1A and 5-HT7 receptors in the sub-chronic PCP-induced executive function deficit in C57BL/6 mice

Authors: *L. RAJAGOPAL[1], B. W. MASSEY[1], E. E. MICHAEL[1], Y. OYAMADA[1,2], M. MIYAUCHI[1,2], M. HUANG[1], H. Y. MELTZER[1];
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Abstract: Introduction: The atypical antipsychotic drug (APD), lurasidone, a dopamine D2, serotonin (5-HT)2A, and 5-HT7 receptor antagonist, and tandospirone, a 5-HT1A partial agonist, has been reported to improve some domains of cognitive function in schizophrenia, including executive functioning, a predominantly cortical function. Executive functioning can be studied in an operant reversal learning (ORL) task in rodents. To approximate the hypoglutamatergic deficit which may contribute to the cognitive deficit in schizophrenia, the N-methyl-d-aspartate antagonist, phencyclidine (PCP) was administered sub-chronically to C57BL/6 mice followed by withdrawal. Sub-chronic PCP has been shown to impair performance in reversal learning deficits in rats. However, there has been a paucity of studies in this model in mice, despite the growing use of mouse as a subject in genetic and molecular studies of schizophrenia. Hence, in the present study, we aimed to evaluate the effects of sub-chronic PCP treatment (10 mg/kg) in

C57BL/6 mice and to investigate the role of 5-HT1A and 5-HT7 receptors in this model. Materials and methods: Two cohorts of C57BL/6 male mice and one cohort of 5-HT7 knock-out (KO) (-/-) mice were used as subjects. Both C57BL/6 and 5-HT7KO(-/-) mice were trained to perform ORL, following which, C57BL/6 mice received sub-chronic PCP (10 mg/kg) or vehicle intraperitoneally (i.p.) twice daily for 7 days, followed by 7-day washout. In experiment 1, PCP treated C57BL/6 mice received acute lurasidone (1 or 3 mg/kg; i.p.), tandospirone (5 mg/kg; i.p.), or co-administration of the 5-HT1A antagonist, WAY100635 (0.6 mg/kg) + lurasidone (3 mg/kg; i.p.). In experiment 2, PCP treated mice received the 5-HT7 antagonist, SB269970 (0.5 or 4 mg/kg), and the 5-HT7KO(-/-) received saline. Results: Sub-chronic PCP significantly impaired the percentage correct responding, total incorrect trials, and total incorrect response in the reversal phase performance ($P<0.05$), with no effect in the initial phase. Parenteral administration of acute lurasidone 3 mg/kg, but not 1 mg/kg, tandospirone 5 mg/kg, or SB269970 4 mg/kg, but not 0.5 mg/kg reversed the PCP-induced deficit in ORL in C57BL/6 mice. Co-administration of the 5-HT1A antagonist, WAY100635, blocked the ability of lurasidone to restore ORL. 5-HT7 receptor knock-out mice showed no deficit in ORL, and performed similarly to vehicle controls. Conclusion: These results indicate that 5-HT1A partial agonism and 5-HT7 antagonism may restore ORL in sub-chronic PCP-treated mice, similar to results we and others have reported for declarative memory, a temporal lobe measure.

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Poster

051. Schizophrenia and Bipolar Disorder

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 51.14/T11

Topic: C.15. Schizophrenia and Bi-polar Disorder

Title: Acute phencyclidine-induced increase in locomotor activity, cognitive deficit and cortical and striatal glutamate and serotonin efflux is suppressed in 5-HT7 knockout mice

Authors: *M. HUANG, L. RAJAGOPAL, S. KWON, E. E. MICHAEL, H. Y. MELTZER; Psychiatry and Behavior Sci., Northwestern Univ. Feinberg Sch. of Med., Chicago, IL

Abstract: Background: Phencyclidine (PCP), a N-methyl-D-aspartate receptor (NMDAR) non-competitive antagonist, is the basis for a widely investigated model of cognitive impairment and psychosis in schizophrenia. There is extensive preclinical evidence supporting 5-HT_7 receptor antagonism as a promising mechanism for the treatment of cognitive deficits in schizophrenia as well as depression. The selective 5-HT_7 receptor antagonist SB-269970, as well as the atypical antipsychotic drugs (APDs), lurasidone and amisulpride, which along with other pharmacologic properties, are also potent 5-HT_7 receptor antagonists, have been found to improve the cognitive deficit induced by sub-chronic administration of PCP in rodents. The purpose of this study was to examine the effect of 5-HT_7 receptor blockade on acute PCP-induced hyperlocomotion, cognitive deficit (novel object recognition test, NOR) and efflux of monoamines and amino acids in mouse cortex and dorsal striatum (dSTR). **Method:** The effects of SB-269970 and PCP on locomotor activity, NOR deficit and the efflux of multiple neurotransmitters were studied in medial prefrontal cortex (mPFC) and dSTR by microdialysis following pretreatment in C57Bl/6 wild type (WT) and 5-HT_7 receptor constitutive knockout mice. The selective 5-HT_7 antagonist, JNJ18038683 to acutely reverse the deficit NOR produced by subchronic PCP treatment in WT mice was also studied. **Results:** Acute PCP (10 mg/kg, ip) induced hyperlocomotor activity, NOR deficit, and increased dopamine (DA), serotonin (5-HT), norepinephrine (NE), glutamate (Glu) and acetylcholine (ACh) in the mPFC and dSTR of wild type mice. The effects of PCP on locomotor activity and 5-HT and Glu, but not DA, NE or ACh, efflux, were suppressed in both regions of 5-HT_7 receptor knockout mice or after systemic pre-treatment with SB 269970 (3.0 mg/kg) in WT mice. JNJ18038683 acutely reversed the effect of subchronic PCP on NOR. C**onclusion:** These findings suggest that 5-HT_7 receptor blockade is an important target for improving cognitive impairment and possibly reducing psychotic symptoms. 5-HT_7R blockade can be incorporated into multi-receptor blocking agents, e.g. lurasidone and amisulpride, or added on, e.g. JNJ18038683. The reduced locomotor activity and NOR deficit induced by PCP after 5-HT_7R blockade or in 5-HT_7 receptor knockout mice, may be due to suppression of glutamatergic or serotonergic neurotransmission, or both.

Disclosures: **M. Huang:** None. **L. Rajagopal:** None. **S. Kwon:** None. **E.E. Michael:** None. **H.Y. Meltzer:** None.

Poster

051. Schizophrenia and Bipolar Disorder

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Scottish Funding Council Innovation Voucher

Title: Effects of phencyclidine in social groups of rats: Homecage monitoring of rodent behaviour

Authors: *R. R. BRETT[1], B. ALLISON[2], J. D. ARMSTRONG[3,2], J. A. PRATT[1];
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Abstract: Testing the effects of manipulations of animals on behaviour in the homecage in established social groups is limited by the difficulty of automatically recognising and monitoring individual animals and depends on time-consuming manual scoring. Using a prototype system which pairs high-quality video recording under infrared lighting with synchronised reads from a radio-frequency identification (RFID) baseplate using a grid of antennae reading at approximately 2Hz, we recorded group-housed animals continuously in their homecage. From location data, we derived each subject's position for each frame of video, giving rise to measures of locomotion and relative position between the animals. 18 male Sprague-Dawley rats (10-12 wks) housed in groups of 3 were implanted with RFID transponders. After habituation to the cage, environment and ip injection, rats were monitored for 72 hrs following an initial saline injection 15 min before lights out. At 24 hrs, one rat per cage received 5 mgkg-1 phencyclidine (PCP) ip and the others saline; at 48 hrs all rats received 5 mgkg-1 PCP. PCP administered to all animals induced a modest increase in locomotor activity over the first 2 hrs (mean distance moved/5 min after saline 14.9 ± 0.6 m, after PCP 16.8 ± 0.6 m, $p=0.05$) and a small but highly significant ($p<0.00001$) decrease in mean separation between cagemates. In the mixed treatment condition, we inferred which animal had received PCP, using a Wald test on a logistic regression with predictors for distance, separation and occupancy of centre zones. Decreased distance moved was modestly predictive ($p=0.078$); centre cage occupancy was highly predictive ($p<0.00001$). In this condition, acute PCP (15-45 min after injection, 1st 30 mins of dark period) significantly altered behaviour; by contrast with saline-treated rats, grooming and resting was virtually absent (resting: saline 685s/30min ± 184s; PCP 10s/30min ± 6s; grooming: saline 488s/30min ± 137s; PCP 19s/30min ± 9s), and treated rats showed head-weaving (76s/30min ± 15s) and rotation behaviour (41s/30min ± 13s). Interestingly, in established social groups of this age, little social behaviour was manifest at this time. We have demonstrated the feasibility of monitoring social groups of animals in the homecage over several days, with automatic scoring of locomotor and derived behaviours and manually scoring of social interactions of individual

animals. Work is ongoing to develop algorithms for automatic scoring of social behaviours to provide a platform for investigation of preclinical models of social deficit relevant to schizophrenia and autism research.

Disclosures: **R.R. Brett:** 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.; Actual Analytics. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Actual Analytics. **B. Allison:** A. Employment/Salary (full or part-time):; Actual Analytics. **J.D. Armstrong:** A. Employment/Salary (full or part-time):; Actual Analytics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Actual Analytics. **J.A. Pratt:** 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.; Actual Analytics. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Actual Analytics.

Poster

051. Schizophrenia and Bipolar Disorder

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 51.16/U1

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: Grant-in-Aid for Young Scientists B

Title: Glutamate stimulates astrocyte release of ATP: A potential mechanism for riluzole's antidepressant action

Authors: *T. YAMANASHI[1], M. KUSUNOSE[1], T. YAMAUCHI[1], K. T. OTA[2], M. IWATA[1], R. S. DUMAN[2], K. KANEKO[1];
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Abstract: Stress decreases neurogenesis and synaptogenesis in the adult hippocampus, leading to depressive-like behavior; however the mechanism by which stress causes neuronal damage is unknown. We previously demonstrated that stress increases ATP in the hippocampus, which stimulates the release of interleukin-1β (IL-1β) and causes decreased neurogenesis and depressive behavior. Those changes are ameliorated by the purinergic P2X7 receptor (P2X7R) antagonist (A-804598), indicating that ATP is critical for stress-induced depression. Here, we investigated the source of ATP induced by stress. Glutamate, an excitatory neurotransmitter, is another molecule that we have previously confirmed to be increased by stress. The released glutamate is taken up by the surrounding astrocytes and transformed into glutamine to maintain homeostasis of the synapse. Thus, we hypothesized that stress increases glutamate that is sensed by astrocytes, which in turn release ATP as a gliotransmitter. To test this hypothesis, we used rat astrocyte primary cell culture to examine ATP release. We found that glutamate (2 to 10 μM) releases ATP in astrocyte cell culture; thus the excess glutamate is a potential stimulus for stress-induced changes in ATP and inflammatory responses in the brain. We next investigated whether inhibition of excess glutamate can ameliorate the stress response induced by immobilization stress. Cortisol is a well-known stress marker, and we first confirmed that P2X7R antagonist (A-804598) suppresses the increase of cortisol, indicating that ATP regulates the increase of cortisol. Cortisol is easy to measure by peripheral blood, so we employed it as an indicator of stress reaction. Riluzole is a drug used for the treatment of amyotrophic lateral sclerosis, and it is thought to prevent glutamate release from presynaptic terminals and stimulate glutamate uptake in the synapse. We thus employed riluzole to reduce glutamate and then measured cortisol levels.

Riluzole was administered intraperitoneally one hour prior to 40 minutes of immobilization stress. The concentration of cortisol in serum was measured by ELISA. Riluzole decreased the increase of cortisol caused by immobilization stress. Together, the results support the hypothesis that stress increases glutamate, which is sensed by astrocytes and induces ATP release, which in turn induces pro-inflammatory responses, including up-regulation of cortisol. Riluzole is thought to be a potential drug for depression, and these mechanisms may contribute to its antidepressant effects.

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Poster

051. Schizophrenia and Bipolar Disorder

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Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: Singapore National Medical Research Council Translational and Clinical Research Program NMRC/TCR/003-GMS/2008 (X.Z)

Duke-NUS Block Funding (X.Z.)

Title: Pregnenolone sulfate normalizes schizophrenia-like behaviors in dopamine transporter knockout mice through AKT/GSK3β signaling via the NMDA pathway

Authors: Y. SZE, P. WONG, C. CHANG, *X. ZHANG;
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Abstract: Schizophrenia is a complex disorder with multiple pathophysiologies. Apart from anti-psychotics, there have been recent advances in the treatment of schizophrenia with alternative pharmacological agents such as neurosteroids. Pregnenolone sulfate (PregS), an endogenous neurosteroid in the central nervous system, is a positive allosteric modulator of the NMDA receptor and plays a role in the modulation of learning and memory. It was found that acute treatment of 80 mg/kg PregS could rescue hyperactivity, stereotypic activity and pre-pulse inhibition deficits in dopamine transporter knockout (DAT KO) mice, an established mouse model of schizophrenia. Additionally, ten day 40 mg/kg PregS treatment also rescued the cognitive deficits of DAT KO mice in the novel object recognition test and social transmission of

food preferences test. To elucidate the pathway in which PregS exerts its effects on, wild type (WT) mice were treated with 0.1 mg/kg MK-801, a known NMDA receptor antagonist. Acute PregS treatment (80 mg/kg) was found to completely abrogate MK-801-induced hyperactivity and pre-pulse inhibition deficits in WT mice, which suggests that the efficacy of PregS relies on NMDA receptor signaling. Since it is known that schizophrenic patients have impaired AKT/GSK3β signaling and that DAT KO mice have decreased GSK3β phosphorylation, we further investigated the possible involvement of the NMDA-mediated AKT/GSK3β signaling pathway in the action of PregS. WT and DAT KO mice were treated with 80 mg/kg PregS for 15, 30, and 60 min, and the phosphorylation levels of AKT and GSK3β in the striatum were measured by Western blotting. An increase in AKT and GSK3β phosphorylation levels, persistent for up to 60 min after drug administration, was observed in PregS-treated DAT KO mice. Moreover, long-term treatment with 40 mg/kg PregS also increased expression levels of NR1 subunit of the NMDA receptor in the hippocampus of DAT KO mice. Thus, it is likely that PregS rescues behavioral anomalies of DAT KO mice by directly exerting its effect through the NMDA receptor-mediated, AKT/GSK3β signaling pathway, and is therefore another potential alternative therapy in the treatment of schizophrenia.

Disclosures: **Y. Sze:** None. **X. Zhang:** None. **P. Wong:** None. **C. Chang:** None.

Poster

051. Schizophrenia and Bipolar Disorder

Location: Halls A-C

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Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: USPHS grant P50 MH103222

Swedish Medical Research Council 2013-2838

The AstraZeneca-Karolinska Institutet Joint Research Program in Translational Science

Title: Behavioral and electrophysiological abnormalities in mice deficient in kynurenine 3-monooxygenase; relevance to schizophrenia

Authors: *L. SCHWIELER[1], A. POCIVAVSEK[3], X. LIU[1], F. GIORGINI[4], P. J. MUCHOWSKI[5], G. ENGBERG[2], P. SHEPARD[3], S. ERHARDT[1], R. SCHWARCZ[3]; [2]Dept of Physiol. & Pharmacol., [1]Karolinska Inst., Stockholm, Sweden; [3]Maryland Psychiatric

Res. Ctr., Univ. of Maryland Sch. of Med., Baltimore, MD; [4]Dept. of Genet., Univ. of Leicester, Leicester, United Kingdom; [5]Neurolog. Dis., Gladstone Inst., San Fransisco, CA

Abstract: Dysregulation of the kynurenine pathway of tryptophan degradation has been implicated in the pathophysiology of schizophrenia and depression. Kynurenine 3-monooxygenase (KMO) is a key metabolic and regulatory enzyme in a pathway that includes several neuroactive metabolites including kynurenic acid (KYNA), an endogenous antagonist of α7 nicotinic acetylcholine and NMDA receptors (Sathyasaikumar et al., Schizophr. Bull., 2011). KMO activity is reduced in the brains of individuals with schizophrenia, and a polymorphism in the KMO gene has been linked to cognitive endophenotypes in the disorder (Wonodi et al., Arch. Gen. Psych., 2011). The decrease in cerebral KMO activity in schizophrenia is paralleled by increased brain levels of KYNA. Elevated KYNA levels are associated with behavioral deficits in rodents and may contribute to cognitive dysfunction. Mice (FVB/N background) with a targeted deletion in the KMO gene were recently generated in our laboratories (Giorgini et al., JBC, 2013). Homozygous (Kmo-/-) animals show chronically elevated brain KYNA levels comparable to those observed in post-mortem tissue from patients with schizophrenia. In the present study, we assessed and compared matched wild-type (WT), heterozygous (Kmo+/-), and Kmo-/-mice in 3 behavioral paradigms and measured the electrophysiological responses of dopaminergic neurons in the ventral tegmental area (VTA). Kmo+/- and Kmo-/- mice displayed deficits in a passive avoidance task including a significant reduction in avoidance latency during the retention trial (WT: 73 ± 9 sec; Kmo+/-: 43 ± 9 sec; Kmo-/-: 33 ± 9 sec; $P<0.05$). Kmo-/- mice also spent significantly more time in the closed versus the open arm of an elevated plus maze (WT: 13.75 ± 5.81 %, Kmo-/-: 4.33 ± 1.98 %, $P < 0.05$). Although Kmo+/- mice did not differ from WT mice in the elevated plus maze, they took longer to acclimate to an open field and showed an increase in rearing after an amphetamine challenge (2 mg/kg, i.p.). Extracellular single unit recordings obtained from anesthetized mice revealed an increase in the spontaneous firing rate of putative VTA dopamine neurons in Kmo+/- (5.6 ± 1.1 Hz) and Kmo-/- mice (5.6 ± 1.0 Hz) relative to WT mice (3.0 ± 0.4 Hz). These results indicate that reduction or elimination of KMO in mice is associated with deficits in contextual memory and an increase in anxiety accompanied by elevated activity in the mesolimbic dopamine system. Collectively, these findings provide new insights regarding the domains of psychopathology likely to be altered as a consequence of a dysregulated kynurenine pathway.

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Poster

051. Schizophrenia and Bipolar Disorder

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 51.19/U4

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: Danish Council for Strategic Research (COGNITO), the NOVO Nordisk Foundation, and the Lundbeck Foundation

Title: Positive allosteric modulators (PAMs) of the alpha7 nicotinic receptor potentiate the mesolimbic regulation of prefrontal glutamate release: Differential effects of Type I and Type II PAMs

Authors: *D. M. BORTZ[1], J. D. MIKKELSEN[3], J. P. BRUNO[2];
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Abstract: Research on cognition-enhancers has focused on drugs that act as agonists at the alpha7 nicotinic acetylcholine receptor (α7nAChR). More recently, effects of positive allosteric modulators (PAMs) of the α7nAChR have been investigated. PAMs are considered to better preserve cholinergic transmission because, unlike direct agonists, PAM are only effective when endogenous ACh/choline are present in concentrations able to activate the α7nAChR . NMDA stimulation of the nucleus accumbens shell (NACsh) in rats produces dose-dependent increases in PFC glutamate release that is mediated by increased activity at the α7nAChR, and this cortical nicotinic transmission is considered reduced in patients with schizophrenia (SZ). Because stimulation of the NACsh evokes the release of both ACh and glutamate, as well as promotes performance in a sustained attention task, we tested the hypothesis that PAMs would potentiate PFC glutamate release, but the magnitude of this potentiation would depend upon the dose of NMDA. We also tested compounds that are different in their ability to reduce α7nAChR desensitization (type II) or not (type I). Adult male Wistar rats were implanted with infusion cannulae into NACsh and a glutamate-sensitive biosensor in the ipsilateral mPFC. Rats received either vehicle (5% DMSO/8% solutal/saline), AVL3288 (type I), or PNU120596 (type II) for 3 consecutive test days. On a test day, rats were infused with NMDA at one concentration (vehicle, 0.05, or 0.30 µg/0.5 µL) and extracellular levels of glutamate were measured. Vehicle injections into NACsh did not evoke glutamate release and neither PAM affected this. Type I PAMs: The low dose of AVL (1 mg/kg) potentiated, relative to vehicle, the glutamate release seen after both the low (0.05 µg; 33.4% increase) and high (0.30 µg; 68.3% increase) doses of NMDA. The high dose of AVL (3 mg/kg) enhanced (152%) the low dose of NMDA but had no effect on the higher dose. Type II PAM: the lower dose of PNU (3 mg/kg) failed to potentiate either dose of NMDA. The higher dose of PNU (9 mg/kg) amplified (154%), relative to vehicle conditions, the low dose of NMDA but had no effect on the higher dose of NMDA. These *in vivo* data demonstrate that

the effects of PAMs on glutamate release in PFC vary as a function of the activity of local α7nAChRs. They also suggest important differences in the potentiation by Type I and II PAMs, making further comparisons very important in order to determine the conditions in which Type I or II would be indicated as a strategy for cognition enhancement.

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Poster

051. Schizophrenia and Bipolar Disorder

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 51.20/U5

Topic: C.15. Schizophrenia and Bi-polar Disorder

Title: Activation of nucleus accumbens stimulates the release of glutamate and dopamine in prefrontal cortex: Role of local nicotine receptors

Authors: *V. VALENTINI[1], D. M. BORTZ[2], V. PERRA[1], G. P. SERRA[1], G. DI CHIARA[1], J. P. BRUNO[2];
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Abstract: Reciprocal interactions, within a distributed neural system containing the nucleus accumbens (NAC), basal forebrain (BF), medial dorsal thalamus, and prefrontal cortex (PFC) are dysregulated in several neuropsychiatric disorders (e.g. schizophrenia, ADD, drug addiction). Previously we demonstrated that stimulation of the NACshell with NMDA evoked ACh release in PFC. This release was cognitively beneficial, increasing resistance to distractors in a sustained attention task. We also demonstrated, using a biosensor with sec-to-sec resolution, that such NMDA activation elevates prefrontal glutamate levels. The goals of the present experiments were to determine if a) NAC-evoked glutamate levels were also observed using more traditional microdialysis methods; b) NAC activation also increased DA release in PFC; and c) nicotinic (and alpha7) receptor activation, from the enhanced cholinergic transmission, was necessary for elevations in glutamate and DA levels. Adult male Wistar rats were implanted with an infusion cannula into the shell region of the NAC and either a biosensor (MEA) or microdialysis probe in the ipsilateral mPFC. NMDA (0.05, 0.15, or 0.30 μg/0.5 μL) was infused and extracellular levels of glutamate and DA were measured. In a separate group of animals, the role of nicotinic (alpha7) receptors in this stimulated release was determined following local perfusions of mecamylamine (10.0 or 100.0 μM) or α-bungarotoxin (α-BGT; 1.0 μM). Intra-NAC infusions of

NMDA produced dose dependent increases in extracellular glutamate as measured with the MEA (2.01± 0.32, 3.34 ± 0.37, and 4.56 ± 0.42 μM above baseline for each of the 3 doses, respectively). The evoked glutamate signal was rapidly cleared to basal levels in ~30 sec. Microdialysis-based measures also revealed an NMDA (0.30 μg) stimulation of glutamate efflux in PFC (75% increase). These levels were not cleared to baseline until 20 min later. Intra-NAC NMDA also increased prefrontal DA levels in PFC (100% increase). Evoked levels of glutamate and DA efflux (microdialysis) were dependent upon activation of nicotinic receptors (alpha7) as mecamylamine (10 μM) or α-BGT blocked the increases. Using two different methods, the results confirm the mesolimbic stimulation of prefrontal glutamatergic transmission. The marked difference in clearance times suggest different pools of glutamate may be sampled by the MEA vs the dialysis probe. Activation of NAC also stimulates DA release in PFC. The evoked release of glutamate and DA appear to be secondary to the release of ACh and a subsequent activation of local nicotinic (alpha7) receptors on glutamate and DA terminals.

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Poster

051. Schizophrenia and Bipolar Disorder

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Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: Centers of the National Institute for Translational Medicine (INCT-TM)

Center of Excellence in Applied Neurosciences of Santa Catarina (NENASC)

CNPq

Title: MAPK signaling correlates with the antidepressant effects of ketamine

Authors: ***G. Z. RÉUS**[1], F. G VIEIRA[2], H. M ABELAIRA[2], M. MICHELS[3], M. B DOS SANTOS[2], D. D LEFFA[4], D. B TOMAZ[2], A. S CARLESSI[2], F. PETRONILHO[4], J. QUEVEDO[4];
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Abstract: Studies have pointed to a relationship between MAPK kinase (MEK) signaling and the behavioral effects of antidepressant drugs. So, in the present study we examined the behavioral and molecular effects of ketamine, an antagonist of the N-methyl-Daspartate receptor (NMDA), which has been shown to have an antidepressant effect after the inhibition of MEK signaling in Wistar rats. Our results showed that acute administration of the MEK inhibitor PD184161, produced depressive-like behavior and stopped antidepressant-like effects of ketamine in the forced swimming test. The phosphorylation of extracellular signal-regulated kinase 1/2 (pERK 1/2) was decreased by PD184161 in the amygdala and nucleus accumbens, and the effects of ketamine on pERK 1/2 in the prefrontal cortex and hippocampus were inhibited by PD184161. The ERK 2 levels were decreased by PD184161 in the nucleus accumbens; and the effects of ketamine were blocked in this brain area. The p38 protein kinase (p38MAPK) and proBDNF were inhibited by PD184161, and the MEK inhibitor prevented the effects of ketamine in the nucleus accumbens. In addition, ketamine increased pro-BDNF levels in the hippocampus. In conclusion, our findings demonstrated that an acute blockade of MAPK signaling lead to depressive-like behavior and stopped the antidepressant response of ketamine, suggesting that the effects of ketamine could be mediated, at least in part, by the regulation of MAPK signaling in these specific brain areas.

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Poster

051. Schizophrenia and Bipolar Disorder

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Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: NIH Grant NS050658-01A1

Title: Inhibition of DOI-induced head twitch response in male DBA/2j mice by D3 dopamine receptor selective compounds

Authors: *S. A. GRIFFIN[1], C. RANGEL-BARAJAS[2], M. MALIK[2], R. H. MACH[3], R. R. LUEDTKE[2];
[2]Pharmacol. & Neurosci., [1]Univ. North Texas Hlth. Sci. Ctr., FORT WORTH, TX; [3]Radiology, Univ. of Pennsylvania Sch. of Med., Philadelphia, PA

Abstract: It continues to be a challenging task to a) identify animal models that can mimic and b) identify reliable *in vivo* methods for screening novel chemical entities for antipsychotic activity. Behavioral pharmacology studies have indicated that hallucinogenic drugs are capable of inducing psychotic episodes and cognitive deficits in humans that resemble some of the symptoms observed in schizophrenia patients. We previously reported on the ability of D2-like and D2 dopamine receptor selective compounds to attenuate the head twitch response (HTR) in DBA/2J mice. For these studies the HTR was induced by the administration of the hallucinogen 2,5-dimethoxy-4-methylamphetamine (DOI), which is an agonist of 5-HT2a and 5-HT2c serotonin receptors. In these studies, D3 dopamine receptor selective compounds of varying binding selectivity and intrinsic efficacy were evaluated for the ability to attenuate the DOI-dependent HTR. DOI (5 mg/kg i.p.) was administered 5, 60, 180 and 360 minutes after a test compound. The HTR was then monitored for 30 min after DOI administration. The effect of the *N*-phenylpiperazine WC 10, which is a D3 dopamine receptor antagonist that exhibits 40-fold binding selectivity for D3 vs. D2 dopamine receptors, was investigated. WC 10 administration produced a dose-dependent decrease in the DOI-induced HTR (IC50 = 3.7 mg/kg). WC 44, a full D3 receptor selective agonist, also inhibited the DOI-induced HTR (IC50 = 5.1 mg/kg). The effect of two partial agonists, WW-III-55 and LAX-4-136, (800- and 150-fold D3 vs. D2 binding selectivity, respectively), were also evaluated. Both compounds inhibited the DOI-induced HTR with similar potency but with different maximum efficacies. At a dose of 5 mg/kg WW-III-55 produced a HTR inhibition of 95%, while LAX-4-136 produced 50% inhibition. Finally, an assessment of the motor and coordination effects of our test compounds was performed using a rotarod test. None of the test compounds caused significant alterations in the motor activity and coordination. These results suggest that the D3 dopamine receptor selective phenylpiperazine analogs may represent a class of novel antipsychotic/antihallucinogenic drugs devoid of the motor side effects associated with the blockade of the D2 dopamine receptor subtype.

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Poster

051. Schizophrenia and Bipolar Disorder

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Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: Statutory Funds of the International Institute of Molecular and Cell Biology

Title: In lithium treated mice β-catenin translocates to nuclei of thalamic neurons in TCF7L2-dependent manner

Authors: K. MISZTAL[1], N. BROZKO[1], A. NAGALSKI[1], L. SZEWCZYK[1], M. B. WISNIEWSKA[1,2], *J. KUZNICKI[1];
[1]IIMCB, Warsaw, Poland; [2]Lab. of Mol. Neurobio., Ctr. of New Technologies, Warsaw Univ., Warsaw, Poland

Abstract: Lithium has been used as a common mood stabilizer and the most effective treatment of bipolar disorder. Yet, its mechanism of action in the brain remains obscure. *In vitro* lithium stabilizes β-catenin level in the cytoplasm by inhibiting glycogen synthase kinase α/β (GSK3α/β), what leads to translocation of β-catenin to the nucleus and subsequent activation of LEF1/TCFs dependent gene transcription. Here we analyze how chronic lithium treatment affects brain β-catenin *in vivo*. C57BL/6 mice were given LiCl in drinking water for 7 days which resulted in the serum and brain levels of lithium known to be therapeutic for patients. By immunoblotting of cellular fractions we detected in the thalamus two-fold increase in nuclear β-catenin level. However, no changes in β-catenin were observed in nuclear fractions from the hippocampus and cortex. Using primary neuronal cultures we investigated the mechanism underlying activation of β-catenin in the thalamus. We hypothesized that TCF7L2, a transcription factor that is highly expressed in thalamic neurons, promotes its nuclear translocation. Using shRNA technology we found that TCF7L2 is indispensable for β-catenin nuclear localization in thalamic cells. Even when β-catenin accumulated upon proteasome inhibition, it stayed in the cytoplasm in TCF7L2 knocked-down cells. On the other hand, in cortical neurons where β-catenin is normally undetectable in the nucleus, LiCl treatment and the ectopic expression of TCF7L2 stabilized β-catenin and induced its transfer to nuclei. Thus, under experimental conditions, TCF7L2 transcription factor is indispensable for β-catenin nuclear localization in neurons. We earlier showed that TCF7L2 is highly expressed in the thalamus and acts as a regulator of several genes for voltage-gated ion channels and neurotransmitter receptors that are predominantly expressed in the thalamus. We conclude that *in vivo* lithium treatment can activate β-catenin signaling pathways specifically in the thalamus to maintain excitability of thalamic neurons.

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Poster

051. Schizophrenia and Bipolar Disorder

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Program#/Poster#: 51.24/U9

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: Stanley Medical Research Institute (TLP)

Massachuetts General Hospital Executive Committee on Research (MPL)

Title: Does the bipolar disorder risk gene ankyrin 3 have a synaptic function?

Authors: *M. P. LEUSSIS[1], O. DURAK[2], M. SAITO[3], E. M. BERRY-SCOTT[4], F. CALDERON DE ALDA[2], L.-H. TSAI[2], T. L. PETRYSHEN[3];
[1]Psychology Dept., Emmanuel Col., BOSTON, MA; [2]MIT, Cambridge, MA; [3]Massachusetts Gen. Hosp., Boston, MA; [4]The Broad Inst., Cambridge, MA

Abstract: Ankyrin 3 (ANK3) is a significant risk gene for bipolar disorder identified in recent genome-wide association studies (GWAS). Investigating the disease-relevant function of ANK3 in brain may increase our understanding of the pathological processes implicated in bipolar disorder. We hypothesize that ankyrin G has a synaptic function in the mammalian brain, perhaps through scaffolding critical proteins to the post-synaptic membrane, similar to its role at the axon initial segment. This study sought to establish the presence of ankyrin G at the post-synaptic density. Further, it evaluated whether reductions in Ank3 expression could alter synapse-associated markers including PSD-95 and assessed dendritic spine density of neurons in mice with decreased Ank3 expression by either RNA interference or conventional transgenic knockout of Ank3. We also conducted Scholl analyses to evaluate differences in dendritic branching patterns following Ank3 reduction in mouse brain. We detected several isoforms of ankyrin G in postsynaptic fractions prepared from C57BL/6 mouse forebrain, indicating that ankyrin G is present at the synapse and may have several functions mediated by different isoforms. Decreased ankyrin G levels, by RNA interference or in Ank3+/- or -/- mice, were associated with altered dendritic synaptic spine density and expression of the synapse associated proteins PSD95 (postsynaptic) and synapsin (presynaptic) in the hippocampus. The changes in spine density were dependent on the location along the dendritic arbor, suggesting these changes may have different effects on neuronal activity. Further, alterations in synaptic proteins were largely normalized by chronic treatment with the mood stabilizer lithium. Mice with reduced Ank3 also exhibited a decrease in dendritic branching complexity in neurons of the granule cell layer of the hippocampal dentate gyrus. The presence of ankyrin G at the postsynaptic density implies a synaptic role for this protein in this dynamic neuronal structure. This is further supported by the changes observed in synaptic-associated proteins in mice with reduced Ank3 expression. The importance of these findings to bipolar disorder is yet to be determined, however reversal of the synaptic changes by chronic lithium treatment strongly supports such a potential role. Synaptic disturbances have been increasingly implicated in numerous psychiatric diseases

including bipolar disorder. Extending the known functions of ANK3 in the brain may help elucidate the neurobiological processes contributing to bipolar disorder.

Disclosures: **M.P. Leussis:** None. **O. Durak:** None. **M. Saito:** None. **E.M. Berry-Scott:** None. **F. Calderon de Alda:** None. **L. Tsai:** None. **T.L. Petryshen:** None.

Poster

051. Schizophrenia and Bipolar Disorder

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 51.25/U10

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: TV Tran, DK Dang, THT Tu, and Y Nam are involved in BK21 PLUS program, National Research Foundation of Korea

A grant (14182MFDS979) from Ministry of Food and Drug Safety in 2014, Republic of Korea

Title: Lithium or valproate attenuates mania-like behaviors induced by d-amphetamine via modulating PKC δ, prodynorphin, and substance P

Authors: ***Y. NAM**[1,2], T.-V. TRAN[2], D.-K. DANG[2], J. CHEONG[3], T.-H. TU[2], E.-J. SHIN[2], Q. WANG[4], J.-S. HONG[4], H.-C. KIM[2];
[1]Dept. of Pharmacology, Col. of Med., Chun-Ang Univ., Seoul, Korea, Republic of; [2]Neuropsychopharm. and Toxicology Program, Col. of Pharm., Kangwon Natl. university, Chunchon, Korea, Republic of; [3]Col. of Pharm., Samyook Univ., Seoul, Korea, Republic of; [4]3Neuropharmacology Section, Lab. of Toxicology and Pharmacology,, Natl. Inst. of Envrn. Hlth. Sci., Research Triangle Park, NC

Abstract: Bipolar disorder (BD) is a type of mental illness, specifically a mood disorder, characterized by episodes of an agitated mood known as mania, with or without distinct episodes of depression. There is an emerging body of data suggesting that BD is associated with the dysregulation of PKC signaling cascade as well as dynorphin opioid neuropeptide system. In this context, the present study aims to investigate the effect of mood stabilizers lithium (LiCl) and valproate (VPA) on the protein expression of phospho-PKC δ and mRNA expressions of the prodynorphin and substance P in the prefrontal cortex and hippocampus of mice undergoing treatment with the pro-maniac agent d-amphetamine (d-AMP). Mice were pretreated with LiCl

or VPA for consecutive five days, then were given d-AMP for ten days continuously. Locomotor activity was assessed directly 30 minutes or 24 hours after the last dose of d-AMP and expressions of phosho-PKC δ, prodynorphin, and substance P were examined in prefrontal cortex and hippocampus. LiCl or VPA significantly attenuated d-AMP-induced hyperlocomotion. Increases in protein expression of phospho-PKC δ, mRNA expression of substance P, while decreases in mRNA expression of prodynorphin induced by d-AMP was also attenuated by treatment of LiCl or VPA. To confirm the role of PKC δ, prodynorphin and substance P in d-AMP-induced mania model, we applied PKC δ knockout and prodynorphin knockout mice, or in combination with neurokinin 1 (substance P) receptor antagonist L-733060**.** D-AMP-induced hyperlocomotion was significantly attenuated in PKC δ knockout and prodynorphin knockout mice, as well as in combined treatment with L-733060. Taken together, the results of this study suggest that PKC δ, prodynorphin, or substance P gene plays a mechanistic role in the pathogenesis of BD. [TV Tran, DK Dang, THT Tu, and Y Nam are involved in BK21 PLUS program, National Research Foundation of Korea. This research was supported by a grant (14182MFDS979) from Ministry of Food and Drug Safety in 2014, Republic of Korea]

Disclosures: **Y. Nam:** None. **T. Tran:** None. **D. Dang:** None. **J. Cheong:** None. **T. Tu:** None. **E. Shin:** None. **Q. Wang:** None. **J. Hong:** None. **H. Kim:** None.

Poster

051. Schizophrenia and Bipolar Disorder

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 51.26/U11

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: NIH RC2 Grant MH090011-1

Title: Identification of possible bipolar 1 disorder related brain areas and lithium drug targets via iPSC derived neurons

Authors: *C. D. PERNIA, B. TOBE, A. CRAIN, A. WINQUIST, E. SNYDER;
The Sanford Burnham Med. Res. Inst., La Jolla, CA

Abstract: Bipolar 1 Disorder (BD1) is a psychiatric illness that affects 2.6% of the adult US population and is characterized by severe fluctuations between manic and depressive episodes. One of the foremost therapeutics for BD1 is lithium, which interestingly is not efficacious for

any other major psychiatric disorder. Lithium is a potent mood stabilizer but clinicians reluctantly prescribe it due to its serious medical side effects. How and where in the brain lithium acts to treat BD1 has yet to be elucidated. Psychiatric disorders have historically been difficult to study at the cellular and molecular level because of the lack of relevant animal behavioral models, and the ethical dilemma of studying human subjects. We hypothesized that studying the lithium response pathway in mixed neuronal cultures derived from human BD1 iPSCs would reveal novel insights into the mechanism of action of lithium in BD1, and by utilizing expression data of lithium sensitive targets indicate regions of the human brain involved with BD1. This study utilizes a comparative proteomic approach, sensitive to post-translational modifications, combined with human iPSC technologies for understanding the therapeutic mechanism of lithium on BD1. Our *in vitro* screen identified at least 15 proteins that are affected by lithium treatment in human BD1 derived neurons, which we cross-referenced with brain structure microarray data from the Allen Brain Atlas to identify areas of the brain where therapeutic action, and perhaps regions of lithium action and or BD1 pathology, could be most pertinent. Four brain areas were identified by their high expression levels of these proteins of interest, which we compared to the expression profiles of other brain areas postulated to be associated with BD1. Our findings substantiate previous human fMRI studies' assertions of specific brain areas being implicated in BD1, and identifies new areas that have yet to be systematically studied in BD1, and also provides a list of relevant drug targets for future research.

Disclosures: **C.D. Pernia:** None. **B. Tobe:** None. **A. Crain:** None. **A. Winquist:** None. **E. Snyder:** None.

Poster

052. Schizophrenia: Mutant Models

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 52.01/U12

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: NIH Grant MH094268

Title: mTOR hyperactivation and behavioral deficits in a Notch1 mouse model: Implication in major mental illness, especially schizophrenia

Authors: *H. JAARO-PELED[1], M. A. LANDEK-SALGADO[1], T. CASH-PADGETT[1], S. LEE[1], H. HIYAMA[1,2], K. NI[2], A. SAWA[1];

[1]Psychiatry and Behavioral Sci., Johns Hopkins Univ., Baltimore, MD; [2]Neurosci., Astellas Pharma Inc., Tsukuba-shi, Japan

Abstract: The Notch pathway is one of the major cell-cell signaling pathways regulating cell differentiation and development. The role for Notch during brain development is fully established, but the significance of Notch signaling in adult brain remains elusive. In the present study, we initially came across the Notch cascade as a pathway robustly down-regulated in olfactory “neuronal” epithelium in schizophrenia patients compared with normal controls. Similar down-regulation was also observed in the prefrontal cortex of a mouse model of psychosis elicited by sub-chronic administration of phencyclidine. Based on these foundations, we have studied Notch1 haploinsufficient mice with emphasis on prefrontal cortex-dependent behaviors. We found that although Notch1 males behaved normally in the sociability phase of the three-chamber social interaction test, they did not show a preference towards a novel mouse in the social novelty phase of the test. Since the Mechanistic target of rapamycin (mTOR) pathway has been shown to be involved in perseverative behavior in autism mouse models, we assayed mTOR activity in the prefrontal cortex of the Notch1 mice and detected hyperactivation. Hence we treated the mice with the mTOR complex 1 inhibitor rapamycin during the three habituation days to the three-chamber apparatus. This treatment rescued the social novelty deficit. We are currently testing whether antipsychotics, which in general are not very efficient against negative and cognitive symptoms of schizophrenia, can also rescue this behavior. Interestingly, females Notch1 mice seem to behave normally in the social novelty test. Thus we are currently also assessing the mTOR pathway in female Notch1 mice. To conclude, the ability to rescue the social behavior by short treatment in adulthood suggests that the impaired social behavior is dependent on the real-time function of mTOR downstream of Notch1 and not on the developmental roles of Notch1.

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Poster

052. Schizophrenia: Mutant Models

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 52.02/U13

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: NIH grant R01MH05190

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Japanese grant: Strategic young researcher overseas visits program for accelerating brain circulation

Title: Subchronic pharmacologic nmda receptor antagonism with MK801 activates Akt signaling pathways

Authors: S. TAKAGI[1,2], D. T. BALU[1], *J. T. COYLE[1];
[1]McLean Hosp., Belmont, MA; [2]tokyo medical and dental university, Tokyo, Japan

Abstract: NMDA receptor (NMDAR) hypofunction is a powerful hypothesis for the pathophysiology of schizophrenia, because in part, NMDAR antagonists like phencyclidine and MK801 cause symptoms in healthy subjects that are similar to schizophrenia. Therefore, NMDAR antagonists have been used as a tool to induce NMDAR hypofunction in animals as a pharmacologic model of schizophrenia. Our laboratory has previously generated serine racemase-null mutant (SR-/-) mice, which display constitutive NMDAR hypofunction due to the lack of the NMDAR co-agonist, D-serine. SR-/- mice have deficits in multiple pathways, including V-akt murine thymoma viral oncogene (Akt) signaling and glycogen synthase 3 kinase (GS3K), which parallel what is observed in schizophrenia. Although some pharmacological NMDAR hypofunction models utilize sub-chronic NMDAR antagonist administration (5-7 days), our SR-/- mice have life-long NMDAR hypofunction. Thus, we analyzed intracellular signaling pathways in MK801 sub-chronically (0.15 mg/kg; o.d; 5 days) treated adult wild-type mice that are reduced in SR-/- mice and schizophrenia. We found that in contrast to SR-/- mice, the phosphorylation (activated) states of Akt1, GS3K, and mammalian target of rapamycin (mTOR) were increased in MK801 treated mice. Furthermore, there is a notable age-dependent change in the behavioral reaction of people to NMDAR antagonists. We therefore administered the same dosing regimen of MK801 to juvenile mice (3-4 weeks old) and compared them to juvenile SR-/- mice. Our findings demonstrate that sub-chronic, pharmacologic NMDAR antagonism has different effects on Akt/GS3K/mTOR signaling than constitutive NMDAR hypofunction caused by a deficit in D-serine. Considering the concordance with schizophrenia, our results suggest that SR-/- mice are a more accurate NMDAR hypofunction model of schizophrenia.

Disclosures: **S. Takagi:** None. **D.T. Balu:** None. **J.T. Coyle:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified

mutual funds); A patent owned by Massachusetts General Hospital for the use of D-serine as a treatment for serious mental illness could yield royalties for Dr. Coyle. F. Consulting Fees (e.g., advisory boards); served as a consultant for EnVivo, and Abbvie in the last 2 years.

Poster

052. Schizophrenia: Mutant Models

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 52.03/U14

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: NARSAD Independent Investigator Award

Title: A double hit mouse model of schizophrenia: Chronic stress impairs latent inhibition in CHL1 deficient mice

Authors: *J. D. OBRAY, B. Z. YANG, B. GUERCIO, M. BUHUSI, C. V. BUHUSI;
Dept. of Psychology, Utah State Univ., Logan, UT

Abstract: Latent inhibition (LI) is an adaptive attentional phenomenon in which preexposure to a neutral stimulus decreases the future associability of the preexposed stimulus. LI is attenuated in patients with schizophrenia, and is reinstated by administration of typical antipsychotic medications. A number of genetic mutations and gene polymorphisms have been found to be associated with an increased risk for the development of schizophrenia. The close homolog of L1 (CHL1) adhesion molecule is involved in brain development and plasticity. CHL1-deficient mice exhibit anatomical changes also observed in individuals with schizophrenia. In individuals with a genetic predisposition to schizophrenia it may be necessary for a precipitating event (such as stress) to occur, constituting a double hit, for symptoms of schizophrenia to appear. In this study, we have evaluated the effects of stress on inducing behavioral deficits characteristic of schizophrenia (impaired latent inhibition) in CHL1 knockout mice (KO), heterozygous mice (HET), and wild type littermate controls (WT). Mice were divided into two groups: mice in the STRESS condition received 6 weeks of chronic mild stress beginning at about 6 weeks of age, while NO-STRESS mice were not manipulated. Upon reaching adulthood (12 weeks of age), all mice were tested in the LI paradigm as follows: Mice were repeatedly exposed to a preexposed (PE) stimulus. Following preexposure, a foot shock was paired with both the PE stimulus and a new, non-preexposed (NPE) stimulus. Conditioned freezing during the PE and NPE stimuli was then assessed two days following conditioning, and the results were analyzed statistically. The results indicate that preexposure reliably decreases freezing, and that the preexposure effect is

reliably larger in the NO-STRESS condition. Most importantly, analyses also indicated a reliable interaction between stress and genotype: While KO mice in the NO-STRESS condition showed reliable LI (significant decrease in freezing to the PE relative to the NPE stimulus), KO mice in the STRESS condition did not show LI (no difference in freezing to the PE and NPE stimuli), indicating an interaction between genotype and stress in latent inhibition, thus providing support for a double hit model of schizophrenia.

Disclosures: **J.D. Obray:** None. **B.Z. Yang:** None. **B. Guercio:** None. **M. Buhusi:** None. **C.V. Buhusi:** None.

Poster

052. Schizophrenia: Mutant Models

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 52.04/U15

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: MOST 102-2420-H-002-008-MY2 to WS Lai

MOST 102-2628-H-002-003-MY3 to WS Lai

grants Drunken Moon Lake Integrated Scientific Research Platform from NTU to WS Lai

grants Aim for Top University Project from NTU to WSLai

Title: The effect of lithium on the alleviation of neuromorphological and behavioral deficits in AKT-promoted P19 embryonal carcinoma cells and Akt1 mouse model of schizophrenia

Authors: *C.-Y. CHANG[1], D.-Z. DA[1], T.-W. WANG[4], W.-S. LAI[1,2,3];
[1]Psychology, [2]Grad. Inst. of Brain and Mind Sci., [3]Neurobio. and Cognitive Sci. Ctr., Natl. Taiwan Univ., Taipei, Taiwan; [4]Dept. of Life Sci., Natl. Taiwan Normal Univ., Taipei, Taiwan

Abstract: Schizophrenia is a severe neuropsychiatric disorder with a strong genetic predisposition. Accumulating evidence from human genetic and animal studies suggests that AKT1 is a susceptibility gene for schizophrenia. Emerging evidence indicates that a sex-specific role of Akt1 in the modulation of methamphetamine induced hyperlocomotion, depression-like behavior, sensorimotor gating function, and neuromorphology. Our recent study also revealed that Akt1-deficit mice are insensitive to antipsychotic drugs, but GSK3 (a key downstream kinase for Akt1) inhibitor could have therapeutic potential. Given the fact that lithium is a GSK3

inhibitor and a mood-stabilizing drug for the treatment of bipolar disorder, it is of great interest to evaluate the therapeutic potential of lithium on the alleviation of Akt1-related deficits. Taking advantage of P19 embryonal carcinoma cells and Akt1 heterozygous mutant (Akt1+/-) mice as our models, a series of experiments was conducted *in vitro* and *in vivo*. In study 1, using Ascl1 to differentiate P19 embryonal carcinoma cells into neurons, we examined the effect of AKT1/2 inhibitor on the neuromarphological alterations in DIV2 and the rescue effect of lithium *in vitro*. Quantitative analyses of Tuj1-immunostained P19-derived neurons revealed that AKT1/2 inhibitor resulted in a 33% reduction of neurite length but no difference was found in the number of differentiated neurons. The reduction of neurite outgrowth was rescued by the treatment of Lithium (0.5 & 1 mM). In study 2, based on our previous and current findings, Akt1+/- female mice and their wild-type littermate controls were used and they received chronic treatment of lithium (100 mg/kg, i.p., twice per day) or saline. A set of 3 behavioral tasks were performed. Compared to WT controls, chronic treatment of lithium alleviated behavioral impairments in the tail suspension task and acoustic PPI in Akt1+/- mice. The treatment of lithium also dampened methamphetamine-induced stereotypic behaviors in both groups compared to their saline controls. Collectively, out findings imply the therapeutic potential of lithium for the treatment of schizophrenia and the importance of GSK3 as a new therapeutic target.

Disclosures: **C. Chang:** None. **D. Da:** None. **T. Wang:** None. **W. Lai:** None.

Poster

052. Schizophrenia: Mutant Models

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 52.05/U16

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: NARSAD (AL)

Title: The effects of fingolimod administration in dysbindin-1 null-mutant mice, a genetic model for cognitive deficits

Authors: *D. D. BECKER-KRAIL[1], A. LAVIN[2];
[2]Neurosci., [1]Med. Univ. of South Carolina, Charleston, SC

Abstract: Schizophrenia is a complex, heritable neuropsychiatric disorder characterized by a range of positive, negative, and cognitive type symptoms. Schizophrenia related deficits in sociability and memory may be associated with diminished expression of the dystrobrevin-

binding protein 1 (dysbindin-1). The lab has previously shown lacking dysbindin-1 reduces glutamate release in the prefrontal cortex (PFC) through decreases in the ready releasable pool of synaptic vesicles, decreased rates of exo- and endocytosis, and diminished expression of L- and N-type Ca^{2+} channels. Fingolimod, a drug historically used to treat multiple sclerosis and rett syndrome, is known to increase endogenous levels of brain derived neurotrophic factor (BDNF), and in turn, it has been shown that BDNF increases N-type Ca^{2+} channels. To explore a potential means of restoring glutamate release, and perhaps improving the cognitive deficits, we investigate the effects of fingolimod using a dysbindin-1 null mutant mouse. The mice were divided into two groups: saline or fingolimod treatment (1 mg/kg, IP injection; 7 days). We assessed sociability and memory across three genotypes (WT, HET, and MUT) using both the social choice/approach and preference for social novelty tasks. For both groups, we assayed BDNF concentration in PFC homogenate using an ELISA, and analyzed presynaptic intracellular $[Ca^{2+}]$ in a crude PFC synaptosome preparation using a Fluo-4 Ca^{2+} assay. Relative to WT mice, non-treated dysbindin-1 MUT mice demonstrated impairments in novel social interaction, deficits in memory as measured through preference for social novelty, and a lower presynaptic intracellular $[Ca^{2+}]_{PFC}$. However, fingolimod treated null MUT mice show a significant increase in social interaction with novel mice, significantly improved memory as measured through preference for social novelty, higher $[BDNF]_{PFC}$, and an increase in presynaptic intracellular $[Ca^{2+}]_{PFC}$. These results show promise for counteracting social and cognitive deficits associated with schizophrenia, and may illuminate the possible role of dysbindin-1 in symptom pathogenesis.

Disclosures: **D.D. Becker-Krail:** None. **A. Lavin:** None.

Poster

052. Schizophrenia: Mutant Models

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

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Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: NIH Grant DA015036

NIH Grant MH51290

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Internal Grant from the O'Keefe Family

ONDCP Contract Number DBK39-03-C-0075

Title: Magnetic resonance imaging reveals neuroanatomical and neurochemical homologies between the serine racemase knockout mouse and schizophrenia

Authors: *M. D. PUHL, D. MINTZOPOULOS, J. E. JENSEN, T. E. GILLIS, G. T. KONOPASKE, M. J. KAUFMAN, J. T. COYLE;
Psychiatry, Harvard Med. School, McLean Hosp, Belmont, MA

Abstract: Schizophrenia results from a complex combination of genetic, epigenetic, and environmental factors. A prominent hypothesis proposes that activity of the N-methyl-D-aspartate receptor (NMDAR) is decreased in individuals with schizophrenia compared to healthy controls. This NMDAR hypofunction may, in part, be due to decreased availability of D-serine, an NMDAR co-agonist. In fact, the genes encoding serine racemase (SR; the synthetic enzyme for D-serine), D-amino acid oxidase (DAAO; the degradation enzyme for D-serine), and the DAAO activator G72 are risk genes for schizophrenia. Evidence suggests that one of the consequences of NMDAR hypofunction is a compensatory increase in glutamate and/or glutamine in the medial prefrontal cortex (mPFC), which, in turn, may lead to overstimulation of fast-firing, parvalbumin-positive γ-aminobutyric acid (GABA) interneurons and increased GABA levels. It is thought that these abnormalities directly relate to the negative symptoms and cognitive impairments that are hallmarks of the disorder. Furthermore, schizophrenia is characterized by neuroanatomical abnormalities, including cortical atrophy accompanied by ventricular enlargement. Given the complexity of the disease, it is essential that valid animal models are developed so that neural substrates and novel therapeutic targets can be identified. Our laboratory has developed a transgenic mouse line with a constitutive deletion of exon 1 of the SR gene, which encodes the catalytic domain of the enzyme. Null mutants (SR-/-) from this line exhibit NMDAR hypofunction. Using ultra-high magnetic field (9.4 Tesla) *in vivo* magnetic resonance structural imaging (MRI) and ultra-short echo time proton magnetic resonance spectroscopy (MRS), the current study investigated whether the neuroanatomical and neurochemical abnormalities similar to those evident in schizophrenia also occur in SR-/- mice. SR-/- mice and wildtype (WT) controls were anesthetized with isoflurane for MRI and MRS scans. Compared to WT controls, SR-/- mice exhibited an increase in ventricular volume ($p<0.05$). Additionally, in a 15 µl mPFC voxel, SR-/- mice exhibited significantly increased GABA/creatine and GABA/water ratios ($ps<0.01$), similar to findings in treated and untreated humans with schizophrenia. Collectively, these data demonstrate *in vivo* neuroanatomical and neurochemical homologies between the SR-/- NMDAR hypofunction mouse model and humans with schizophrenia.

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come to an institution.; NARSAD, PhotoThera, Inc., Michael J. Fox Foundation for Parkinson's Research, Air Products and Chemicals, Inc. **J.T. Coyle:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); A patent owned by MGH for the use of D-serine to treat serious mental illness could yield royalties.. F. Consulting Fees (e.g., advisory boards); Abbott, Jansen Pharmaceutical, Puretech, En Vivo.

Poster

052. Schizophrenia: Mutant Models

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 52.07/U18

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: Roche Postdoc Fellowship Program

Title: D2 and DTNBP1 genetic interaction: Relevance to schizophrenia

Authors: *S. GUADAGNA[1], H. HUANG[1], E. BORRELLI[2], T. BALLARD[3], F. PAPALEO[1]; [1]Dept. of Neurosci. and Brain Technol., Italian Inst. of Technol., Genoa, Italy; [2]Dept. of Microbiology and Mol. Genet., Univ. of California, Irvine, CA; [3]Neurosci. Discovery, pRED, F. Hoffmann-La Roche Ltd, Basel, Switzerland

Abstract: Schizophrenia is a complex polygenic disorder with a strong genetic component. Despite other systems possibly being implicated, the pathophysiology of schizophrenia, its treatment and aspects of the associated cognitive deficits have been consistently linked to dysregulation of dopaminergic neural transmission and in particular of dopamine D2 receptor pathways. Two distinct isoforms of D2 receptors are encoded by the D2 gene: the short form D2S and the long form D2L. Interestingly, recent results indicate that functional genetic variants in the D2 gene might modulate schizophrenia-related phenotypes by modifying D2S/D2L ratio. Dystrobrevin-binding protein 1 (DTNBP1) is one of the leading candidate susceptibility genes for schizophrenia. DTNBP1 gene expression and its protein levels are reported to be significantly reduced in patients with schizophrenia. Moreover, genetic variations reducing DTNBP1 expression impact cognitive abilities and lead to up-regulation of D2 receptors on the neural surface. Based on this biological evidence, we generated a novel mouse model bearing selective mutations of both the D2 and DTNBP1 schizophrenia-susceptibility genes. We then predict that synergistic effects of a reduced dysbindin-1 (dys) expression and an increased D2S/D2L ratio in the same subject might alter the dopamine/D2 signaling triggering cognitive- and schizophrenia-

relevant symptoms. Using this model we are finding alterations of the startle response and prepulse inhibition in the dys/D2L double heterozygous knock-out (KO) mice but not in single heterozygous KO mice. Preliminary tests with a 5-choice serial reaction time task paradigm revealed also increased impulsivity in these mice compared to wild type and single dys and D2L heterozygous KO mice. Taken together, these results represent the first evidence suggesting a functional interaction between mutations in the DTNBP1 and D2 genes in the expression of behavioral phenotypes relevant to schizophrenia.

Disclosures: **S. Guadagna:** 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.; F. Hoffmann-La Roche Ltd. **H. Huang:** None. **E. Borrelli:** None. **T. Ballard:** A. Employment/Salary (full or part-time):; F. Hoffmann-La Roche Ltd. **F. Papaleo:** 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.; F. Hoffmann-La Roche Ltd.

Poster

052. Schizophrenia: Mutant Models

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

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Topic: C.15. Schizophrenia and Bi-polar Disorder

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102-2628-H-002-003-MY3 from the Ministry of Science and Technology in Taiwan

Drunken Moon Lake Integrated Scientific Research Platform and Aim for Top University Project from National Taiwan University

Title: The involvement of Akt1 in the modulation of immune responses and behavioral consequences in Akt1 deficient mice after neonatal poly(I:C) challenge

Authors: *W.-R. WONG[1], C.-H. HUANG[2], W.-S. LAI[1,2,3];
[1]Grad. Inst. of Brain and Mind Sci., [2]Dept. of Psychology, [3]Neurobio. and Cognitive Sci. Ctr., Natl. Taiwan Univ., Taipei, Taiwan

Abstract: Schizophrenia appears to be a multifactorial disorder with a strong genetic predisposition. Accumulating evidence from human and animal studies suggests that AKT1 (protein kinase Bα) may contribute to susceptibility to schizophrenia and Akt1-deficient mice displayed neuromorphological and behavioral abnormalities as well. In addition to genetic predisposition, immuno-precipitated neurodevelopmental mouse models displayed aberrant behavioral functions and altered Akt1 expression after prenatal immune challenge. However, the precise role of Akt1 in the modulation of neonatal immune responses and behavioral consequence remains unclear. The objective of this study is to examine the effect of early infection alone and its interaction with Akt1 deficiency on the alterations of neonatal immune responses and behavioral consequence in both neonate and adulthood. Akt1 heterozygous pups and their wild-type littermate controls received daily injections of polyriboinosinic-polyribocytidylic acid (poly(I:C)) from postnatal days 2 to 6. Using cytometric bead array, our cytokine analysis revealed a 30% reduction of TNFα level in Akt1 deficient pups right after the last daily poly(I:C) challenge. Behavioral result from neonatal developmental milestones also revealed that male Akt1 deficient pups had decreased body weight and their performance on negative geotaxis reflex was impaired after neonatal poly(I:C) challenge. Besides, a battery of behavioral tasks, including open field, Y maze, social preference, social recognition, USVs playback approaching paradigm, tail suspension, and prepulse inhibition tests, were conducted in another batch of adult mice. In consideration of sex difference, an interaction between genotype and treatment in social recognition task was found in male mice, suggesting that the ability of social recognition in adulthood might be affected by Akt1 deficiency and neonatal poly(I:C) challenge. An impairment of sensorimotor gating function was also found in Akt1-deficent females. Collectively, our data indicated that neonatal poly(I:C)-induced immune response can be altered by Akt1 deficiency, especially during neonatal period. But it appears to have minor impact on behavioral performance in adulthood. Further neurochemical analysis is still in progress. Findings from this study will provide clues to understanding the involvement of Akt1 in the early infection-induced model of schizophrenia.

Disclosures: **W. Wong:** None. **C. Huang:** None. **W. Lai:** None.

Poster

052. Schizophrenia: Mutant Models

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 52.09/U20

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: NIMH Grant MH097803

Title: Htr2a expression responds rapidly to environmental stimuli in an Egr3-dependent manner suggesting a functional link between two schizophrenia susceptibility genes

Authors: *A. M. MAPLE, X. ZHAO, D. I. ELIZALDE, A. L. GALLITANO;
BMS, Univ. of Arizona Col. of Medicine-PHX, Phoenix, AZ

Abstract: The serotonin system has been implicated in the etiology of schizophrenia, however the mechanisms by which serotonin influences susceptibility to this severe mental illness are poorly understood. The serotonin 2A receptor (5-HT_{2A}R) has been of particular interest as agonists of this receptor cause psychosis in normal individuals and antagonism of the 5-HT_{2A}R is thought to be an important function of atypical antipsychotics. In addition, *Htr2a*, the gene that encodes the 5-HT_{2A}R, is one of the most well-replicated schizophrenia candidate genes in genetic association studies, and numerous *in vivo* and post-mortem studies have found decreased 5-HT_{2A}Rs in the brains of schizophrenia patients. We recently reported that 5-HT_{2A}R binding levels are decreased by approximately 70% in the prefrontal cortex of mice lacking the immediate early gene (IEG) early growth response 3 (*Egr3*), paralleling 5-HT_{2A}R deficits in schizophrenia patients. We have previously shown that mice lacking *Egr3* display schizophrenia-like behaviors and responses to antipsychotics. In humans, *EGR3* has been associated with schizophrenia risk and *EGR3* mRNA is reduced in the brains of schizophrenia patients. These findings suggest that *Egr3* may regulate expression of *Htr2a*. As an environmentally induced transcription factor that is required for synaptic plasticity, *Egr3* may influence both the environmental and the genetic contributions to schizophrenia risk. In this study, we tested the hypothesis that the 5-HT_{2A}R is also regulated in response to environmental stimuli, and that this regulation requires the activity of *Egr3*. To determine whether *Htr2a* can be regulated an environmental stressor, we used a sleep deprivation paradigm in mice. We found that this stimulus increased *Htr2a* mRNA expression in the cortex, using quantitative RT-PCR. Next, we tested whether this activation required *Egr3*, (which is induced by sleep deprivation). We found that the activation of *Htr2a* by sleep deprivation was not observed in *Egr3*-/- mice. These data suggest *Egr3* may rapidly modulate *Htr2a* in response to the acute environmental stimulus of sleep deprivation. Furthermore, they provide a functional link between two schizophrenia candidate genes and a possible explanation of how genetic and environmental factors influence risk for schizophrenia.

Disclosures: **A.M. Maple:** None. **X. Zhao:** None. **D.I. Elizalde:** None. **A.L. Gallitano:** None.

Poster

052. Schizophrenia: Mutant Models

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 52.10/U21

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: NARSAD Independent Investigator Award

Title: A dual hit mouse model for schizophrenia: Neural correlates of stress and latent inhibition

Authors: *B. M. GUERCIO[1], D. O'BRAY[2], C. BUHUSI[1], M. BUHUSI[1];
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Abstract: Latent inhibition (LI) is an adaptive attentional phenomenon in which preexposure to a neutral stimulus decreases the future associability of the preexposed stimulus. LI has been shown to be attenuated in patients with schizophrenia, although the cause is not fully understood. A number of genetic mutations and gene polymorphisms have been found to be associated with an increased risk for the development of schizophrenia, including mutations in the CHL1 gene. The close homolog of L1 (CHL1) adhesion molecule is involved in neuron development and plasticity, particularly neuronal migration, development of neuronal connections, as well as cellular signaling pathways. In mice lacking the CHL1 adhesion molecule, anatomical changes have been found to parallel anatomical changes observed in individuals with schizophrenia. In this study, we have evaluated the effects of stress on inducing behavioral deficits characteristic of schizophrenia (impaired latent inhibition) in CHL1 knockout mice (KO), and wild type littermate controls (WT). Mice were divided into two groups: mice in the STRESS condition received 6 weeks of chronic mild stress beginning at about 6 weeks of age, while NO-STRESS mice were not manipulated. Upon reaching adulthood (12 weeks of age), all mice were tested in the LI paradigm as shown in the poster "A double hit mouse model of schizophrenia: Chronic stress impairs latent inhibition in CHL1 deficient mice," in which LI was found to be impaired in stressed KO but not WT mice, as predicted by the dual hit hypothesis. Following LI testing c-fos immunohistochemistry stainining was performed to assess neuronal activation in the prelimbic cortex (PrL), infralimbic cortex (IL), basal lateral amygdala (BLA), nucleus accumbens shell (NacSH), and nucleus accumbens core (NacC), which are all known to contribute to LI. We found that c-fos activation was increased in the IL, and NacSH in the stress condition. Additionally KO mice showed significantly more activation than WT mice in the IL. These results enhance current understanding of the neural circuits involved in attentional deficits induced by stress and genetic predisposition in models of schizophrenia.

Disclosures: **B.M. Guercio:** None. **D. O'bray:** None. **C. Buhusi:** None. **M. Buhusi:** None.

Poster

052. Schizophrenia: Mutant Models

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 52.11/U22

Topic: C.15. Schizophrenia and Bi-polar Disorder

Title: Impairment of synaptic functions and local network activity in dysbindin-1 deficient mice

Authors: *J. ZHAO[1], J. NORDMAN[1], S. KOLACHANA[1,2], Z. LI[1];
[1]NIMH/NIH, Bethesda, MD; [2]Thomas Jefferson High Sch. for Sci. and Technol., Alexandria, VA

Abstract: Dysbindin-1 has been reported as a susceptibility gene of schizophrenia. Both miRNAs and proteins of dysbindin-1 are significantly decreased in the hippocampus of schizophrenia patients. In neurons, dysbinidin-1 is distributed at both presynaptic and postsynaptic sites, suggesting that it is involved in synaptic regulation. In mice deficient in dysbindin-1 expression, dopamine D2 receptors (D2R, a member of the D2-like subfamily of dopamine receptors) are increased on the cell surface. D2R dysfunction has long been recognized to play an important role in schizophrenia. In schizophrenia brains, D2R density is increased. In hippocampal, cortical and striatal neurons, brief activation of D2R inhibits NMDA currents. However, whether D2R-mediated changes in synaptic transmission affect the structure of synapses (such as dendritic spines, tiny dendritic protrusions) has not been experimentally tested. Neural oscillation has been implicated in information integration and cognitive processing by electrophysiological recordings of individual neurons, local field potentials and EEG. Clinical studies suggest that abnormal perception and cognition of schizophrenia patients may be associated with abnormal neural synchrony activity. The role of dysbindin in neural oscillation has not been investigated. In this study, we used the sandy mouse (carrying a deletion mutation in dysbindin-1 and expressing no dysbindin-1 proteins) as a genetic model of schizophrenia. We measured the strength of synaptic transmission by analyzing miniature excitatory postsynaptic currents (mEPSCs) with whole-cell voltage clamp. In adolescent sandy mice, mEPSC frequency was significantly reduced, whereas its amplitude was comparable with that in wild-type mice. The reduction in mEPSC frequency in sandy mice was abolished by injecting lentivirus expressing D2R siRNAs, indicating that it is caused by D2R overactivation. We further study whether synaptic dysfunctions influence on neural network synchronization in sandy mice. By analyzing the local field potentials in sandy mice hippocampus, we found gamma band oscillation was marked decreased. These results indicate the impairment of local neuronal

network function were associated with dysbindin-1. In the further, we will focus on the mechanism of neuronal network activity impairment associated with dysbindin pathway.

Disclosures: **J. Zhao:** A. Employment/Salary (full or part-time):; NIMH/NIH. **J. Nordman:** None. **S. Kolachana:** None. **Z. Li:** None.

Poster

052. Schizophrenia: Mutant Models

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 52.12/U23

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: Canadian Institute of Health Research (CIHR)

Title: Constant light exacerbates behavioral deficits in the dysbindin-1 mutant mouse model of schizophrenia

Authors: ***S. BHARDWAJ**, K. STOJKOVIC, N. CERMAKIAN, L. K. SRIVASTAVA; Douglas Mental Hlth. Univ. Institute, McGill Univ., Montreal, QC, Canada

Abstract: Alterations in sleep and circadian rhythms are often reported in schizophrenia. However, the mechanisms underlying such disruptions and their relationship with schizophrenia symptoms remain elusive. Here, we assessed the impact of constant light, a condition known to disrupt circadian rhythms, in sdy mice with a mutation in the schizophrenia susceptibility gene dysbindin-1. First, we recorded running wheel activity of homozygous mutants (sdy) and wild-type (WT) male mice under different lighting conditions: 12h light:12h dark (LD), constant darkness (DD) and constant light (LL; 20 or 200 lux). Parameters such as free-running period and distribution of activity over the 24h day were evaluated. While the free-running period in LL was longer than in DD, the increase was even larger in sdy mice. Moreover, we observed a higher subjective day/subjective night ratio of activity in sdy mice. We then assessed if the schizophrenia-relevant behavioral abnormalities described in sdy mice are affected under LL. Spontaneous locomotor activity, prepulse inhibition (PPI) of acoustic startle and anxiety-like behavior (elevated plus maze) were assessed first under baseline (2 weeks in LD), then in LL (3 weeks, 200 lux), and then again in LD (3 weeks). Spontaneous locomotor activity was not significantly different between sdy and WT at baseline; however, following LL exposure, sdy animals showed an increase compared to WT animals, and this enhanced activity persisted even after 3 weeks of LD exposure. PPI data showed a similar pattern of behavioral deficits induced

by LL: PPI was significantly decreased in sdy mutants after LL compared to WT. However, after 3 weeks in LD, while the PPI deficit was still present, it was attenuated. LL exposure led to a significant increase in anxiety-like behavior in WT animals that was fully reversed after 3 weeks in LD. Interestingly, this effect of LL on anxiety-like behavior was not observed in the sdy mutants. Altogether, our data suggest that dysbindin-1 deficiency leads to altered circadian activity in mice, and that constant light causes sustained behavioral deficits in mice with this mutation. Our results reveal lighting condition as a novel environmental factor interacting with genetic susceptibility to potentiate schizophrenia-like symptoms.

Disclosures: **S. Bhardwaj:** None. **K. Stojkovic:** None. **N. Cermakian:** None. **L.K. Srivastava:** None.

Poster

052. Schizophrenia: Mutant Models

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 52.13/U24

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: KAKENHI 24791244

KAKENHI 20585690

Title: Altered regulation of cAMP/PKA signaling by PDE10A and PDE4 in DISC1 mutant mice

Authors: ***A. NISHI**[1], M. KUROIWA[1], T. SHUTO[1], N. SOTOGAKU[1], Y. HANADA[1], M. MORITA[2], A. SAWA[3], T. HIKIDA[2];
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Abstract: A transgenic mouse model with a putative dominant-negative DISC1 under expression control of the prion protein promoter, DISC1-DN-Tg-PrP, is susceptible to social isolation stress, resulting in alteration of dopamine signaling and psychosocial behaviors (Niwa et al., 2013). In this study, we investigated whether PDE10 and/or PDE4 are involved in alteration of cAMP/PKA signaling in the striatum (dorsal) and nucleus accumbens (NAc, ventral striatum) of DISC1 mutant mice without or with isolation stress for 3 weeks. Treatment of striatal slices with a PDE10A inhibitor, papaverine, increased the phosphorylation of GluA1 and

DARPP-32 at PKA-sites in a dose-dependent manner. The effect of papaverine was larger in DISC1 mutant mice than in wild-type mice. After isolation stress, the effect of papaverine was largely reduced only in DISC1 mutant mice. The altered responses to papaverine in the striatum were not observed in the NAc. As predicted, the stimulatory effect of a D1 receptor agonist, SKF81297, on the phosphorylation of PKA-sites in the striatum of DISC1 mutant mice was smaller, but became comparable with wild-type mice after isolation stress. In contrast to the results with papaverine, analysis using a PDE4 inhibitor, rolipram, revealed the alteration of cAMP/PKA signaling in the NAc of DISC1 mutant mice after isolation stress. The stimulatory effects of rolipram on the phosphorylation of PKA-sites (GluA1 and DARPP-32) in the NAc were similar in wild-type and DISC1 mutant mice, but largely enhanced only in DISC1 mutant mice after chronic stress. Such altered responses to rolipram were not observed in the striatum. Isolation stress enhanced the stimulatory effects of SKF81297 on DARPP-32 phosphorylation in wild-type mice, but the enhancement was smaller in DISC1 mutant mice. Thus, PrP-dnDISC1 mice shows the alteration of PDE10A function in the striatum before isolation stress, but the alteration of PDE4 function in the NAc becomes dominant after isolation stress, leading to the derangement of dopamine/cAMP/PKA signaling in DISC mutant mice.

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Poster

052. Schizophrenia: Mutant Models

Location: Halls A-C

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Program#/Poster#: 52.14/U25

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: KAKENHI 23120011

KAKENHI 23680034

Title: Social isolated DISC1 mutant mice displayed high sensitivity to chronic cocaine exposure and rolipram treatment

Authors: ***T. HIKIDA**[1], M. MORITA[1], M. NIWA[2], M. KUROIWA[3], A. SAWA[2], A. NISHI[3]; [1]Med. Innovation Ctr., Kyoto Univ. Grad. Sch. of Med., Kyoto, Japan; [2]Dept. of Psychiatry, Johns Hopkins Univ. Sch. of Med., Baltimore, MD; [3]Dept. of Pharmacol., Kurume Univ. Sch. of Med., Kurume, Japan

Abstract: Development of drug addictive behaviors is modulated by both genetic and environmental risk factors. To address the role of gene-environment interaction (GXE) in drug addiction, we combined a transgenic mouse model with a putative dominant-negative DISC1 under expression control of the prion protein promoter, DISC1-DN-Tg-PrP as a genetic model (G) and adolescent social isolation stress as an environmental factor (E), established in Niwa et al., 2013. We studied cocaine-related bahaviors of four groups of mice: wild-type mice without isolation (control); wild-type mice with isolation (E group); DISC1-DN-Tg-PrP without isolation (G group); and DISC1-DN-Tg-PrP with social isolation stress (GXE group). Acute and chronic cocaine exposure induced significantly higher locomotion in GXE group than in other groups. In conditioned place preference (CPP) test, GXE group mice exhibited significant place preference conditioned with lower dose of cocaine than other groups. These results indicate that the gene-environment interaction enhanced sensitivity to chronic cocaine exposure and lead to development of cocaine addictive behaviors. Next, we examined molecular and biochemical changes in nucleus accumbens of GXE model mice and found significant increases in mRNA expression level and enzyme activity of phosphodiesterase 4 (PDE4). Then, we addressed whether PDE4 could be involved in the development of cocaine addictive behaviors. When we injected PDE4 inhibitor rolipram before place conditioning of mice with cocaine in the CPP test, rolipram completely inhibited conditioned place preference induced by chronic cocaine exposure in GXE group. These results suggest the utility of GXE model to analyze the pathophysiology of drug addiction.

Disclosures: **T. Hikida:** None. **M. Morita:** None. **M. Niwa:** None. **M. Kuroiwa:** None. **A. Sawa:** None. **A. Nishi:** None.

Poster

052. Schizophrenia: Mutant Models

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Support: This work was performed in conjunction with the Institut de Recherches Servier and the Innovative Medicine Initiative (NEWMEDS) consortium under Grant Agreement no. 115008.

Title: Targeting Hippocampus-Prefrontal cortex pathway in the Df(22q11.2)/+ mouse model of Schizophrenia

Authors: A. TRIPATHI[1], E. SCHENKER[2], M. SPEDDING[2], *T. M. JAY[1];
[1]INSERM U894, Physiopathologie Des Maladies Psychiatriques, Univ. Paris Descartes, Paris, France; [2]Inst. de Recherches Servier, Suresnes, France

Abstract: Modelling neuropsychiatric disorders such as schizophrenia (SCZ) have proved to be extremely difficult due to the diverse symptoms that characterized the disease: positive symptoms, negative symptoms and cognitive deficits. However, probable genetic causation of SCZ and involvement of copy number variations provide us a means to create better animal models for investigation of underlying abnormalities and potential pharmacological approaches. Deletion in 22q11.2 loci has been reported to be the largest genetic risk factor for SCZ. A transgenic mouse with syntenic deletion at this locus, the Df(h22q11)/+ mice(1), therefore lends itself to be a good model to investigate the anomalous functional connectivity in prefrontal networks and their disruption that has emerged to be of special importance in psychiatric diseases, particularly in SCZ and thought to underlie the cognitive abnormalities that characterize the disease. Following our recent characterization of the ventral hippocampus (vHipp) input to the medial prefrontal cortex (mPFC) in C57 BL/6J mice, we here investigated in Df(h22q11)/+ mice the functional connectivity of this pathway, essential for transmitting information about mnemonic and emotional processing. *In vivo* mPFC responses to low and high frequency stimulation (HFS) of the vHipp were recorded using excitatory postsynaptic field potentials (PSPs). In wild type animals, an increase in PSP amplitude was obtained and stabilized for an hour after HFS application. This increase in PSP amplitude after HFS was significantly reduced in Df(h22q11)/+ mice as compared to the wild type mice indicating a decrease in long term potentiation (LTP) i.e. mPFC synaptic plasticity. Besides, deficits in long term synaptic plasticity, Df(h22q11)/+ mice also showed a decrease in the number of Parvalbumin (PV) positive interneurons in layers 5 and 6 of the mPFC as compared to the wild type mice. The reduction in the number of PV inhibitory neurons in mPFC may contribute to physiological deficits in Df(h22q11)/+ mice and add further credibility to this mouse model of SCZ. Further physiological and behavioral studies that are in progress in these mice will be presented. The present work will serve as a framework for assessing the role of the vHipp-mPFC in expanded phenotyping of this and related models of SCZ. The observed behavioral and systems-level activity changes may lead to a better understanding of this complex disease and open new therapeutic strategies. (1) Df(h22q11)/+ mice were created and provided by by H. Lundbeck A/S.

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Poster

052. Schizophrenia: Mutant Models

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Topic: C.15. Schizophrenia and Bi-polar Disorder

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Evelyn F. McKnight Brain Institute

Title: Alterations in the E to I balance in PGC-1α deficient mice are caused by dysfunction of inhibition from perisomatic targeting interneurons in the hippocampus

Authors: *A. F. BARTLEY[1], Q. LI[1], R. M. COWELL[2], J. J. HABLITZ[1], L. E. DOBRUNZ[1]; [1]Neurobio., [2]Psychiatry and Behavioral Neurobio., Univ. of Alabama At Birmingham, BIRMINGHAM, AL

Abstract: Complex brain disorders such as schizophrenia and autism are disorders of circuit function, caused by an imbalance between excitatory and inhibitory synaptic transmission (E/I). Short-term plasticity differentially alters synaptic response of excitatory and inhibitory synapses; as a result, the E/I ratio is dynamic, continuously changing as a function of stimulus frequency. However, nothing is known about how the dynamics of the E/I ratio are affected by changes in inhibition as seen in disorders such a schizophrenia. Transcriptional dysregulation in interneurons, particularly those containing parvalbumin, is a consistent pathophysiological feature of schizophrenia. PGC-1α (peroxisome proliferator activated receptor γ coactivator 1α) is a transcriptional co-activator localized to interneurons. Loss of PGC-1α results in decreased parvalbumin expression and alterations in GABAergic inhibition. PGC-1α-/- mice are therefore a model of interneuron transcriptional dysregulation that mimics some molecular aspects of schizophrenia. In this study, PGC-1α-/- mice are used to investigate the effects of interneuron transcriptional dysregulation on the dynamic E/I balance at the synaptic and circuit level in the CA1 region of hippocampus. In particular, we recorded from CA1 pyramidal cells in acute hippocampal slices from young adult wildtype and PGC-1α-/- mice. We find that loss of PGC-1α causes a decrease in the E/I ratio in response to Schaffer collateral stimulation, and that the underlying mechanism is enhanced basal inhibition. This decreases the spread of activation in CA1 measured with voltage-sensitive dye imaging, and dramatically limits the spiking of CA1 pyramidal cells, thereby reducing the output from hippocampus. Our data indicate that these alterations are mainly mediated by dysfunction in perisomatic targeting interneurons, specifically parvalbumin basket cells. However, the E/I ratio and impaired circuit function are partially restored at higher frequencies, caused by reduced recruitment of interneurons. These studies will greatly advance our understanding of the dynamics of E/I ratio and provide insights into new

strategies for correcting E/I imbalances and circuit dysfunction. Interestingly, PGC-1α -/- mice have been shown to have reduced inhibition in cortex, suggesting that the effects of PGC-1α on circuit function are region specific. Developing a complete understanding of the effects of interneuron transcriptional dysregulation on circuit function in different brain regions will allow us to develop therapeutic targets to overcome the communication breakdown seen in complex brain disorders.

Disclosures: **A.F. Bartley:** None. **Q. Li:** None. **R.M. Cowell:** None. **J.J. Hablitz:** None. **L.E. Dobrunz:** None.

Poster

052. Schizophrenia: Mutant Models

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Program#/Poster#: 52.17/U28

Topic: C.15. Schizophrenia and Bi-polar Disorder

Title: Vasoactive intestinal peptide receptor 2 deficient mice exhibit dopamine D1 receptor upregulation

Authors: *D. R. GEHLERT, M. MORIN;
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Abstract: Recent human genetic studies have associated duplications of the upstream region of the gene encoding Vasoactive Intestinal Peptide Receptor 2 (VIPR2) with significant risk for schizophrenia[1]. Increased VIPR2 transcription and signaling were reported in lymphocytes isolated from patients exhibiting these duplications, however, little is known about the consequences of VIPR2 expression or deletion in the brain. Conventional antipsychotic drugs work through antagonizing brain dopamine receptors and have no known interaction with VIPR2 receptors. Therefore, we conducted studies to understand the potential link between alterations in VIPR2 expression and the dopaminergic system in mice. In the present studies, VIPR2 deficient mice[2] and age matched controls were sacrificed and slide mounted coronal sections of fresh frozen brain tissue were obtained. Consecutive sections were labeled for D1-like receptors (^{3}H-SCH23390), D2-like receptors (^{3}H-Raclopride) and the dopamine transporter (^{3}H-WIN 35,428, DAT). The binding was detected and quantitated using standard autoradiographic techniques. In these studies, no statistically significant difference was noted in the expression of D2-like receptors or DAT in any of the brain regions evaluated. However, there were statistically significant increases in the expression of ^{3}H-SCH23390 binding in the Caudate-Putamen

(45.5±5.2 nCi/mg vs. 22.5±3.1 nCi/mg, $p<0.05$), Nucleus Accumbens Core (16.7±1.9 nCi/mg vs. 9.8±1.6 nCi/mg, $p<0.05$) and Olfactory Tubercle (32.6±3.3 nCi/mg vs. 13.3±1.5 nCi/mg, $p<0.05$) of VIPR2 knockout mice when compared to wild type controls. No differences in D1 expression were noted in the Shell of the Nucleus Accumbens or the Lateral Septum. These results are consistent with a role for VIPR2 receptor expression in the normal development and expression of the D1 receptors in specific brain regions in mice. Additional studies will be undertaken to assess the functional consequences of the increased binding densities. [1]Vasic et al., Nature 471: 499, 2011; [2]Asnicar et al., *Endocrinology* **143**: 3994–4006, 2002.

Disclosures: **D.R. Gehlert:** A. Employment/Salary (full or part-time):; Eli Lilly and Company. **M. Morin:** A. Employment/Salary (full or part-time):; Eli Lilly and Company.

Poster

052. Schizophrenia: Mutant Models

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Program#/Poster#: 52.18/U29

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: MH083269

DE019580

Title: Deconstructing the BLOC-1 complex: Effects of pallid and dysbindin gene mutation on recognition memory

Authors: *K. H. KARLSGODT[1], S. SPIEGEL[2], A. S. JAMES[3], J. JENTSCH[3];
[1]Psychiatry Res., Feinstein Inst. for Med. Res., Glen Oaks, NY; [2]Thomas Jefferson Sch. of Med., Philadelphia, PA; [3]Univ. of California Los Angeles, Los Angeles, CA

Abstract: Numerous studies have implicated DTNBP1, the gene encoding dystrobrevin-binding-protein or dysbindin, as a candidate risk gene for schizophrenia, though this relationship remains somewhat controversial. Variation in dysbindin and in its location on chromosome 6p have been shown to influence cognitive processes, including those relying on a complex system of glutamatergic and dopaminergic interactions. Dysbindin is a part of the larger Biogenesis of Lysosome-related Organelles Complex 1 (BLOC-1) which includes seven other protein subunits: palladin, muted, cappuccino, snapin, BLOS1, BLOS2, and BLOS3. Data suggests that multiple sububit proteins must be present in order to form a functional oligomeric BLOC-1 complex, such

that dysbindin protein levels are lower in pallid mice, while pallid levels are lower in dysbindin mice. Accordingly, a mutation leading to underexpression of either of these proteins should show phenotypic effects. Palladin is normally expressed in the brain, and a null model of palladin mutation has been used to study the BLOC-1 complex. Although dysbindin and palladin have similar distribution patterns in the mouse and human hippocampi, including in axon terminal fields of glutamatergic neurons in dentate, CA2 and CA3, cognition has been investigated as related to alterations in dysbindin but not palladin. Therefore, we sought to test whether palladin was associated with neurocognitive deficits similar to those associated with dysbindin, which would indicate that any disruption of BLOC-1 function may result in impairment. We employed two tests designed to assess the rodent analogue of recognition memory, the Novel Object Recognition Task (NORT) and Social Novelty Recognition Task (SNRT). Our overall findings indicate that mice with a mutation in either the palladin or dysbindin genes show an appreciable memory deficit on both SNRT and NORT when compared to dysbindin heterozygous (dys-/wt) and wild type (wt/wt) mouse lines. The pallid and null mutant (dys-/dys-) mice lack any preference for novel stimuli, as shown by a ratio of novel/familiar object exploration ≈1. This is compared to the wt/wt and dys-/wt ratio of ≈2, indicating increased preference for the novel mouse or object. We have shown deficits in recognition memory across tasks, indicating that this impairment represents a generalized recognition memory deficit. This novel finding holds significant implications about the role of the palladin gene in relation to dysbindin. Both the SNRT and NORT showed this memory deficit. This leads us to infer that disruption of the BLOC-1 complex, from either palladin or dysbindin mutation, has an effect on memory.

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Poster

052. Schizophrenia: Mutant Models

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Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: K-GRL (2Z03990)

Global Frontier R&D Program (2011-0031525)

National Science Foundation (1064912)

Title: Using auditory steady state responses to characterize neural connectivity in mice models of schizophrenia

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Abstract: Abnormal neural oscillations can reflect dysfunction between neural pathways in the brain and can be characteristic of certain neurological disorders. It has been shown that patients with schizophrenia can exhibit EEG abnormalities in event-related potentials, spectral power, neural synchrony, and brain connectivity. In particular the 40 Hz auditory steady state response (ASSR) in schizophrenic subjects has been shown to have reduced power and phase synchronization to the auditory stimulus. The ASSR is an auditory-evoked potential that can be elicited by a click train and is observed in various parts of the brain including the auditory and frontal cortex. The goal of the study is to investigate ASSR interrelations between EEG channels which is expected to further elucidate differences in neural pathologies associated with schizophrenia. In this study EEG data was obtained from two PLC-beta1 mutant mice as models of schizophrenia, as well four wild control mice. Four channels of scalp EEG were recorded: two over the left and right auditory cortex and two over the left and right frontal cortex. The mice were presented with separate auditory stimuli at 20, 30, 40 and 50 Hz. The power spectrum of the controls showed the expected characteristic frequency modulated pattern at the respective stimulus frequencies. Such distinct and consistent spectral patterns were not observed in the mutants. Various connectivity measures were examined including phase-locking value (PLV), which quantifies the degree of phase synchrony between two EEG channels but does not provide any information about the causal relationships and direction of information flow between different brain regions. Therefore, partial-directed coherence (PDC) was also evaluated. The various connectivity measures indicate a generally weaker neural synchronization and connectivity in mutants compared to the controls, particularly the fronto-temporal relationships. These results support the notion that synchrony of gamma oscillations is disturbed in schizophrenia and that this deficit is related to clinical symptoms such as auditory hallucinations.

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Poster

052. Schizophrenia: Mutant Models

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Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 52.20/U31

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: PHS Award P01 NS048120-08

Title: Altered spatial learning in mice with a deficit in hippocampal parvalbumin-expressing GABA-ergic interneurons leads to apparent cognitive inflexibility

Authors: *S. P. PASKEWITZ[1], S. WEISS[2], B. INBAR[1], M. E. ROSS[4], H. MOORE[3];
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Abstract: Expression of parvalbumin - a calcium-binding protein characteristic of certain classes of GABA-ergic interneurons - is reduced in the hippocampi of people with schizophrenia (Konradi et al., 2011). The resulting disregulation of hippocampal processing may lead to excess activity in the mesencephalic dopamine system (Floresco et al., J Neurosci, 2001). We have recently modeled these functional outcomes of a deficit parvalbumin-expressing (PV+) interneurons using mice lacking the gene cyclin D2 (Ccnd2 KO). These mice display a partial (approximately 40%) PV+ interneuron deficit in the hippocampus, but no changes in other interneuron populations. Ccnd2 KO mice display hippocampal disinhibition, impaired hippocampus-dependent fear-conditioning and altered midbrain dopamine neuron activity (Glickstein et al., Development, 2007; Gilani et al., Proc Natl Acad Sci USA, 2014). In this experiment we examined cognitive flexibility and different forms of spatial navigation in Ccnd2 KOs (c.f. Ragozinno et al., J Neurosci, 1999). Mice - starting on different trials from the "east" and "west" arms of a cross-maze - were trained to find sucrose-pellets hidden in the "north" and "south" arms according to either a egocentric rule (e.g. "turn left") or an allocentric rule (e.g. "turn north"). After mice achieved a criterion level of performance, the rule was switched. We predicted that the hippocampal dysfunction of Ccnd2 KO mice would hinder learning of the allocentric rule (Packard and McGaugh, Neurobiol Learn Mem, 1996), while excess ventral striatal dopamine might impair cognitive flexibility (Haluk and Floresco, Neuropsychopharmacology, 2009). Ccnd2 KO mice initially learned both rules as rapidly as wild type controls (WTs). However, they took longer to switch from an egocentric rule to an allocentric one. This effect is explained by the fact that WT, but not KO, mice showed accelerated learning of the allocentric rule when it came second in order. We propose that WT mice automatically encoding a spatial map while learning the egocentric rule, a process impaired by hippocampal PV+ interneuron deficits. Further, Ccnd2 KO mice showed signs of increased perseveration when switching from an allocentric rule to an egocentric one. These results suggest that Ccnd2 KO mice learn allocentric rules in a different, less flexible manner than WTs (c.f. O'Keefe and Nadel, The Hippocampus as a Cognitive Map, 1978).

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Poster

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Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: Autism Speaks Grant#7647

Title: Mitochondrial deficits in fast-spiking interneurons lead to elevated anxiety, impaired sensory gating and social dysfunction

Authors: ***M. INAN**[1], M. ZHAO[2], M. MANUSZAK[1], M. V. BOBOILA[3], I. DINCHEVA[4], V. PICKEL[1], F. S. LEE[4], A. M. RAJADHYAKSHA[3], T. H. SCHWARTZ[2], P. GOLDSTEIN[5], G. MANFREDI[1];
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Abstract: Patients affected by mitochondrial disease often develop neuropsychiatric symptoms. At the same time, deficits in the function of parvalbumin-expressing, "fast-spiking" cortical interneurons (PV cINs) have been associated with neuropsychiatric disorders. PV cINs have high bioenergetic demands, making them highly susceptible to mitochondrial impairment. Thus, there is a logical convergence between mitochondrial metabolism and PV cINs, and their mutual involvement in the pathogenesis of neuropsychiatric symptoms in mitochondrial diseases. Here, we link the mitochondrial dysfunction and interneuronopathy hypotheses of neuropsychiatric symptoms by studying transgenic mice with conditional ablation of Cytochrome c oxidase, a key enzyme of the oxidative phosphorylation (OXPHOS) machinery, restricted to PV neurons (PV CKO). First we found that PV cINs have the highest mitochondrial content among other cIN populations, consistent with their fast-spiking activity. Further, analyses of PV CKO mice revealed a progressive decline in OXPHOS function. In parallel with the biochemical phenotype, inhibitory synaptic transmission was reduced and excitability of the cortical circuitry was progressively increased. At the behavioral level, cortical function defects correlated with lack of sensory gating, increased anxiety and impaired sociability, all of which are hallmark symptoms of neuropsychiatric disorders, such as schizophrenia and autism spectrum disorders. Taken together, our results support the hypothesis that bioenergetic impairment in PV cINs can

contribute to the pathogenesis of neuropsychiatric symptoms. Furthermore, from these findings, mitochondria in PV cINs emerge as potential therapeutic targets for neuropsychiatric symptoms.

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Poster

052. Schizophrenia: Mutant Models

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Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: CREST

GASR(C)25430077

Title: Contrasting expression patterns for immaturity and maturity marker genes in the dentate gyrus between mouse lines with 'immature dentate gyrus' and mice overexpressing glucocorticoid receptor

Authors: *H. KOSHIMIZU[1,2], H. HAGIHARA[1,2], K. TAKAO[3,2], T. MIYAKAWA[1,2]; [1]ICMS, Fujita Hlth. Univ., Toyoake, Japan; [2]CREST, JST, Kawaguchi, Japan; [3]Ctr. for Gene. Anal. of Behav., NIPS, Okazaki, Japan

Abstract: Adequate maturation of hippocampal neurons is thought to be crucial for normal cognitive function and emotional behavior, and dysregulation of cellular maturity in the hippocampus could be involved in mental disorders. Previously, we identified the 'immature dentate gyrus (iDG)' phenotype, in which almost all the granule cells in the dentate gyrus (DG) exhibit a pseudo-immature status, in several gene-targeted mouse lines that display behavioral abnormalities related to schizophrenia. On the other hand, the research group led by Huda Akil has reported that overexpression of glucocorticoid receptor (GR) in the forebrain throughout the lifetime and in early life causes increased depression-like and/or anxiety-like behaviors in mice (Wei et al., PNAS, 2004; Biol. Psychiatry, 2012), raising the possibility that mice overexpressing GR (GRov mice) may represent a potential animal model for mood disorders, such as depression and anxiety disorder. In addition, overexpresson of GR causes an 'aging-like' neuroendocrine phenotype and mild cognitive dysfunction in young mice (Wei et al., J. Neuroscience, 2007). In

this study, we evaluated the transcriptional maturation status of GRov mouse DG by using a bioinformatics tool, NextBio, and publicly available microarray data sets. The DG of GRov mice exhibited an contrasting patterns of immaturity and maturity marker gene expression to those in the developing DG (P14 compared to P30) in wild-type (WT) mice, while the gene expression patterns of those marker genes in iDG of Schnurri-2 knockout (Shn2 KO), alpha CaMKII heterozygous KO, and forebrain-specific calcineurin KO mice are similar to those in the developing DG (P14 compared to P30) of WT mice. Contrasting gene expression patterns were found between the DG of GRov mice and the mouse lines showing iDG phenotype. These observations indicated that maturation status of the DG in GRov mice may be changed potentially toward 'over-maturity'.

Disclosures: **H. Koshimizu:** None. **H. Hagihara:** None. **K. Takao:** None. **T. Miyakawa:** None.

Poster

052. Schizophrenia: Mutant Models

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Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: NIMH00023

Title: Knockout of the schizophrenia candidate gene neuregulin 3 impairs synaptic transmission and behavior

Authors: *D. J. FIGUEIREDO, C. SHEN, X. SUN, W.-C. XIONG, L. MEI;
Neurosci., Georgia Regents Univ., Augusta, GA

Abstract: Neuregulin 3 (Nrg3) is a member of the Neuregulin family of growth factors that are encoded by six individual genes. The EGF-like domain of Nrg3, located in the N-terminal extracellular region, is ~30% identical in amino acid sequence to Nrg1. In human, the Nrg3 gene is positioned at 10q22-23, a locus associated with schizophrenia. Many single nucleotide polymorphisms (SNP's) of the Nrg3 gene, especially in the first intronic region, are associated with schizophrenia and with smoker cessation success rate.Nrg3 is thought to act by activating the receptor tyrosine kinase ErbB4. However, unlike Nrg1, a well-characterized schizophrenia susceptibility gene, little is known about the function of Nrg3. We have characterized Nrg3 expression the brain. Western blot analysis indicated that in adult mice, Nrg3 was primarily in

the brain,but not detectable in the liver, heart, bone or skeletal muscle. In the brain, it was detectable in various regions, relatively high in the cortex and midbrain. The expression of Nrg3 is developmentally regulated, being highest at P16. Studies of cortical cells in culture indicated that Nrg3 was detectable in neurons, but not astrocytes. To study its function *in vivo*, we generated floxed Nrg3 mice. Brain-specific knockout by hGFAP-Cre, led to reduction of Nrg3 level in the brain. The mutant mice were viable and fertile, without gross anatomic deficits. However, they appeared to spend less time in the center in open-field tests, suggesting increased anxiety. Preliminary electrophysiological studies indicate abnormal transmission and synaptic plasticity in mutant hippocampal slices. These observations support the hypothesis that Nrg3 may play a role in regulating neural activity. Further characterization of mutant phenotypes and investigation of underlying mechanisms may shed light on pathophysiological mechanisms relevant to schizophrenia or nicotine addiction.

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Poster

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Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: NIH Grant 1RO1MH098534

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Title: Alterations in regulation of inhibition by dopamine D4 receptors in a mouse model of interneuron transcriptional dysregulation

Authors: *L. BRADY, A. F. BARTLEY, L. E. DOBRUNZ;
Neurobio., Univ. of Alabama At Birmingham, Birmingham, AL

Abstract: GABAergic interneurons that express parvalbumin play an essential role in proper network activity. Parvalbumin is regulated by the transcriptional coactivator PGC-1α, and PGC-1α-/- mice have decreased expression of parvalbumin in interneurons, similar to that observed in schizophrenia postmortem tissue. Pilot studies from our lab have shown that the inhibition/excitation (I/E) ratio is increased at the Schaffer collateral (SC) pathway in PGC-1α-/-

mice. This is caused by enhanced inhibition, which reduces the spread of activation in CA1 as measured by voltage-sensitive dye imaging. The mechanism for the enhanced inhibition is not yet known. Acute application of haloperidol, an antipsychotic that blocks dopamine D2-like receptors, has been shown to increase excitatory synaptic transmission in CA1. Consistent with this, we observed that haloperidol increased the spread of activation in wildtype (WT) slices. We tested whether haloperidol could alleviate the I/E imbalance in slices from PGC-1α-/- mice, but found instead that haloperidol significantly decreased the spread of activation. Haloperidol appears to make the I/E imbalance worse, suggesting that there are alterations in dopamine regulation of synaptic transmission in PGC-1α-/- mice. To date, it is not known whether PGC-1α has a direct regulatory effect on the dopamine system in hippocampus, or how this might affect GABAergic inhibition. We investigated alterations in the regulation of GABAergic inhibition by the dopamine system using field potential recordings in hippocampal slices from PGC-1α-/- mice. We found that dopamine has a disinhibitory effect in the SC-CA1 pathway, although there was no difference in the magnitude between WT and PGC-1α-/-. However, dopamine activates multiple receptors with opposing effects. Surprisingly, there was no difference in the effects of a D2 receptor antagonist between WT and PGC-1α-/-. However, blocking D4 receptors caused an increase in the field potential in PGC-1α-/- slices due to disinhibition, which was not seen in WT slices. These data suggest that there is a tonic effect of D4 activation that enhances feed-forward inhibition in PGC-1α-/- slices, which may contribute to the enhanced I/E ratio in these mice. Alterations in the dopamine system's modulation of inhibition, through changes in D4 receptor effects, may therefore be involved in the circuit dysfunction caused by loss of PGC-1α. These results may lead to a greater understanding of circuit dysfunction in disorders of I/E imbalance such as schizophrenia, and provide information for the development of alternate pharmacotherapeutic approaches.

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Poster

052. Schizophrenia: Mutant Models

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Title: Disruption of Nrg1-ErbB4 signaling in specific GABAergic interneurons alters cortical activity and sensory processing

Authors: *R. BATISTA-BRITO[1], J. MOSSNER[1], U. KNOBLICH[1], J. A. CARDIN[1,2]; [1]Neurobio., [2]Kavli Inst., Yale Univ., New Haven, CT

Abstract: Neuregulin1 (Nrg1) is a diffusible trophic factor in the brain that activates a tyrosine kinase receptor, ErbB4. Genetic studies have shown a strong correlation between schizophrenia and mutations in the genes Nrg1 and ErbB4. Studies in mice further suggest that loss of Nrg1 or ErbB4 results in schizophrenia-like phenotypes. Previous work suggests that Nrg1-ErbB4 signaling is necessary for the function of GABAergic, parvalbumin-expressing inhibitory interneurons (PV-INs) in the brain. However, the impact of ErbB4 disruption on cortical network function and the balance of excitation and inhibition *in vivo* remain unknown. Furthermore, it is unclear whether Nrg1-ErbB4 signaling is important for the normal function of non-PV expressing interneurons. In order to understand the role of ErbB4 in inhibitory function in cortical networks we removed ErbB4 from either all interneurons or in specific interneuron subtypes, including PV-, SST- and VIP-expressing interneurons. To assess how Nrg1-ErbB4 signaling affects cortical activity patterns, we recorded spontaneous and visually evoked activity in the primary visual cortex (V1) of awake behaving mice. We used arrays of tetrodes to simultaneously measure local field potentials and make single-unit recordings of large numbers of neurons in V1. Waveform analysis of clustered units allowed us to identify many putative pyramidal cells and fast-spiking (FS) interneurons with high fidelity. In a subset of experiments, we used Cre-dependent optogenetic tagging to record the activity of target interneuron populations. We observed altered spontaneous and visually evoked LFP activity when ErbB4 was removed from all interneurons, as well as from PV and VIP interneurons specifically. ErbB4 deletion from PV and VIP interneurons also altered firing rates and visually evoked spike responses. In contrast, ErbB4 deletion from SST interneurons had no impact on V1 activity at the LFP or spike levels. Our findings suggest that the Nrg1-ErbB4 signaling pathway is important for both the excitatory-inhibitory balance and patterned activity of cortical networks *in vivo*.

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Poster

052. Schizophrenia: Mutant Models

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Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: PHS Award 2P01 NS048120

Sidney R. Baer Jr Foundation

Title: Interrogation of dysregulated hippocampal activity in a mouse model of psychosis

Authors: *M. O. CHOHAN[1,2], H. MOORE[1,2];
[1]Integrative Neurosci., New York State Psychiatric Inst., New York, NY; [2]Psychiatry, Columbia Univ., New York, NY

Abstract: Deficits in the number or function of cortical GABAergic interneurons have been hypothesized as a core pathophysiology in schizophrenia (SCZ). GABAergic interneuron hypofunction may contribute to a resting hypermetabolic state in the hippocampus (HIPP), a phenotype that predicts psychosis, which may underlie cognitive and other behavioral impairments seen in SCZ. We previously tested this hypothesis in mice with a null mutation of the cyclin D2 gene (Ccnd2). Ccnd2 is expressed within the medial ganglionic eminence (MGE) of the embryonic brain where it regulates early development of the parvalbumin-expressing interneurons (PV+IN) of the cortex. Ccnd2 nulls show partial and selective decrease in the density of HIPP PV+IN and a corresponding increase in *in vivo* HIPP metabolic activity, an increase in midbrain dopamine (DA) neuron activity and response to amphetamine, and disrupted cognition. Transplanting interneuron precursors derived from the MGE into the adult HIPP normalized these psychosis relevant phenotypes. Here, we aimed to determine the effects interneuron precursor transplants had on HIPP milieu by characterizing HIPP neuronal sub-type activity. Using well-established in-vivo extracellular single-unit recording methods in anesthetized mice, we recorded HIPP neuronal activity from excitatory projection and inhibitory neurons. Ccnd2 nulls showed increased excitatory neuron spiking and a decrease in the density of spontaneously active INs. Preliminary analyses showed no statistically significant differences in spiking activity of detectable INs or the density of excitatory projection neurons; however, the sample size must be increased before we can be confident that these characteristics are unchanged. These findings establish the plausibility of a causative link between hippocampal IN deficits, hippocampal hyperactivity and DA system dysregulation in psychotic disorders.

Characterization of HIPP neuronal activity following IN precursor transplants is ongoing which will further help elucidate mechanisms used by these transplanted cells that help normalize hyper-metabolic state in the hippocampus in these mice.

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Poster

052. Schizophrenia: Mutant Models

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Title: Region-specific dendritic spine loss of pyramidal neurons in dopamine transporter knockout mice

Authors: *Y. KASAHARA[1,2], Y. ARIME[2,3], F. S. HALL[4], G. R. UHL[4], H. TOMITA[1,2], I. SORA[2,5];
[1]Dept. of Disaster Psychiatry, Intl. Res. Inst. of Disaster Scien, Sendai, Japan; [2]Dept. of Biol. Psychiatry, Tohoku Univ. Grad. Sch. of Med., Sendai, Japan; [3]Dept. of Biol. Psychiatry and Neurosci., Dokkyo Med. Univ. Sch. of Med., Mibu, Japan; [4]Mol. Neurobio. Br., Natl. Inst. on Drug Abuse, Intramural Res. Program, NIH/DHSS, Baltimore, MD; [5]Dept. of Psychiatry, Kobe Univ. Grad. Sch. of Med., Kobe, Japan

Abstract: Dopamine transporter (DAT) knockout (KO) mice show numerous behavioral alterations, including hyperlocomotion, cognitive deficits, impulsivity and impairment of

prepulse inhibition of the startle reflex (PPI), phenotypes that may be relevant to frontostriatal disorders such as schizophrenia. Dendritic spine changes of pyramidal neurons in the dorsolateral prefrontal cortex (DLPFC) are among the most replicated of findings in postmortem studies of schizophrenia. The mechanisms that account for dendritic changes in the DLPFC in schizophrenia are unclear. Here, we report basal spine density of pyramidal neurons in the medial prefrontal cortex (mPFC), the motor cortex, the CA1 region of the hippocampus, and the basolateral amygdala in DAT KO mice. Pyramidal neurons were visualized using DAT KO mice crossbred with a Thy1-GFP transgenic mouse line. We observed a significant decrease in spine density of pyramidal neurons in the mPFC and the CA1 region of the hippocampus in DAT KO mice compared to that in WT mice. On the other hand, no difference was observed in spine density of pyramidal neurons in the motor cortex or the basolateral amygdala between DAT genotypes. These results suggest that decreased spine density could cause hypofunction of the mPFC and the hippocampus, and contribute to the behavioral abnormalities observed in DAT KO mice, including cognitive deficits. This might suggest that aberrant dopaminergic signaling may trigger dystrophic changes in dendrites of hippocampal and prefrontocortical pyramidal neurons in schizophrenia.

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Poster

052. Schizophrenia: Mutant Models

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Howard University RCMI program

Howard University Institutional Support

NSF-Alliance for Graduate Education and the Professoriate (AGEP)

Title: Biochemical and physiological deficits in gabaergic signaling in a disc1 model of schizophrenia

Authors: S. N. REID[1], M. C. GONDRÉ-LEWIS[1], *K. S. JONES[2];
[1]Anat., Howard Univ. Sch. of Med., Washington, DC; [2]Biol., Howard Univ., Washington, DC

Abstract: The etiology and pathophysiology of schizophrenia (SZ) are unclear. Increasing evidence suggests that mutations in the disrupted-in-schizophrenia-1 (DISC1) gene can dramatically increase the risk for developing SZ or bipolar disorder by as much as 50-fold. Postmortem brain tissue from SZ patients show deficits in biochemical markers of several components of GABA signaling, but how these deficits impact inhibitory neurophysiology remain unknown. Here we examine putative alterations in biochemistry and physiology of GABAergic neurotransmission in subcortical regions of a mouse model which expresses a truncated version of the human DISC1 protein [hDISC1] in excitatory forebrain neurons (Pletnikov, et al 2010). Biochemical and immunohistochemical data suggest expression of hDISC1 appears to reduce expression of the calcium binding protein, parvalbumin, and glutamate receptor subunits, NR1 and GluR1, in the hippocampus and amygdala. These data imply alterations in the function of parvalbumin-expressing, GABA-ergic fast-spiking interneurons (FSIs) and reduced excitatory receptor transmission at glutamatergic synapses may mediate DISC1 pathology. Since FSIs are highly implicated in the etiology of schizophrenia, the impact of hDISC1 expression on cortical and subcortical FSIs was examined using physiological approaches. Whole-cell patch-clamp recordings demonstrate that dnDISC1 expression reduced the frequency of spontaneous excitatory post-synaptic current (sEPSC) of FS cells and these sEPSCs were less sensitive to blockade by an NMDA receptor antagonist. The electrophysiology data corroborate our biochemical findings and support the notion that NMDA signaling may be impaired in hDISC1 mice. Because the hDISC1 transcript is expressed predominantly in principal cells, the biochemical and physiological deficits we observe maybe mediated via non-cell-autonomous mechanisms, which at present are undefined.

Disclosures: **S.N. Reid:** None. **M.C. Gondré-Lewis:** None. **K.S. Jones:** None.

Poster

052. Schizophrenia: Mutant Models

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Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: 31-116689

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51AU40_125759

Title: Involvement of the receptor for advanced glycation end-product (RAGE) in redox dysregulation and neuroinflammation in an animal model of schizophrenia

Authors: D. DWIR[1], J.-H. CABUNGCAL[1], P. STEULLET[1], R. TIROUVANZIAM[2], *K. Q. DO[1];
[1]Ctr. for Psychiatric Neurosci., Prilly-Lausanne, Switzerland; [2]Res. Emory Univ. Sch. of Med., Atlanta, GA

Abstract: Schizophrenia is a major psychiatric disease which involves both genetic and environmental factors. Glutathione (GSH), an important cellular antioxidant and redox regulator, is decreased in CSF and brain of patients. The key GSH synthesizing genes present polymorphisms associated with the disease. Thus, a redox dysregulation during neurodevelopment is a critical risk factor for schizophrenia, on which converge genetic impairments of glutathione synthesis and environmental vulnerability factors generating oxidative stress. Increasing evidence also points to immune dysregulation in schizophrenia. Anomalies in peripheral immune cells as well as dysregulation of immune-related genes have been reported in schizophrenia brain. However the causes and the underlying mechanisms of this subclinical, inflammatory-like state are still unclear. As oxidative stress is known to induce inflammatory processes, the latter were studied in a transgenic animal model with GSH deficit (GCLM-/-). RAGE represents one potential link between oxidative stress and inflammatory process, as it is activated by ROS and induces inflammatory gene expression. We compared by immunohistochemistry proteins involved in RAGE pathway and microglia activation markers in the anterior cingulate cortex between GCLM-/- and WT mice at P40 and at P90 following oxidative stress induction by a dopamine uptake blocker between P30 and P40 or between P80 and P90. At both time-points, the number of Iba1-immunoreactive (IR), CD11b-IR and CD68-IR cells were increased in GCLM-/- compared to WT at basal level with no further increase after an additional oxidative stress. Interestingly, microglia activation was found only in regions where oxidative stress was increased in the GCLM-/-, suggesting a pro-inflammatory state induced by oxidative stress. RAGE shedding was to be induced in neurons in both genotypes. At P40, RAGE shedding was increased in GCLM-/- while it was decreased following oxidative stress induction compared with WT at basal level. However, at P90, RAGE shedding was decreased in GCLM-/- compared to WT. In addition, S100b, a ligand of RAGE, followed the same pattern as RAGE shedding at both time points, suggesting a feedback regulation of this ligand. RAGE shedding might be induced by MMP9, as this metalloproteinase was increased in GCLM-/- at P40. Finally, we investigated NFkB activation by RAGE using an Adeno-Associated Virus containing GFP under a promoter activated by NFkB. In this work we propose that an interaction between redox dysregulation and pro-inflammatory condition via RAGE is a potential trigger of structural and morphological impairments related to schizophrenia.

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Poster

053. Alcohol: Neural Mechanisms and Behavior I

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Topic: C.17. Drugs of Abuse and Addiction

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Title: Ethanol inhibits GABA neurons in the ventral tegmental area and dopamine release in the nucleus accumbens via presynaptic α6 nicotinic receptors on GABA terminals

Authors: *T. J. WOODWARD[1], S. I. SHIN[1], J. K. MABEY[1], A. C. NELSON[1], N. D. SCHILATY[1], D. H. TAYLOR[2], J. WU[2], M. MCINTOSH[3], S. C. STEFFENSEN[1]; [1]Brigham Young Univ., Provo, UT; [2]Barrow Neurolog. Inst., Phoenix, AZ; [3]Univ. of Utah, Salt Lake City, UT

Abstract: The prevailing view is that enhancement of dopamine (DA) transmission in the mesocorticolimbic system underlies the rewarding properties of alcohol and nicotine (NIC). Dopamine neurotransmission is regulated by inhibitory ventral tegmental area (VTA) GABA neurons, whose excitability is a net effect of glutamate (GLU) and GABA neurotransmission that are modulated by NIC cholinergic receptors (nAChRs) on afferent terminals. We have shown previously that VTA GABA neurons are inhibited by EtOH (EtOH), and adapt to chronic EtOH exposure, evincing marked hyperexcitability during withdrawal. The aim of this study was to evaluate the role of α6 nAChRs in EtOH effects on VTA GABA neurons as well as DA release in the nucleus accumbens (NAc). In electrophysiology studies, superfusion of low doses of EtOH (5 mM) enhanced the frequency (25%) and amplitude (24%) of mIPSCs recorded in acutely dissociated VTA GABA neurons from GAD GFP mice. The α6 nAChR antagonist α-conotoxin P1A (10 nM) did not affect mIPSCs, but prevented the EtOH-induced increase in mIPSC frequency. In the slice preparation of the VTA, superfusion of low doses of EtOH (5 mM) increased eIPSC amplitude in VTA GABA neurons (52%). The α6 nAChR antagonist α-conotoxin MII (100nM) did not affect eIPSCs, but prevented the eIPSC changes caused by EtOH. Additionally, in slice preparation of the VTA, superfusion of low doses of EtOH (5 mM) decreased the firing rate in VTA DA neurons (54%) while high doses (50 and 100 mM) increased VTA DA firing rate (40% and 42%, respectively). The α6 nAChR antagonist α-

conotoxin MII (100nM) did not affect firing rate, prevented the decrease seen at low doses, but was without effect at EtOH enhancement of firing rate at high doses of EtOH. These findings have been further supported by selective stimulation of GABA inputs using optogenetic VGAT-ChR2-YFP BAC transgenic mice. CPP studies have further shown the involvement of α6 nAChR in EtOH modulation, as α6 KO mice evinced less preference for the EtOH compartment. Using FSCV in slice preparation, EtOH inhibited DA release in the NAc. Superfusion of α-conotoxin MII did not affect DA release, but prevented EtOH inhibition of DA release. These findings suggest that EtOH enhancement of GABA inhibition of VTA GABA neurons is mediated by α6 nAChRs, which have been shown to be located on GABA terminals. In addition, GABA inhibition of DA release at terminals in the NAc may be mediated by α6 nAChRs on terminals from projecting VTA GABA neurons. Results from this study could provide a preclinical pharmacologic rationale for considering drugs that act selectively on nAChRs as therapeutic agents for the treatment of alcohol dependence and alcohol and NIC co-dependence.

Disclosures: **T.J. Woodward:** None. **S.I. Shin:** None. **J.K. Mabey:** None. **A.C. Nelson:** None. **N.D. Schilaty:** None. **D.H. Taylor:** None. **J. Wu:** None. **M. McIntosh:** None. **S.C. Steffensen:** None.

Poster

053. Alcohol: Neural Mechanisms and Behavior I

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Topic: C.17. Drugs of Abuse and Addiction

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Title: Neurosteroids block the inhibitory effects of ethanol through GABA(A) receptors on dopamine terminals in the nucleus accumbens

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Abstract: GABA(A) receptors are ligand-gated chloride-ion channels of a wide variety in the central nervous system (composed of a variety of five subunits of α, β, γ, δ, ε, π, θ, or ρ), with the most common composition being an α2β2γ subunit construct. Given the purported evidence of the δ-subunit on GABA regulation of dopamine (DA) release in the nucleus accumbens (NAc), it

is likely that this is a possible avenue for ethanol modulation of DA release. Neurosteroids have long been known to act on GABA(A) receptors. It has been theorized that the δ-subunit may have a neurosteroid binding site. Thus, using fast scan cyclic voltammetry (FSCV), we performed experiments on brain slices in the NAc by superfusing various GABA(A)R-modulating neurosteroids - allopregnanolone, DHEAS, and estrone sulfate. We also tested trilostane, which enhances the endogenous expression of DHEAS via block of 3 β-hydroxysteroid dehydrogenase. DHEAS (20 μM) enhanced DA release in the NAc by 20 %, while allopregnanolone, estrone sulfate, and trilostane did not significantly alter DA release. Ethanol (20 - 160 mM, IC50 = 80 mM) reduced DA release in wild type (WT) mice. Superfusion of DHEAS (20 μM) significantly attenuated ethanol inhibition of DA release, however, the other neurosteroids tested only had partial effects on attenuating the inhibition of DA release by ethanol. The selective GABA δ-subunit antagonist Ro15-4513 (10 μM) enhanced DA release by 26 % and reduced ethanol inhibition of DA release to 91 % of baseline levels. In addition, we found a lack of attenuating effects of ethanol (20 - 160 mM) on DA release with GABA δ-subunit knockout mice (δKO) as compared to controls. Based on this pharmacological and genetic data, we conclude that ethanol is partially acting in the NAc via GABA δ-subunit receptors on DA terminals, and that indirect antagonism of GABA(A) receptors (via DHEAS or Ro15-4513) in the NAc enhances DA release.

Disclosures: **N. Schilaty:** None. **D.M. Hedges:** None. **A.W. Perez:** None. **T.D. Okelberry:** None. **S.C. Steffensen:** None.

Poster

053. Alcohol: Neural Mechanisms and Behavior I

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Topic: C.17. Drugs of Abuse and Addiction

Support: PHS NIH AA020919

Title: Functional switch in GABA(A) receptors on VTA GABA neurons by chronic ethanol

Authors: ***A. NELSON**[1], T. J. WOODWARD[1], J. K. MABEY[1], S. I. SHIN[1], R. TING-A-KEE[2], H. VARGAS-PEREZ[2], D. VAN DER KOOY[2], S. C. STEFFENSEN[1];
[1]Brigham Young Univ., Provo, UT; [2]Univ. of Toronto, Toronto, ON, Canada

Abstract: Past research has demonstrated that the motivational effects of opiates and ethanol switch from a dopamine (DA)-independent to a DA-dependent pathway when the animal is in a drug-dependent state. A corresponding change occurs in ventral tegmental area (VTA) GABA(A) receptors in opiate-dependent animals, which switch from a GABA-induced hyperpolarization of VTA GABA neurons to a GABA-induced depolarization. The aim of this study was to evaluate VTA GABA neuron excitability, GABA synaptic transmission to VTA GABA neurons and GABA-mediated DA release in the nucleus accumbens (NAc) under ethanol-naïve and dependent conditions. To accomplish these studies, we used standard whole-cell and attached-cell mode electrophysiological techniques to evaluate acute and chronic ethanol effects on VTA GABA neurons in CD-1 GAD GFP mice, which enabled the visual identification of GABA neurons in the slice preparation. In naïve animals, superfusion of ethanol (IC_{50} = 30 mM) and the GABAA receptor agonist muscimol (IC_{50} = 100 nM) decreased VTA GABA neuron firing rate in a dose-dependent manner. Compared to either saline-injected or air-exposed controls, in animals made dependent on ethanol by twice daily injections of 2.0 g/kg ethanol or ethanol vapor (200 mg% BAL), neither ethanol nor muscimol significantly affected VTA GABA neuron firing rate on average. Indeed, some cells were excited by ethanol and muscimol, while others remained partially inhibited, producing the average of no change in firing rate of the whole population. While microdialysis studies typically show that ethanol enhances DA release in the NAc, we and others have found that ethanol decreases DA release at terminals, as measured by fast scan cyclic voltammetry. We have recently reported that ethanol inhibition of DA release at terminals in the NAc of ethanol-naïve animals is mediated by GABA, possibly from VTA GABA neurons that project to the NAc. Thus, we evaluated the effects of ethanol on DA release in the same ethanol-dependent animals. Compared to air-exposed controls, superfusion of ethanol did not significantly affect DA release. Taken together, these findings suggest that VTA GABA neurons undergo a switch in GABA(A) receptor function in ethanol-dependent animals, similar to opiate-dependent animals, which results in a corresponding switch in DA release, perhaps resulting from adaptations in VTA GABA neuron input to the NAc.

Disclosures: **A. Nelson:** None. **T.J. Woodward:** None. **J.K. Mabey:** None. **S.I. Shin:** None. **S.C. Steffensen:** None. **R. Ting-A-Kee:** None. **H. Vargas-Perez:** None. **D. van der Kooy:** None.

Poster

053. Alcohol: Neural Mechanisms and Behavior I

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Topic: C.17. Drugs of Abuse and Addiction

Title: Transcriptome analysis on ethanol-induced brain injury among primary cultures of brain cells and slices

Authors: *K. SUGIMOTO[1], H. TANAKA[2], R. KATADA[1], K. IGARASHI[1], M. YOSHIDA[1], H. MATSUMOTO[1];
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Abstract: Most of alcoholics have cognitive deficiencies such as learning, memory and motor skills. These functions constitute signal transduction, neural transmission, and so on via molecules and receptors such as dopamine, GABA and glutamate. However, any roles of brain cells in ethanol-induced brain injury remain unclear. In the present study, we evaluated the effects of ethanol on the mRNA expression in the rat brain slices and primary cultures of astrocytes, microglia and neuron from the rat. First, the experiment with organotypic brain slice cultures was performed to examine the effects of ethanol on various genes expression. Male Wistar rats (8 weeks old) were carbon dioxide-anesthetized and quickly decapitated. After dissection of the brain and removal of the frontal and occipital poles (including the cerebellum), the specimens were sliced into 200 μm thick sections on a LEICA VT1000S tissue slicer. After culturing for 3 hours, the slice was incubated in the medium with or without 50-mM ethanol for 12 hours. Thereafter, the expression of various mRNAs in the specimens was revealed by a quantitative real-time RT-PCR system. Ethanol treatment significantly increased the expression of oxidative stress-, ion channel- and glutamate receptor-related genes compared as non-treatment with ethanol. Of course, these organotypic brain slices consist of neurons, astrocytes, microglial cells, NG2 glial cells, oligodendrocytes and so on. Then, we investigated what kind of brain cells might contribute to ethanol-induced expression of mRNAs in the brain slice experiments. Neurons and glial cells were isolated from the hippocampus of E18-19 rat embryos and the forebrain of newborn rats, respectively. We confirmed the differences in the ethanol-induced expression of mRNA among these cells. Therefore, these findings suggested differential effects of ethanol on the brain injury among brain cells.

Disclosures: **K. Sugimoto:** None. **H. Tanaka:** None. **R. Katada:** None. **K. Igarashi:** None. **M. Yoshida:** None. **H. Matsumoto:** None.

Poster

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Support: Developmental Exposure Alcohol research Center (NIAAA: P50 AA017823)

Center for Development and Behavioral Neuroscience at Binghamton University

Title: A comparison of neuroimmune alterations produced by acute ethanol exposure in late adolescent, adult, and aged Fisher 344 rats

Authors: *A. GANO, T. L. DOREMUS-FITZWATER, T. DEAK;
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Abstract: Prior studies have established that the aging brain transforms toward a more inflamed state during senescence, as evidenced by increased microglial activation and increased cytokine response. Our recent work has shown that acute ethanol exposure (4 g/kg) increased expression of interleukin-6 (Il-6) in both the hippocampus (HPC) and paraventricular nucleus of the hypothalamus (PVN) 3 hr after ethanol, whereas interleukin-1 (Il-1) and tumor necrosis factor α (Tnfα) were suppressed at this time point. The goal of the present study was to examine whether ethanol challenge would differentially alter the cytokine response in aged Fisher 344 rats. In this 3 (age group: late adolescent (~PND50), young adult (~PND100), aged (~18 month) x 3 (drug exposure: ethanol, vehicle, homecage) between-subjects design (N = 48, n = 4-6 per group), rats received acute intraperitoneal injections of either 3.5 g/kg 20% v/v ethanol, or equivolume saline, or remained unmanipulated in the homecage. Brain tissue and blood were collected 3 hours later for analysis of blood ethanol concentrations (BECs), plasma corticosterone (CORT), and gene expression of cytokines in the PVN and HPC. As an initial test of behavioral sensitivity to ethanol, Loss of Righting Reflex (LORR) was assessed between exposure to drug and tissue collection. Although BECs did not vary across ages (range: 337-361 mg/dl), adolescents recovered LORR substantially faster than adults and aged rats. Elevated basal concentrations of CORT were observed in aged rats (relative to adolescent) prior to ethanol, yet ethanol exposure produced an equivalent increase in CORT in all age groups. These effects were mirrored by ethanol-induced C-fos expression in the PVN (but not hippocampus). Ethanol increased Il-6 in the PVN and HPC of all rats regardless of age. Interestingly, the pattern of cytokine changes in the hippocampus largely recapitulated what we have observed in adult Sprague Dawley rats (increased Il-6; decreased Il-1 and Tnfα), whereas in the PVN ethanol increased Il-6, Il-1 and Cd14, with Tnfα being suppressed. Overall, age-related increases in Il-1 in the hippocampus and Cd14 in the PVN indicated typical aging-associated increases in neuroimmune status. These findings provide an important foundation for understanding how heightened neuroimmune status in the aging brain might impact the evoked neuroimmune response produced by ethanol challenge during senescence.

Disclosures: **A. Gano:** None. **T.L. Doremus-Fitzwater:** None. **T. Deak:** None.

Poster

053. Alcohol: Neural Mechanisms and Behavior I

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Topic: C.17. Drugs of Abuse and Addiction

Support: Intramural Programs of NIAAA

NINDS ZIA-AA000421

Title: Activity of the striatal indirect pathway neurons alters ethanol seeking behaviors in mice

Authors: *M. B. BLEGEN, R. BOCK, M. F. ADROVER, V. A. ALVAREZ;
Section on Neuronal Structure, Natl. Inst. on Alcohol Abuse and Alcoholism, Rockville, MD

Abstract: Investigating the neural circuitry that mediates ethanol seeking and drinking is a critical step in understanding and preventing the development of alcohol use disorders. The nucleus accumbens (NAc), a key region in the mesolimbic reward circuit, is involved in alcohol seeking behaviors. Medium spiny neurons (MSNs) form the main output of the NAc and consist of two populations: direct and indirect pathway MSNs. Indirect-pathway MSNs (i-MSNs) are thought to inhibit striatal output and facilitate behavioral inhibition, but the role of this pathway in alcohol seeking and drinking remains unclear. The goal of this study was to develop a model of operant ethanol self-administration in mice and test how manipulating the activity of i-MSNs changes operant ethanol drinking behaviors. Mice (*Adora2a-cre*$^{+/-}$ and *Adora2a-cre*$^{-/-}$, males and females, 5-8 weeks old) were given intermittent access to 20% ethanol for 4h during the dark cycle for 5 days/week for 3 weeks. Subsequently, mice were trained on a cued operant self-administration task to earn 20% ethanol (no sucrose) as a reinforcer in 2h sessions for 4 weeks. Most mice acquired the behavior within 7 sessions during which the ratio of active to inactive nose-pokes increased and plateaued at 4.6. Mice earned an average of 50 rewards/2h, which corresponded to 2.25 ± 0.07 g/kg/day of ethanol. Blood ethanol concentrations (BECs) averaged 59 ± 6.8 mg/dl during the intermittent access phase and 68 ± 2.9 mg/dl during the operant phase, ranging from 23 to 172 mg/dl. When ethanol was substituted with water and cues were removed during extinction sessions, the ratio of active to inactive pokes fell to 2. Together, these results show that ethanol, even without sucrose, is a potent reinforcer in mice and that pairing intermittent ethanol access with operant ethanol drinking is a good model from which to study circuitry controlling ethanol seeking and drinking. Manipulations of i-MSNs were performed using Designer Receptor Exclusively Activated by Designer Drug (DREADD) to selectively

inhibit i-MSN output with Gi–coupled DREADDs or activate i-MSN output with Gq-coupled DREADDs. Inhibition of i-MSNs had no effect on operant ethanol seeking or consumption. However, when paired with an aversive stimulus, quinine adulteration, inhibition of the i-MSNs appeared to increase operant ethanol seeking while activation of the i-MSNs caused a robust 60% decrease in active pokes for quinine adulterated ethanol. These results provide strong evidence that indirect pathway neurons play an important role in regulating alcohol seeking under conditions that require behavioral inhibition, which engage this circuit.

Disclosures: **M.B. Blegen:** None. **R. Bock:** None. **M.F. Adrover:** None. **V.A. Alvarez:** None.

Poster

053. Alcohol: Neural Mechanisms and Behavior I

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIH DICBR

Title: Alcohol and the prescription opioid analgesic, oxycodone, disrupt opioid peptide-mediated long-term depression of excitatory transmission in the dorsal striatum

Authors: *B. K. ATWOOD, D. LOVINGER;
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Abstract: Prescription opioid analgesic and alcohol abuse are two forms of drug abuse that present profound socioeconomic burdens in the United States. Understanding the long-term effects of opioid and alcohol use on brain function is increasingly important. Both opioid analgesics and alcohol alter the function of brain opioid receptors, but the consequences of these alterations on synaptic plasticity are unknown. The dorsal striatum is a brain region that is important for goal-directed and habitual action selection and is a site of importance for drug-induced neuronal plasticity. We recently reported evidence that exogenously applied and endogenously released opioids produce long-term depression of excitatory inputs to the dorsal striatum (OP-LTD) in mice and rats (Atwood et al., 2014). OP-LTD is mediated by three different receptors: the mu, delta and kappa opioid receptors. Mu and delta OP-LTD are both presynaptically expressed and occur throughout the dorsal striatum. However these forms of LTD are dissociable in that they summate, differentially occlude endocannabinoid-LTD, and inhibit different striatal inputs. Mu OP-LTD inhibits thalamostriatal inputs, but not inputs from

motor cortex. Conversely, delta OP-LTD inhibits motor cortex inputs but does not affect inputs from thalamus. Kappa OP-LTD shows a unique subregional expression within striatum and has a less clear site of action. A single *in vivo* exposure to the opioid analgesic, oxycodone, disrupts mu OP-LTD and endocannabinoid-LTD, but not delta or kappa OP-LTD. The effect of oxycodone on mu OP-LTD is long-lasting as it is disrupted for three days following a single *in vivo* exposure. Similar to the effects of oxycodone on endocannabinoid-LTD, a recent report indicates that two weeks of chronic intermittent ethanol exposure followed by three days of withdrawal disrupts endocannabinoid-LTD (DePoy et al., 2013). Using a similar ethanol administration paradigm, we find that ethanol exposure also disrupts mu OP-LTD. These data reveal novel, opioid-mediated forms of long-term striatal plasticity that are likely important mediators of striatal-dependent learning and behavior. In addition we find that both the opioid analgesic oxycodone and alcohol have similar negative impacts on mu OP-LTD and endocannabinoid-LTD. The effects of these drugs on these forms of striatal plasticity may underlie some components of the transition from casual drug use to drug abuse and addiction.

Disclosures: **B.K. Atwood:** None. **D. Lovinger:** None.

Poster

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Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq 2496/13-5)

International Brain Research Organization (IBRO)

Title: Ethanol decreases the spontaneous firing rate of a specific subset of Globus Pallidus neurons

Authors: *K. P. ABRAHAO, D. M. LOVINGER;
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Abstract: The Globus Pallidus external segment (GPe) is a sub-cortical brain structure containing neurons that generate high-frequency autonomous pacemaker activity which helps to control basal ganglia output. The GPe receives strong GABAergic afferent input from the

striatum as well as glutamatergic innervation from the subthalamic nucleus. Both inhibitory and excitatory components control the spontaneous firing rate of GPe neurons. Alcohol is one of most widely abused drugs and has known actions on brain GABAergic and glutamatergic synaptic transmission. Ethanol can also alter spontaneous neuronal firing in midbrain neurons. However, little is known about ethanol actions in GPe. Thus, we performed whole-cell patch-clamp recordings from neurons in mouse GPe brain slices to examine acute ethanol actions. Different studies describe specific electrophysiological and biochemical characteristics for different GPe neuron populations. We were able to identify three clusters of GPe neuronal types based on the distribution of the firing rates recorded in slices from 21-58 day old C57BL/6J male mice. Type 1, 2 and 3 neurons exhibited firing rates of (mean ± standard deviation) 9.49 ± 4.02, 27.50 ± 5.46 and 69.45 ± 22.49, respectively. The firing rate was significantly positively correlated with the resting membrane potential (r=0.37) and the voltage sag at hyperpolarized potentials (r=0.41), but negatively correlated with the input resistance (r=-0.38). Bath application of 40 mM ethanol decreased the firing rate of the type 1 GPe neurons by ~25% but it did not alter the firing rate of type 2 or 3 neurons. Ongoing studies are examining the effects of other doses of ethanol as well as the effect of *in vivo* chronic ethanol exposure on the firing rate of GPe neurons. We are also identifying biochemical markers of the type 1 neurons, and examining what mechanisms contribute to ethanol-induced decreases in the firing rate of these neurons. This knowledge may provide better understanding of the mechanisms contributing to ethanol effects on the CNS and behavior.

Disclosures: **K.P. Abrahao:** None. **D.M. Lovinger:** None.

Poster

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Topic: C.17. Drugs of Abuse and Addiction

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Title: Rapid adaptation of dopamine D2 receptors in brain and blood following acute ethanol administration

Authors: *E. JANG, R. J. FOLSOM, J. R. LINZEY, L. FRIEND, C. F. BURNETT, S. H. BURNETT, S. C. STEFFENSEN;
Psychology, Brigham Young Univ., Provo, UT

Abstract: Dopamine (DA) D2 receptor expression parallels DA levels in the brain and chronic ethanol exposure alters the expression of these autoreceptors in the brain. We have previously shown that DA D2 receptor expression following chronic ethanol exposure is down-regulated in the ventral tegmental area (VTA) of the midbrain where DA neurons originate and in their terminals in the nucleus accumbens (NAc), likely a result of lowered DA levels. All five subtypes of DA receptors, including D2 receptors, also have been discovered on T-lymphocytes in mice. The aim of this study was to evaluate short-term D2 receptor adaptation in the brain and blood by acute ethanol in anesthetized rats. First, we measured DA release following injection of ethanol (2.5 g/kg, IP), the D2 agonist quinpirole (0.1 mg/kg, IV), and the D2 antagonist eticlopride (1.0 mg/kg, IP) in the NAc using *in vivo* fast scan cyclic voltammetry (FSCV). Second, we determined that there is a population of DA D2 receptors found on activated monocytes. Activated monocytes are a type of immune cell that leaves the blood and enters interstitial fluid. We sampled blood at hourly intervals in anesthetized rats before and after systemic administration of saline, ethanol, quinpirole, or eticlopride and measured D2 receptor expression using RT-PCR. Systemic administration of eticlopride markedly enhanced (250%) the amplitude of the evoked DA signal, which remained elevated for 2-3 hrs. The expression of D2 receptor mRNA in blood decreased at 2 hrs by 35-64% following ethanol or eticlopride injection. In contrast to both groups, injection of quinpirole increased the expression of D2R mRNA by 176% at 2 hrs. These findings suggest that acute doses of drugs not only yield rapid changes in central D2 receptor expression, but elicit changes in peripheral D2 receptor expression as well. These results have significant clinical potential as changes in D2 receptor expression could be monitored and used for the treatment of addiction as well as other diseases involving DA including Parkinson's disease.

Disclosures: **E. Jang:** None. **R.J. Folsom:** None. **J.R. Linzey:** None. **L. Friend:** None. **C.F. Burnett:** None. **S.H. Burnett:** None. **S.C. Steffensen:** None.

Poster

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Title: Social isolation stress affects dopamine signaling and kappa opioid receptor system in the nucleus accumbens and the basolateral amygdala

Authors: *A. KARKHANIS, J. ROSE, B. A. MCCOOL, J. L. WEINER, S. R. JONES;
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Abstract: Chronic early life stress, such as neglect during childhood, results in increased risk for alcohol use disorders during adulthood. Similarly, rats reared in social isolation (SI) during adolescence show increased ethanol (EtOH) intake compared to group housed controls (GH). Stress elevates dynorphin levels, a kappa opioid receptor (KOR) ligand, which regulates dopamine (DA). Activation of KORs inhibits DA release in the NAc and BLA. NAc and BLA are highly interconnected and play integral roles in the neurobiology of stress, anxiety, and reward-seeking behavior. Recent literature shows that the kappa opioid receptor (KOR) system regulates drug seeking following chronic exposure to ethanol. Therefore, in order to understand the potential underlying mechanisms driving the isolation-induced increased EtOH intake, the effects of acute EtOH on DA in the presence and absence of nor-binaltorphimine (norBNI), a KOR antagonist, were examined in the NAc and the BLA. Moreover, the sensitivity of KORs was examined in NAc slices of SI and GH rats. DA in the NAc and BLA was measured using dual-probe *in vivo* microdialysis in freely moving rats that were either housed in groups (4 rats/cage) or individually (1 rat/cage) for six weeks (PD 28 - 74). Acute EtOH (1 or 2 g/kg, i.p.) was administered after establishing stable DA baselines. A separate group of rats was pre-treated with norBNI (10 mg/kg; i.p.) 24 hrs prior to EtOH challenge. To examine the sensitivity of KORs, *ex vivo* voltammetry was used to assess the effects of U50,488 (10 - 1000 nM), a KOR agonist, on DA release in NAc slices from GH and SI rats. The baseline levels of DA were not different in the NAc of SI and GH rats, but significantly lower in the BLA of SI compared to GH rats. KOR activation increased baseline DA levels in NAc and BLA. The SI rats showed increased DA responses to EtOH (2 g/kg) in both NAc (200% of baseline) and BLA (280% of baseline) and this increase was significantly greater in BLA compared to NAc. EtOH augmented DA responses in the NAc of SI rats pre-treated with norBNI, and attenuated it in the BLA. The inhibitory effects of U50,488 on DA release were enhanced in the NAc of SI compared to GH rats suggesting that chronic stress sensitizes KORs. Increased DA elevations after EtOH in both regions of SI rats is consistent with the stimulant literature, however the mechanisms behind the distinct effects of EtOH-induced DA in presence and absence of norBNI in NAc and BLA are unclear. It is possible that these differences may explain the effects of EtOH on behaviors related to specific brain areas, e.g., augmentation of DA in the NAc results in increased reinforcement, whereas augmentation in DA in the BLA may contribute to decreased anxiety.

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Poster

053. Alcohol: Neural Mechanisms and Behavior I

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Title: CRF-R1 modulation of extracellular serotonin and dopamine in mice given intermittent access to alcohol

Authors: ***L. S. HWA**, E. HOLLY, A. SHIMAMOTO, J. F. DEBOLD, K. A. MICZEK; Psychology, Tufts Univ., Medford, MA

Abstract: Excessive and harmful alcohol drinking can be characterized by recurring cycles of binge drinking and withdrawal. Intermittent access to 20% alcohol (IAA) and water in C57BL/6J mice can generate dependent-like drinking and withdrawal, approximating the human condition. Our previous findings have shown that IAA can cause an activation of corticotropin-releasing factor type 1 receptors (CRF-R1) in extrahypothalamic sites, including the serotonergic dorsal raphé nucleus (DRN) and the dopaminergic ventral tegmental area (VTA). The present studies investigate the dysregulation of neurotransmitters during the transition to dependence in the IAA schedule. Adult, male C57BL/6J mice were given IAA for at least 4 weeks. Animals were implanted with microcannula into the DRN and microdialysis probes into the medial prefrontal cortex (mPFC) for measurement of extracellular serotonin in an efferent site after intra-DRN microinjection of a CRF-R1 antagonist. Another group of IAA mice were implanted with microcannula targeting the VTA and probes into the nucleus accumbens (NAcc) for measurement of extracellular dopamine in an efferent site after intra-VTA microinjection of a CRF-R1 antagonist. Using *in vivo* microdialysis, mice were tested for either serotonin or dopamine 24 hours after alcohol had been removed, and when the next set of alcohol and water were to be presented in the IAA schedule, three hours into the dark phase. Samples were collected before and after microinjection of a CRF-R1 antagonist, CP-154,526, and analyzed with high performance liquid chromatography. Microinjection of CP-154,526 into the DRN

increased extracellular serotonin levels in the mPFC in mice with a history of IAA. A positive control, naltrexone, did not have a significant on serotonin under the same conditions. Ongoing studies are confirming whether CRF-R1 antagonists blunt increased dopamine in NAcc after ethanol consumption (assessing the effect of CP-154,526 on dopamine in the NAcc). Also, we are exploring the transition to dependence by measuring dopamine after the first IAA episode compared with dopamine in the post-dependent mouse. These findings suggest that CRF-R1 interacts with serotonin neurons in the DRN, perhaps the CRF-R1 antagonist disinhibits 5-HT impulse flow or alternatively, CRF acts upon an alternative mechanism, like CRF-R2, to activate serotonin flow.

Disclosures: **L.S. Hwa:** None. **E. Holly:** None. **A. Shimamoto:** None. **J.F. DeBold:** None. **K.A. Miczek:** None.

Poster

053. Alcohol: Neural Mechanisms and Behavior I

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 53.12/V16

Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grant AA020103

Title: Monoamine oxidase (MAO) inhibitors and knockdown of MAO transcriptional activator, Kruppel-like Factor 11, exhibit neuroprotective effects against ethanol-induced neurotoxicity and cell death in SH-SY5Y cells

Authors: *J. W. DUNCAN[1,2], S. HARRIS[1], X. ZHANG[1], X.-M. OU[1], C. A. STOCKMEIER[1,2,4], J. WANG[1,2,3];
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Abstract: Alcoholism is the 3rd leading cause of preventable death in the U.S. and has an annual cost burden of ~$223.5 billion/year (CDC). Due to the health-related risks and morbidity associated with alcohol dependence, new pharmacological targets to reduce subsequent neurotoxicity and brain cell death from ethanol exposure could be beneficial. We have demonstrated that a 50mM-100mM concentration range (a physiologically relevant blood level observed in alcohol abuse) of ethanol in cell culture models increases Monoamine Oxidase-B (MAO-B) transcriptional activator, Kruppel-like factor 11 (KLF11), MAO-B expression, and

MAO-B catalytic activity which collectively results in neuronal cell death. Much of the literature has suggested the application of MAO-B inhibitors, such as Selegiline and Rasagiline, for the treatment of neurodegenerative diseases as they increase expression of anti-apoptotic Bcl-2 proteins and oxidative stress scavenging enzymes in neurodegenerative cell models. Whether these neuroprotective effects persist in the presence of ethanol is currently being investigated. In the present study, new generation MAO-Is (Rasagiline and M30) were concomitantly administered with ethanol to examine their neuroprotective properties in cultured human neuroblastoma (SH-SY5Y) cells. Furthermore, cells transfected with siRNA targeting KLF11 provided a similar degree of neuroprotection to that of MAO-I administration. Cell viability was significantly enhanced in ethanol-exposed [75mM-150mM] cells treated with KLF11 siRNA or MAO-Is (.025nM - 100nM concentrations) compared to ethanol-exposed control cells. Moreover, western blot analysis revealed a KLF11 knockdown and MAO-I drug effect on the expression of anti-apoptotic proteins and oxidative stress scavenging enzymes, leading to an overall increase in cytoprotective markers. Together, these results further support the efficacy of KLF11 RNA interference (RNAi) and MAO-I pharmacotherapy to combat ethanol-induced neurotoxicity and cell death. Further exploration of these two novel candidate neuroprotective strategies may afford potential avenues to alleviate ethanol-induced neurotoxic consequences.

Disclosures: **J.W. Duncan:** None. **S. Harris:** None. **X. Zhang:** None. **X. Ou:** None. **C.A. Stockmeier:** None. **J. Wang:** None.

Poster

053. Alcohol: Neural Mechanisms and Behavior I

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grant R01AA021505

Title: Activity of D1R MSNs in the dorsomedial striatum is essential for voluntary alcohol consumption

Authors: *J. WANG, Y. CHENG, X. WANG;
Dept. of Neurosci. & Exptl. Therapeut., Texas A&M Hlth. Sci. Ctr., Bryan, TX

Abstract: Dopamine D1 receptor-expressing (D1R) medium spiny neurons (MSNs) in the striatum give rise to the direct pathway that contributes to selection of “Go” actions in rewarding

behaviors. We recently found that excessive alcohol intake enhances synaptic strength in D1R MSNs of the dorsomedial striatum (DMS) in mice. The enhanced synaptic strength in D1R MSNs may contribute to the maintenance of excessive alcohol intake by increasing "Go" actions that are associated with alcohol intake. Here, we examined whether manipulation of D1R MSN activity in the DMS alters voluntary alcohol consumption. We infused in the DMS of drd1a-Cre mice with a viral vector containing Cre-dependent hM4Di. The selective expression of hM4Di in D1R MSNs allows us to downregulate the activity of these neurons and to examine their contribution to alcohol drinking behaviors. Mice were trained to consume high levels of alcohol using the intermittent access 2-bottle choice drinking procedure. After a stable baseline of alcohol intake is achieved, CNO, the ligand of hM4Di, was systemically administered, and alcohol intake was measured for 30 min, 4 hrs, and 24 hrs. We found that CNO administration causes significant reduction of alcohol intake in 30 min and 4 hrs, but not in 24 hrs. The reduction of alcohol intake is accompanied by a decrease in alcohol preference. However, CNO administration does not alter water intake. Together, our results suggest that the activity of D1R MSNs in the DMS, which contribute to "Go" actions, is required for the maintenance of excessive alcohol consumption.

Disclosures: **J. Wang:** None. **Y. Cheng:** None. **X. Wang:** None.

Poster

053. Alcohol: Neural Mechanisms and Behavior I

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Topic: C.17. Drugs of Abuse and Addiction

Support: R01AA021121

T32AA07455

Title: The role of VTA GABA(A) receptors in maladaptive decision making and increased phasic dopamine release following chronic adolescent alcohol intake

Authors: *A. G. SCHINDLER, K. T. TSUTSUI, H. H. N. HOANG, J. J. CLARK;
Univ. of Washington, Seattle, WA

Abstract: Alcohol is the most commonly abused substance among adolescents and shows the highest liability of all abused drugs. We have previously demonstrated that voluntary

consumption of alcohol by adolescent rats results in increased maladaptive risk-taking behavior and nucleus accumbens (NAc) phasic dopamine release in adulthood, as assessed by a probability discounting task and fast scan cyclic voltammetry (FSCV) respectively. These finding suggest that adolescent alcohol exposure-induced changes in NAc dopamine release could bias choice by assigning greater value to the risky option, but the underlying mechanisms remain unknown. GABA(A) receptors located in the VTA are a potential candidate for mediating these effects, and we hypothesize that adolescent alcohol intake confers persistent changes to risk-taking behavior through alcohol-mediated, GABA(A) receptor-induced modulation of NAc dopamine release. Using electrical stimulation of the pedunculopontine nucleus (PPT) afferents to VTA DA neurons, here we demonstrate that adult rats previously exposed to alcohol during adolescence showed increased NAc phasic dopamine release, as compared to controls. Conversely, NAc phasic dopamine release via electrical stimulation of the medial forebrain bundle (MFB) did not differ between alcohol and control rats, suggesting that increased dopamine release following adolescent alcohol intake occurs via changes within the VTA. PPT stimulation elicits GABA(A) receptor dependent current in VTA DA neurons, raising the possibility that adolescent alcohol intake produces this increased phasic dopamine release through downregulation of GABA(A) receptors on VTA DA neurons. Systemic administration of L-838,417, a GABA(A) receptor α2, α3, and α5 subunit allosteric agonist, caused a dose-dependent decrease in phasic DA release elicited by PPT stimulation in control rats, and this decrease was enhanced in rats exposed to alcohol during adolescence. In order to investigate this phenomenon further, imaging flow cytometry was used to measure expression of GABA(A) receptor subunits on individual neurons of the ventral midbrain. Taken together, these results provide unique insight into the potential role that GABAergic modulation of dopamine neurons plays in the maladaptive risk-taking behavior seen following adolescent alcohol intake and highlights new potential therapeutic targets.

Disclosures: **A.G. Schindler:** None. **K.T. Tsutsui:** None. **H.H.N. Hoang:** None. **J.J. Clark:** None.

Poster

053. Alcohol: Neural Mechanisms and Behavior I

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grant AA007680

NIH Grant AA021213

Title: Up-regulation of novel synaptic GABA-A receptor subtypes contributes to altered mIPSC kinetics and pharmacology in rat hippocampus after acute or chronic ethanol intoxication

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[2]Neurobio., Geffen Sch. of Med. at UCLA, Los Angeles, CA

Abstract: Alcohol (EtOH) intoxication causes long-lasting plastic changes of various γ-aminobutyric acid type A receptors ($GABA_A$Rs) in the brain. These alterations contribute to EtOH-induced changes in synaptic and extrasynaptic physiology and pharmacology involving decreased sensitivity to EtOH enhancement of extrasynaptic $GABA_A$R-mediated tonic inhibitory currents and increased EtOH sensitivity of GABAR-mediated miniature inhibitory postsynaptic currents (mIPSCs) (Liang et al., 2006). This is believed to play a crucial role in alcohol dependence and withdrawal symptoms. Previously we showed that in rat hippocampus and other brain regions, acute and chronic EtOH administration to rodents by gavage results in decreased cell surface expression of $GABA_A$R subunits δ and α1 while γ2 and α4 are up-regulated. Modulation of synaptic and extrasynaptic $GABA_A$R-mediated inhibitory currents are consistent with contribution of α to mIPSCs. Here, we report also an increase in surface expressed α2 and γ1 subunits. Co-immunoprecipitation experiments reveal an increase in $GABA_A$Rs composed of α4/β/γ2 and α2/β1/γ1 at synaptic sites. The two subtypes have rather similar BZ pharmacology, consistent with a role for either α 4 or α 2 subunit-containing $GABA_A$Rs in the EtOH-dependent rodent. Two-pulse EtOH experiments were used to identify the receptor subtype showing sensitivity to EtOH application, measuring the temporal sequence of changes in $GABA_A$R cell surface expression and mIPSC sensitivity to EtOH. Studies on α4 KO mice, which reveal increased EtOH drinking in the TBC assay, demonstrate an EtOH-sensitive synaptic current in mice lacking the α 4 subunit, untreated with EtOH. They show elevated α2, γ2, and γ1, suggesting possible involvement of α2-containing receptors in EtOH-sensitivity. Furthermore, mIPSC analysis with an optimally scaled template algorithm is consistent with significant presence of α 2 mIPSCs in rat hippocampus, especially after CIE and in α 4 KO mice, and they appear to be enhanced by acute EtOH. These studies, coupled with human genetic linkage of α2 to AUD, suggest that besides up-regulated α4/β/γ2 receptor subtypes, also α2-containing $GABA_A$Rs are involved in withdrawal syndromes and alcohol dependence including increased drinking.

Disclosures: **R.W. Olsen:** None. **A.K. Lindemeyer:** None. **X.M. Shao:** None. **J. Liang:** None.

Poster

053. Alcohol: Neural Mechanisms and Behavior I

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIH R01 AA013983

Title: Intermittent and binge-like alcohol drinking in Gabra2 knock-in mice with benzodiazepine- or ethanol-insensitive α2 subunits

Authors: *E. L. NEWMAN[1], G. GUNNER[1], P. HUYNH[1], S. MOSS[2], U. RUDOLPH[3], J. F. DEBOLD[1], K. A. MICZEK[1,2];
[1]Psychology, Tufts Univ., Medford, MA; [2]Neurosci., Tufts Univ. Sackler Sch. of Grad. Biomed. Sci., Boston, MA; [3]Lab. of Genet. Neuropharmacology, McLean Hospital, and Dept. of Psychiatry, Harvard Med. Sch., Belmont, MA

Abstract: Benzodiazepines and alcohol may disinhibit drug-taking behavior via positive allosteric modulation of heteromeric GABA-A receptors. Endophenotypic analyses have identified GABRA2 single nucleotide polymorphisms as predictors of an individual's predisposition to alcohol dependence. To further characterize the role of the GABA-A α2 subunit, knock-in mice rendered selectively insensitive to benzodiazepines (H101R) or ethanol (S270H/L277A) were assessed for binge-like or dependence-inducing ethanol intake. In a 4-day drinking in the dark procedure, male mutant and C57BL/6J wild type mice received 2-hour access to 20% ethanol (EtOH) on three consecutive days. On the fourth day, access was extended to 4 hours to measure binge-like EtOH consumption. *Gabra2* (H101R) males with benzodiazepine-insensitive α2 subunits drank significantly more (7.9 g/kg) than WT or *Gabra2* (S270H/L277A) mutants (5.5-6 g/kg) during the 4-hour access period. As a comparison, mutant and WT mice were also evaluated for dependence-inducing alcohol intake in a chronic, intermittent access protocol. On alternating days, EtOH-drinking animals received 24-hour, 2-bottle choice access to 20% EtOH (w/v) and water while water-drinking control mice always received two bottles of water. Under intermittent EtOH conditions, WT and *Gabra2* (H101R) males consistently drank 18-20 g/kg/24h, suggesting that benzodiazepine-sensitive GABA-A α2 subunits are not required for high EtOH intake in this protocol. After eight weeks of chronic intermittent EtOH-drinking, wild type mice were assessed in a social approach protocol 6-8 hours after removal of their 20% EtOH bottles. During withdrawal, these mice expressed

reduced durations of social approach behavior as compared to water-drinking controls. Current investigations assess chronic intermittent alcohol drinking and social measures of withdrawal in *Gabra2* males with benzodiazepine (H101R) - or ethanol (S270H/L277A) -insensitive GABA-A α2 subunits.

Disclosures: E.L. Newman: None. **S. Moss:** None. **U. Rudolph:** None. **G. Gunner:** None. **P. Huynh:** None. **J.F. DeBold:** None. **K.A. Miczek:** None.

Poster

053. Alcohol: Neural Mechanisms and Behavior I

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Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 53.17/V21

Topic: C.17. Drugs of Abuse and Addiction

Title: Zebrafish and conditioned place preference: A translational model of drug reward

Authors: *D. J. ECHEVARRIA[1], A. D. COLLIER[2], E. M. CAMARILLO[2], K. M. KHAN[2]; [1]Dept Psychol, Univ. Southern Mississippi, HATTIESBURG, MS; [2]Univ. of Southern Mississippi, HATTIESBURG, MS

Abstract: Addiction and substance abuse amass hundreds of billions of dollars annually in costs associated with healthcare, crime and lost productivity, solely within the United States. Efficacious treatments remain few in number, the development of which will be facilitated by comprehension of environmental, genetic, pharmacological and neurobiological mechanisms implicated in the pathogenesis of addiction. Animal models such as the zebrafish (Danio rerio) have gained momentum within various domains of translational research, and as a model of complex brain disorders (e.g., drug abuse). Behavioral quantification within the conditioned place preference (CPP) paradigm serves as a measure of the rewarding qualities of a given substance. If the animal develops an increase in preference for the drug paired environment, it is inferred that the drug has positive-reinforcing properties. This study reports the effects of acute (1 day) and chronic (14 days) exposure of ethanol, nicotine and caffeine on CPP behavior in zebrafish.

Disclosures: D.J. Echevarria: None. **A.D. Collier:** None. **E.M. Camarillo:** None. **K.M. Khan:** None.

Poster

053. Alcohol: Neural Mechanisms and Behavior I

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Title: Incubation of alcohol craving is dependent on alcohol cue-activated neurons in the nucleus accumbens core

Authors: *C. MONTANARI[1], N. SUTO[1], T. KERR[1], D. WATRY[1], B. STARR[1], B. T. HOPE[2], P. P. SANNA[1], F. WEISS[1];
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Abstract: The core subregion of the nucleus accumbens (NAcore) has been implicated in relapse-promotion by alcohol cues. We examined the effects of selective disruption of alcohol cue-activated neurons localized within in the NAcore on 1) alcohol cue-activated operant responding or 'alcohol seeking', and 2) time-dependent increases or "incubation" of this behavior (an animal model of progressive enhancement in drug craving observed during protracted abstinence). Selective disruption of 'cue-activated' neurons was accomplished by the Daun02 method in cFos-lacZ rats. In this transgenic rat strain, the inactive prodrug Daun02 is catalyzed into the cytotoxin daunorubicin by beta-galactosidase (ß-gal) only in Fos-positive 'activated' neurons, and thereby induces selective apoptosis. Fos-negative 'non-activated' neurons express neither Fos nor ß-gal such that Daun02 is not converted to daunorubicin in these cells and cellular disruption is prevented. Male cFos-lacZ rats were trained for operant self-administration of alcohol (10%, v/v; oral). Insertion of an 'active-lever' and illumination of a house-light signaled alcohol availability during a single-daily alcohol self-administration session. Each delivery of alcohol (0.1 ml) was paired with illumination of a cue-light. Alcohol self-

administration then was discontinued and rats remained in their home cages until testing for alcohol seeking on the first, third, fourteenth, and forty-second days of the abstinence (D1, D3, D14, and D42). All stimulus conditions during these tests were identical to those in effect during alcohol self-administration, except that sessions were conducted under extinction conditions (with water substituted alcohol). Daun02 or vehicle was bilaterally microinjected into the NAcore on D1 following the first test session. In vehicle-treated rats, a transient, time-dependent increase in alcohol seeking was observed (evident on D14), as reported previously. Daun02-induced selective disruption of alcohol cue-activated neurons on D1 prevented this time-dependent increase, but spared alcohol seeking itself (evident on D3, D14 and D42). Thus, the incubation rather than the execution of alcohol seeking per se appears to be dependent on alcohol cue-activated neurons localized within the NAcore. Supported by NIAAA/NIH and NIDA/NIH: DA033533 (N.S.), AA020960 (P.P.S.), AA021667 (P.P.S.), AA021549 (F.W.), and AA018010 (F.W.).

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Poster

053. Alcohol: Neural Mechanisms and Behavior I

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grant AA020537

NIH Grant AA020930

Title: Genetic influence of Kcnn3 on extinction learning identifies a novel target for enhancing inhibitory learning of alcohol-associated cues

Authors: ***J. T. GASS**, L. JAMES, J. T. MCGONIGAL, P. J. MULHOLLAND; Neurosciences, Med. Univ. South Carolina, CHARLESTON, SC

Abstract: Exposure to alcohol-related cues contributes to high rates of relapse in treatment-seeking alcoholics. The ability to facilitate the extinction of alcohol-associated cues using cognitive enhancers is a promising therapeutic approach to reduce relapse rates. Previous work in our laboratory has shown that we can facilitate the extinction of alcohol-seeking behavior with

the mGluR5 positive allosteric modulator CDPPB. Interestingly, mGluR5 activation produces a long-lasting decrease in small-conductance calcium-activated potassium (KCa2) channel currents. KCa2 channels have been implicated in synaptic plasticity, cognition, and addiction, and modulating these channels can enhance the extinction learning for food-seeking behavior and fear expression. Recent evidence has also demonstrated that genetic factors can influence extinction learning in mice. However, the specific genes that regulate extinction learning have not been identified, and it is currently unknown if modulating KCa2 channels can facilitate extinction of alcohol-associated memories. Thus, the purpose of this study was to determine if the genes that encode KCa2 channels (Kcnn1-3) predict extinction learning in BXD recombinant inbred (RI) strains of mice and if blocking KCa2 channels enhances extinction learning of alcohol cues. Preliminary evidence showed that Kcnn3 transcript levels in the prefrontal cortex (PFC) of BXD RI strains of mice were correlated with the number of trials to extinguish responding for food-related cues ($R2 = 0.607$, $p < .05$; $n = 7$ strains). We found that lower transcript levels of Kcnn3 in the PFC were associated with faster extinction (i.e., enhanced learning). To complement our genetic findings, we examined the ability of apamin, a KCa2 channel allosteric inhibitor, to facilitate extinction of alcohol-seeking behavior. Wistar rats were trained to self-administer 10% EtOH and then exposed to extinction training. Vehicle or apamin was administered 30 min prior to each extinction session. Apamin significantly enhanced the extinction of alcohol-seeking behavior [$F(13,130)=3.8$, $p < 0.001$; $n = 6$]. This was evidenced by significantly reduced responding on multiple days of extinction (p values < 0.05) and fewer sessions required to reach extinction criteria [$t(10)=5.6$, $p <0.001$]. These data indicate that PFC Kcnn3 transcript levels regulate extinction learning and that modulation of KCa2 channels by either direct inhibition or indirect activation of mGluR5 receptors facilitates extinction learning of alcohol-seeking behavior. KCa2 channels may be a novel pharmacogenomic target for enhancing cue exposure therapy in the treatment of alcohol use disorders.

Disclosures: **J.T. Gass:** None. **L. James:** None. **J.T. McGonigal:** None. **P.J. Mulholland:** None.

Poster

054. Cocaine Reinforcement I

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Topic: C.17. Drugs of Abuse and Addiction

Support: RO1 DA03982

P50 DA15369

Title: Synaptic activity through GluN2A-containing NMDA receptors mediates ability of BDNF-TrkB signaling to suppress cocaine-seeking in rats

Authors: *B. GO, J. F. MCGINTY;
Dept. of Neurosciences, Med. Univ. of South Carolina, Charleston, SC

Abstract: Brain-derived neurotrophic factor (BDNF) is an important modulator of excitatory synaptic transmission. BDNF increases cell surface expression of AMPARs and NMDARs through phosphorylation and translocation events. Intra-dorsomedial PFC (dmPFC) infusion of BDNF immediately after the last cocaine self-administration (SA) session suppresses cocaine-seeking and normalizes a cocaine-induced decrease in basal extracellular glutamate levels in the nucleus accumbens (NAc). Such an intra-dmPFC BDNF infusion rescues cocaine SA-induced ERK and CREB dephosphorylation in the dmPFC. TrkB receptors are expressed in postsynaptic density regions of cortical pyramidal neurons and their stimulation leads to ERK activation through interaction with synaptic NMDARs. Therefore, in the present study we examined whether the ability of intra-dmPFC BDNF to suppress cocaine seeking is mediated by BDNF-induced recovery of synaptic activity in the dmPFC. Immediately after the last cocaine SA session (2 hr/day x 2 weeks, 0.2 mg/infusion), a mixture of the AMPA receptor antagonist, CNQX (0.1nmol/0.5ul/side), and the NMDA receptor antagonist, LY235959 (10ng/0.5ul/side) or 1%DMSO, was infused into the dmPFC 30 min before BDNF or PBS. Rats infused with CNQX/LY235959 before BDNF showed significantly higher active lever pressing during a post-abstinence relapse test (PA test) and cue-induced reinstatement test (cue test) than rats that received 1% DMSO followed by BDNF. This suggests that AMPA/NMDA activity is necessary for BDNF's suppressive effect on cocaine-seeking in rats with a cocaine SA history. Then we checked the specific role of GluN2A-containing NMDA receptors in the BDNF-mediated suppression of cocaine seeking. Immediately after the end of cocaine SA), the GluN2A receptor antagonist, TCN-201 (0.1nmol/0.5ul/side) or 2% DMSO, was infused into the dmPFC 20 min before BDNF or PBS. Rats infused with TCN-201 before BDNF showed significantly less active lever pressing during a PA test and cue test than rats that received vehicle followed by BDNF. This suggests that GluN2A-containing NMDARs are required for BDNF's ability to suppress cocaine-seeking in rats with a cocaine SA history.

Disclosures: **B. Go:** None. **J.F. McGinty:** None.

Poster

054. Cocaine Reinforcement I

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grant T32DA007288-21

Title: Role of calcium-permeable AMPA receptors in the mPFC in cue-induced reinstatement of cocaine seeking

Authors: *J. I. PEÑA BRAVO, A. LAVIN;
Neurosci., Med. Univ. of South Carolina, Charleston, SC

Abstract: Drug addiction is characterized by chronic relapse episodes following attempts of abstinence. Vulnerability to relapse in addicts has been attributed to an increase in reactivity to environmental stimuli (previously neutral in nature) that, through repeated compulsive drug use, become strongly associated with drug consumption and trigger drug seeking. A circuit that has been extensively studied in addiction in rodents involves the interplay between cortical and striatal structures such as the medial prefrontal cortex (mPFC) and the nucleus accumbens (NAc) respectively. Of interest to us is the bimodal modulation that the mPFC exerts onto the NAc, with the pre-limbic (PL-mPFC) projections to the core of the NAc driving cocaine seeking and the infralimbic (IL-mPFC) projections to the NAc shell inhibiting cocaine seeking after extinction training. Previous studies describe an accumulation of calcium-permeable AMPA receptors (Cp-AMPARs) in the NAc following a month of withdrawal from extended access cocaine self-administration. We investigated the hypothesis that alterations in Cp-AMPAR plasticity in the mPFC, after cue-induced reinstatement, modulate the drive in cocaine seeking behavior in self-administering rats. In the current study, whole-cell patch clamp electrophysiological recordings of mPFC brain slices were performed using a CsCl-based internal solution containing spermine (0.1mM) to measure AMPAR rectification index (RI). This method allows an indirect measure of Cp-AMPARs levels in the PL-mPFC and IL-mPFC. Preliminary results show that 24 hrs after cue exposure, there is an opposing switch in AMPAR subunit composition between the IL-mPFC (increase in AMPAR RI) and PL-mPFC (decrease in AMPAR RI) projection neurons. These results suggest an experience-dependent reactivation or renewal of the cocaine-cues association memory after extinction training that leads to a delayed increase in IL-mPFC Cp-AMPARs and a corresponding increase in activity of the IL-mPFC to NAc shell projection neurons.

Disclosures: **J.I. Peña Bravo:** None. **A. Lavin:** None.

Poster

054. Cocaine Reinforcement I

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Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 54.03/V26

Topic: C.17. Drugs of Abuse and Addiction

Title: Effects of baclofen on cue-induced cocaine-reinstatement in the rat

Authors: *T. D. WOLINSKY, C. FROGER-COLLÉAUX, C. RONDEAU, S. PAILLARD, V. CASTAGNE;
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Abstract: Treating relapse of drug-seeking behavior is considered the most challenging part of the treatment for addictive disorders. Relapse can be modeled in laboratory animals using reinstatement paradigms, whereby extinguished behavioral responding for a drug is reinstated by different factors, such as cues or stress. Activation of GABAB receptor complexes may be a promising pharmacotherapy for diminishing relapse potential to reinforcing drugs. We therefore assessed the effects of baclofen (a prototypic γ-aminobutyric acid B (GABAB) receptor agonist) on cue-induced cocaine-reinstatement in the rat. Male Sprague Dawley rats, implanted with i.v. jugular catheters, were submitted to 2-hr daily sessions during which they could receive i.v. infusions of cocaine under a fixed ratio-1 (FR1) schedule of lever pressing. At the beginning of each daily session rats received a non-contingent infusion of cocaine. During these sessions a cue light was turned on for 3 sec when rats pressed the active lever. After 2 sec of cue light, a tone signal was turned on for 1 sec together with activation of the infusion pump. Each infusion was followed by a 30-sec timeout. Once cocaine self-administration (SA) behavior was acquired, rats were moved to extinction sessions in which active lever presses no longer had consequences. Rats were considered to have extinguished behavior when they exhibited an average of < 12 active lever presses during 2 consecutive sessions. Rats were tested in the first reinstatement session, the following day after the rats had met the extinction criteria. The rats were then re-submitted to at least 2 further extinction sessions followed by a second reinstatement session. Rats were pretreated 30 minutes before the reinstatement sessions with vehicle or baclofen. The cue reinstatement sessions began with a tone (1 sec) and cue light (3 sec). The remainder of the 2 hr session was identical to the SA session except that cocaine was not delivered. During the first reinstatement session, half of the rats received vehicle and the other half received baclofen. On the second reinstatement session, the inverse was applied. Baclofen 2.5 and 5 mg/kg, dose-dependently decreased the number of active lever presses during cue reinstatement (8.0 ± 2.5 for

baclofen at 2.5 mg/kg versus 16.9 ± 2.1 for vehicle and 6.4 ± 4.5 for baclofen at 5 mg/kg versus 27.3 ± 3.5 for vehicle), producing 81.2 and 99.6% inhibition respectively. These data suggest the potential anti-craving efficacy of baclofen for the treatment of cocaine drug-seeking.

Disclosures: **T.D. Wolinsky:** None. **C. Froger-Colléaux:** None. **C. Rondeau:** None. **S. Paillard:** None. **V. Castagne:** None.

Poster

054. Cocaine Reinforcement I

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Topic: C.17. Drugs of Abuse and Addiction

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NIH grant DA031533

Title: More than a replacement therapy: Amphetamine treatment reverses the behavioral and neurochemical consequences of long-access cocaine self- administration

Authors: *C. SICILIANO[1], E. S. CALIPARI[2], S. R. JONES[1];
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Abstract: Cocaine abuse and dependence is a major health problem with no FDA-approved pharmacotherapies. Although amphetamine (AMPH) has been examined as a potential treatment for cocaine addiction, with promising behavioral results in rodents, monkeys and humans, the mechanism for AMPH-induced decreases in cocaine reinforcement has yet to be elucidated. It has been proposed that AMPH acts as an agonist replacement therapy; however, our data suggest that these effects may be a result of AMPH-induced stabilization of the dopamine (DA) system. Fixed-ratio 1 self-administration of cocaine in daily six-hour sessions (long-access) has been shown to result in increased intake, or "escalation". This escalation is often used as a pre-clinical model of the switch from recreational abuse to addiction. We found that treatment with 5 mg/kg/day AMPH s.c., delivered continuously via osmotic mini-pump for 14 days, significantly attenuated the development of escalation, suggesting that amphetamine treatment has a

protective effect against increased cocaine intake. Because cocaine's reinforcing effects have been shown to be dependent on its ability to inhibit the DA transporter and elevate accumbal DA levels, we used fast scan cyclic voltammetry in brain slices containing the nucleus accumbens core to measure cocaine inhibition of the DA transporter. We found that long-access reduced cocaine potency for DA uptake inhibition, suggesting that tolerance is driving escalation whereby animals increase intake to compensate for reduced subjective effects of the compound. Further, in line with the behavioral data, AMPH treatment blocked the development of cocaine tolerance when administered during cocaine self-administration. Providing more evidence that the effects of AMPH on cocaine intake are independent of agonist replacement, AMPH, when administered after cocaine self-administration, completely reversed cocaine tolerance. Taken together, these data suggest AMPH reduces cocaine self-administration, at least in part, by reversing the cocaine-induced changes in the DA system. Although previous literature has shown promising behavioral results suggesting AMPH is a potentially effective treatment for cocaine abuse, this is, to our knowledge, the first investigation of the neurochemical mechanisms for these results. While agonist therapies for cocaine addiction are controversial and AMPH has a high abuse potential, these data may be able to define a mechanism for some of AMPH's therapeutic effects, which could drive the design of more targeted pharmacotherapies with less abuse liability.

Disclosures: **C. Siciliano:** None. **E.S. Calipari:** None. **S.R. Jones:** None.

Poster

054. Cocaine Reinforcement I

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Topic: C.17. Drugs of Abuse and Addiction

Support: PHS Grant DA033370

Title: Inactivation of the lateral habenula reduces the anxiogenic response of both undrugged and cocaine-treated rats in the elevated plus maze

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Abstract: Solomon and Corbitt's (1974) Opponent Process Theory of motivation states that all affective stimuli produce diametrically opposite and temporally dissociated actions. Human users of cocaine report just such a response after taking the drug; an initial euphoric state followed by a period of dysphoria and anxiety. While the euphoric effects of cocaine have long been studied, the anxiogenic aspects of the drug remain largely unexplained. Recent work has suggested a role for the lateral habenula (LHb) in the behavioral response to anxiogenic stimuli. The current study was therefore devised to examine whether the LHb might play a role in cocaine-induced anxiety. An elevated plus maze was used to measure the anxiogenic response of rats following permanent or reversible inactivation of the LHb. Three groups of male albino rats served as subjects. One group had permanent LHb inactivation produced by bilateral intracranial infusions of kainic acid; another experienced reversible inactivation of the LHb via bilateral intracranial pretreatment with a baclofen/muscimol infusion prior to behavioral testing; and a third group consisted of sham-lesioned control subjects. Each animal was habituated to the elevated plus apparatus during an initial 5-min baseline trial. Testing then consisted of two 5-min sessions on two separate days during which rats each received either a 1.0 mg/kg IV cocaine injection (in volume of 0.1 ml), or a comparable volume of saline in a counterbalanced order. This was dose selected on the basis of prior work demonstrating its dual positive and anxiogenic actions. The dependent measures were latency to enter the open arms of the apparatus, and the total time spent in the open arms during each 5-min test session. Animals that underwent LHb inactivation (either by kainic acid lesion or by IC pretreatment with baclofen/muscimol) were faster to enter, and spent more time in, the open arms of the maze during baseline indicating that LHb activation reduced the subjects' inherent anxiety associated with placement into the unfamiliar apparatus. This effect of LHb inactivation was accentuated when subjects were pretreated with IV cocaine. These results are therefore consistent with a habenular complex involvement in the processing of anxiogenic stimuli, including the administration of cocaine. Supported by PHS grant DA033370 awarded to AE.

Disclosures: **K. Shelton:** None. **K. Bogyo:** None. **M.B. Kurland:** None. **T. Schick:** None. **S. Sved:** None. **S. Janusonis:** None. **A. Ettenberg:** None.

Poster

054. Cocaine Reinforcement I

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIH/NIDA Grant DA033370

Title: Intra-ventral tegmental area infusions of the nonselective CRF receptor antagonist astressin B reduces the anxiogenic response of rats to cocaine in a runway model of drug self-administration

Authors: *S. COTTEN, III, A. K. KLEIN, M. A. BRITO, T. OHANA, B. MARGOLIN, A. WEI, A. ETTENBERG;
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Abstract: In addition to its positive/rewarding effects, cocaine has also been shown to have negative/anxiogenic properties. Much is known about the underlying neuronal mechanisms responsible for the appetitive properties of cocaine, but what of cocaine's negative effects? Of particular relevance to this question has been recent work demonstrating that alterations in the release of the stress hormone corticotropin releasing factor (CRF) within the ventral tegmental area (VTA) can produce alterations in the cocaine-seeking behavior of rats. To further investigate these findings we employed a straight-arm runway model of drug self-administration. Animals running a straight alley once a day for an IV injection of cocaine develop over trials a unique behavior characterized by a rapid approach toward the goal, a stop at the goal threshold, and then an abrupt "retreat" back toward the start box. These retreats have been shown to reflect an approach-avoidance conflict stemming from the subjects' association of the goal box with the mixed positive and negative properties of cocaine. The runway model therefore provides a tool with which to explore the appetitive (approach) and the anxiogenic (avoidance) qualities of cocaine in the same animal on the same trial. In the current study, 3 groups of male rats were trained over 15 consecutive days to run the alley for single daily injections of 1.0 mg/kg IV cocaine delivered upon goal-box entry. Prior to each trial, rats received bilateral intra-VTA infusions of one of three doses of the non-specific CRF receptor antagonist astressin B (0.0, 0.5 or 1.0 ug/side) 10 min after which they were tested in the runway. The latency to leave the start box, the time to enter the goal box (run time), and retreat frequency was recorded on every trial. Start latencies progressively increased over the course of testing for all groups indicating that the positive incentive (appetitive) properties of cocaine remained intact across subjects. The vehicle-pretreated group demonstrated increases in approach-avoidance retreats over trials, while rats pretreated with the CRF antagonist demonstrated reliable reductions in retreats (the high dose attenuated and the low dose completely eliminated the behavior). This effect of the antagonist was not due to some nonspecific action of the peptide since, in a subsequent test, astressin B was found to have no effects on the rats' spontaneous locomotor behavior. These results are therefore consistent with the growing body of literature demonstrating that CRF release within the VTA plays a role in the negative/anxiogenic effects of cocaine. This research was supported by PHS grant DA-033370 awarded to AE.

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Poster

054. Cocaine Reinforcement I

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIDA Grant 031734

Title: Episodic social defeat stress escalates dopamine release and cocaine self-administration in mice

Authors: *X. HAN, L. ALBRECHET-SOUZA, M. R. DOYLE, E. Y. ZHANG, J. F. DEBOLD, K. MICZEK;
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Abstract: Social stress can contribute to the initiation and maintenance of drug use. In preclinical studies, social defeat stress, as generated by repeated confrontations with an aggressive resident, is associated with long-term neural adaptations, which may escalate subsequent drug intake. However, how the intensity and timing of social defeat stress alters these effects have not been well characterized. Here we investigated the effects of distinct social defeat stress intensities and durations (brief vs. moderate) on cocaine self-administration, and explored dopamine (DA) release in the nucleus accumbens shell (NAcSh) by using *in vivo* microdialysis. Adult male CFW mice experienced 10 days of social defeat stress, either brief (15 attack bites in ca. 2 min) or moderate (30 attack bites in ca. 5 min), and compared to controls that were handled daily. Six days after the last social defeat, mice were implanted either with catheters for intravenous cocaine self-administration, or guide cannulae aimed at NAcSh for microdialysis. After recovery, mice were allowed to self-administer cocaine (0, 0.3, 0.6 and 1.0 mg/kg/infusion) according to a FR1 schedule. Another cohort of mice was assessed for DA levels in the NAcSh in response to acute amphetamine (1.5 mg/kg, i.p.) 10 days after the last defeat. Mice in both brief and moderate stress groups self-administered more cocaine (0.3 mg/kg/infusion) than the controls. Moreover, both brief and moderate stress groups showed a larger and more prolonged increase in DA concentration after d-amphetamine challenge than controls did. These findings suggest that the neuroadaptation in the dopaminergic system resulting from repeated social defeat stress may contribute to escalated cocaine intake.

Disclosures: **X. Han:** None. **L. Albrechet-Souza:** None. **M.R. Doyle:** None. **E.Y. Zhang:** None. **J.F. DeBold:** None. **K. Miczek:** None.

Poster

054. Cocaine Reinforcement I

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Title: Taking STEPs to reduce cocaine-seeking behavior

Authors: *B. M. SIEMSEN[1], M. CHATTERJEE[2], P. L. LOMBROSO[2], J. F. MCGINTY[1]; [1]Med. Univ. of South Carolina, Charleston, SC; [2]Child Study Ctr., Yale Univ. Sch. of Med., New Haven, CT

Abstract: Relapse to drug seeking is a main clinical obstacle pertaining to the treatment of cocaine addiction in humans. One hypothesis as to why addicts continually seek cocaine is hypoactivity within the prefrontal cortex during withdrawal, leading to the inability to initiate executive control to inhibit relapse when presented with conditioned stimuli associated with drug use. In rats trained to self-administer cocaine, extracellular regulated kinase 1/2 (pERK1/2) and cAMP response-element binding protein (pCREB) phosphorylation is decreased in the dorsal-medial prefrontal cortex (dmPFC) two hours after the end of cocaine self-administration (early withdrawal), but returns to baseline levels 20 hours later. These dynamic fluctuations in protein expression facilitate context, cue, and cocaine prime-induced relapse to drug-seeking following abstinence and extinction training, respectively. Reversing pERK1/2 and pCREB deficits with a single BDNF infusion into the dmPFC immediately after the last cocaine self-administration session reduces context, cue, and cocaine prime-induced relapse and results in normalized pERK1/2 and pCREB expression in the dmPFC during early withdrawal (Berglind et al., 2007; Whitfield et al, 2011). Additionally, Striatal Tyrosine-Enriched Phosphatase (STEP) is up-regulated in the dmPFC two hours after the final session of cocaine self-administration, and is suspected to result in the reduction of pERK1/2 and its downstream target pCREB during early withdrawal from cocaine (Sun et al., 2013). Recently, 10 mg/kg of the small molecule STEP inhibitor TC-2153 has been shown to increase levels of pERK1/2 in the frontal cortex of mice, and reverse cognitive deficits in an Alzheimer's disease mouse model (Xu et al., 2014). We hypothesized that systemic administration of TC-2153 immediately after the final cocaine self-administration sessions (2 hr/day x 14 days) would attenuate context, cue, and cocaine prime-

induced relapse to drug seeking after abstinence and extinction training through a reversal of ERK1/2 dephosphorylation that occurs during early withdrawal. Preliminary results indicate that TC-2153 (5 mg/kg, i.p.) reduces context-induced cocaine-seeking in rats abstinent from cocaine for one week. Cue and cocaine prime-induced reinstatement tests after extinction training are ongoing. Future experiments will focus on increasing sample size as well as preferentially targeting the dmPFC with TC-2153 microinfusions immediately after cocaine self-administration, and analysis of western blots for pERK, pCREB, and STEP in the dmPFC two hours after cocaine self-administration.

Disclosures: **B.M. Siemsen:** None. **M. Chatterjee:** None. **P.L. Lombroso:** None. **J.F. McGinty:** None.

Poster

054. Cocaine Reinforcement I

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Topic: C.17. Drugs of Abuse and Addiction

Support: P50 DA15369

T32 DA007288

Title: The role of src family kinases in the suppressive effect of prefrontal cortical BDNF on cocaine seeking

Authors: *S. M. BARRY, J. F. MCGINTY;
Neurosci. Inst., Med. Univ. of South Carolina, Charleston, SC

Abstract: Relapse to drug seeking remains a major obstacle in the treatment of cocaine addiction in human addicts. Animal models of relapse have demonstrated that neuroadaptations in reward circuits following cocaine self-administration underlie reinstatement to drug seeking. Specifically, dysregulation of the pathway from the prefrontal cortex (PFC) to the nucleus accumbens (NAc) is implicated in reinstatement. Brain-derived neurotrophic factor (BDNF) is synthesized in PFC pyramidal neurons and antereogradely transported to the NAc where it is the primary source of BDNF in the NAc. Our lab has shown that a single BDNF infusion into the prelimbic cortex following a final cocaine self-administration session results in attenuation of reinstatement to cocaine seeking through normalization of basal and cocaine primed extracellular

glutamate levels in the nucleus accumbens. This attenuating effect can be blocked by blocking either BDNF's receptor TrkB, the members of its associated intracellular signaling cascade, or AMPA/NMDA receptors. These results imply that the interaction between glutamate mediated synaptic activity and TrkB signaling is imperative to BDNF's suppressive effect on drug seeking. Src family kinases are involved in both NMDA/AMPA mediated activation of TrkB and TrkB mediated phosphorylation of NMDA receptors. Thus src family kinases serve as a likely link between these two signaling systems. We hypothesized that infusion of the src family kinase inhibitor PP2 into the prelimbic cortex prior to a BDNF infusion will block BDNF's attenuation of both context and cue induced reinstatement. PP2 blocked BDNF's suppressive effect on context-induced reinstatement, while there was no significant effect for cue-induce reinstatement. Future directions will include analysis of phosho-GluN2B levels to determine if PP2's blocking action occurs from dysregulation of TrkB mediated NMDA receptor activation.

Disclosures: **S.M. Barry:** None. **J.F. McGinty:** None.

Poster

054. Cocaine Reinforcement I

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Title: The role of HDAC3 in the acquisition and extinction of cocaine-cue memories

Authors: *Y. ALAGHBAND[1], J. L. KWAPIS[1], M. ASTARABADI[1], J. D. RAYBUCK[2], D. P. MATHEOS[1], K. M. LATTAL[2], M. A. WOOD[1];
[1]Neurobio. and Behavior and Ctr. for the Neurobio. of Learning & Memory, Univ. of California, Irvine, Irvine, CA; [2]Oregeon Hlth. and Sci. Univ., Portland, CA

Abstract: Environmental cues associated with the rewarding properties of drugs are a major cause of relapse among addicts. Specifically, drug-cue memories are formed as a result of associating cues in the environment with drug taking after repeated pairings of an environmental context with the positive effects of drugs. Conditioned place preference (CPP) is used to study contextual cue-elicited drug-seeking. Because drug-paired cues evoke memories that influence drug-seeking and relapse, a better understanding of the neurobiological basis for this phenomenon will aid the development of novel, biologically-based therapies for drug addicts. Given that drug-cue memories are persistent and stable, it is hypothesized that the same molecular mechanisms responsible for long-term memory formation also underlie the formation and maintenance of drug-cue memories. Chromatin modification is a molecular mechanism for both long-term memory formation and cocaine-induced neuroplasticity and behavior. Histone deacetylases (HDACs) are chromatin modifying enzyme that have been implicated as powerful negative regulators of memory processes. Previous work from our laboratory has shown that inhibition of HDAC3 (the most highly expressed Class I HDAC in the brain) not only facilitates the acquisition of cocaine-CPP, but also modulates the extinction of drug-seeking behavior in a manner that is subsequently resistant to reinstatement (Malvaez et al., 2013; Rogge et al., 2013). Little is known, however, regarding the specific neural loci where HDAC3 plays this critical role in the acquisition and extinction of cocaine-cue memories. In these experiments, we explore the role of HDAC3 in the cocaine-cue memories using a genetic and viral approach. To investigate the role of HDAC3 in the dorsal hippocampus and infralimbic cortex during the acquisition and extinction of cocaine-cue memories using the CPP model, C57BL/6J male mice received infusions of viruses which either overexpressed the Hdac3 gene or had a dominant negative mutation of the gene. Our findings demonstrate a critical role for HDAC3 in the molecular mechanisms underlying cocaine-cue memories.

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Poster

054. Cocaine Reinforcement I

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Title: The role of neuron specific nucleosome remodeling complex subunit BAF53b in cocaine-associated behaviors

Authors: *A. WHITE[1], M. A. WOOD[2];
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Abstract: Recent evidence suggests that epigenetic mechanisms have a critical role in the development of drug-seeking behavior. However, there is currently very little known about the role of nucleosome remodeling, a major epigenetic mechanism, in drug-induced behavioral changes. In this study, we examined the role of BAF53b, a neuron-specific subunit of the nBAF (Neuron-specific Brg1-Associated Factor) nucleosome remodeling complex, in cocaine-associated behaviors. In these studies, we examined Baf53b+/- heterozygous knockout mice as well as transgenic mice expressing a dominant-negative BAF53b (CaMKIIα-BAF53bΔHD). To assess cocaine-seeking behaviors, both types of BAF53b mutant mice (n= 10-15 per group) and their respective wild-type littermates were subject to cocaine-induced conditioned place preference (CPP) using 5mg/kg or 10mg/kg doses. Also, cocaine sensitivity (10mg/kg) was examined in Baf53b+/- heterozygous mice. We demonstrate that BAF53b is necessary for the acquisition/consolidation of some but not all cocaine-induced behaviors. Both the Baf53b+/- heterozygous knockout mice and the CaMKIIα-BAF53bΔHD transgenic mice had deficits in cocaine-induced CPP. In contrast, Baf53b+/- heterozygous mice exhibited normal cocaine sensitization. This study provides the first evidence that a neuron-specific nucleosome remodeling complex (nBAF) has a role in drug-associated behaviors. Using two different genetic manipulations, we established a differential role for BAF53b in cocaine-induced conditioned place preference, but possibly not in cocaine sensitization. These findings demonstrate that nucleosome remodeling, a major epigenetic mechanism, is critically involved in context-cocaine associated memory formation.

Disclosures: **A. White:** None. **M.A. Wood:** None.

Poster

054. Cocaine Reinforcement I

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Topic: C.17. Drugs of Abuse and Addiction

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DA018165

DA025922

Title: A histone deacetylase 3 inhibitor enhances extinction and attenuates reinstatement of self-administration in rats

Authors: *L. N. HITCHCOCK[1], J. D. RAYBUCK[1], M. A. WOOD[2], K. M. LATTAL[1]; [1]Behavioral Neurosci., Oregon Hlth. & Sci. Univ., Portland, OR; [2]Neurobio. & Behavior, Univ. of California, Irvine, Irvine, CA

Abstract: Addiction is a chronic, often relapsing disease that causes compulsive drug seeking. The neurobiological basis of relapse in humans is often studied with an animal model of reinstatement. In the acquisition phase of this paradigm, animals press a lever to receive reinforcing intravenous cocaine infusions. During extinction, reinforcers are then withheld and the animal eventually inhibits this lever-pressing behavior. But like humans, rats will relapse, or renew this drug-seeking behavior once they are removed from the extinction context or exposed to drug-associated cues. Given that there are no long-term therapies for cocaine addiction to date, I investigated whether a novel and selective epigenetic drug (RGFP966) could promote extinction and weaken reinstatement. After rats were trained to self-administer cocaine to stable and high rates, rats were given a subcutaneous injection of RGFP966 (histone deacetylase (HDAC) 3 inhibitor) prior to the first extinction day (no cocaine reinforcers). As a result, RGFP966-injected rats responded significantly less during extinction and reinstatement tests than vehicle-injected rats. These effects were not likely due to a performance deficit or a change in motivation to self-administer cocaine, as injections of RGFP966 had no effect on stable responding during a fixed or progressive ratio schedule of cocaine self-administration in subsequent studies. Results suggest that a systemic injection of RGFP966 enhanced extinction and suppressed reinstatement after cocaine self-administration by inhibiting HDAC3 activity. Future studies will determine whether brain region specific decreases in HDAC3 activity further suppress drug seeking.

Disclosures: **L.N. Hitchcock:** None. **J.D. Raybuck:** None. **M.A. Wood:** None. **K.M. Lattal:** None.

Poster

054. Cocaine Reinforcement I

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Bundesministerium für Bildung und Forschung, NGFN-Plus 01GS08155

Title: Bi-directional role of CRHR1 in control of dopamine signalling during cocaine relapse

Authors: *R. E. BERNARDI[1], L. BROCCOLI[1], A. C. HANSSON[1], J. M. DEUSSING[2], R. SPANAGEL[1];
[1]Psychopharmacology, Central Inst. of Mental Hlth., Mannheim, Germany; [2]Max Planck Inst. of Psychiatry, Munich, Germany

Abstract: The ability of many drugs of abuse, including cocaine, to mediate reinforcement and drug-seeking behaviors is in part mediated by the corticotropin releasing hormone (CRH) system, in which CRH exerts its effects partly via the CRH receptor subtype 1 (CRHR1) in extra-hypothalamic areas. Most of these previous studies have used pharmacological means to address the role of CRHR1 in drug dependence. The current study examined cocaine-induced locomotor sensitization and cocaine self-administration and reinstatement measures in mice with an inducible knockout of the CRHR1 receptor using the Cre-loxP system (ERT2) in either dopamine transporter (DAT)-containing neurons or dopamine receptor 1 (D1)-containing neurons. For sensitization testing, mice received 5 daily injections of cocaine (15 mg/kg IP), with tests for locomotion on days 1, 5, and following a 7-day withdrawal period. For self-administration, mice received daily sessions of self-administration in which presses on the active of two levers resulted in an intravenous infusion of cocaine (0.5 mg/kg per infusion) paired with a cue light. Following 8 days of self-administration, mice were given extinction trials in which the cue was omitted and no cocaine was delivered. After reaching criterion for extinction, mice were given reinstatement sessions as follows: cue reinstatement, yohimbine-induced reinstatement (stress), yohimbine + cue reinstatement, and cocaine reinstatement. Between reinstatement tests, mice were given daily extinction sessions to once again reach criterion. There were no differences in the acute or sensitized locomotor response to cocaine in CRHR1-DATCreERT2 or CRHR1-D1CreERT2 mice and their respective controls. Furthermore, both CRHR1-DATCreERT2 and CRHR1-D1CreERT2 mice reliably self-administered cocaine at the level of wild-type littermates. However, CRHR1-DATCreERT2 mice demonstrated a significant increase in cue-induced reinstatement relative to controls, whereas CRHR1-D1CreERT2 mice demonstrated a significant decrease in cue-induced reinstatement relative to controls. Interestingly, despite the well-known role for CRHR1 in stress responsivity, there were no

differences in stress-induced reinstatement in CRHR1-DATCreERT2 or CRHR1-D1CreERT2 mice and their respective controls. or cocaine-induced reinstatement of drug-seeking behavior. These data further demonstrate the involvement of CRHR1 in cue-induced reinstatement following cocaine self-administration, and implicate a bi-directional role of CRHR1 in presynaptic negative feedback and postsynaptic feed-forward control of dopamine signalling during relapse-like behavior.

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Poster

054. Cocaine Reinforcement I

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Title: One-trial cocaine-induced behavioral sensitization in preweanling rats: role of dopamine and serotonin receptor subtypes

Authors: M. J. STONE, A. MOHD-YUSOF, A. E. GONZALEZ, M. L. BECKER, J. A. MORTOLA, A. VELIZ, *S. A. MCDOUGALL;
Dept. of Psychology, California State Univ., San Bernardino, CA

Abstract: In rodents, accumulating evidence indicates that the underlying processes and neural mechanisms mediating one-trial behavioral sensitization differ markedly between the preweanling period and adulthood. For example, there is abundant evidence that contextual conditioning does not influence the one-trial behavioral sensitization of preweanling rats, whereas drug-environment associations are necessary for the occurrence of one-trial behavioral sensitization in older animals. Additionally, the D_1 antagonist SCH23390 blocks the induction of one-trial cocaine-induced behavioral sensitization in adult rats, while not affecting the sensitized

responding of preweanling rats. In adult rats, the neural mechanisms mediating behavioral sensitization involve multiple receptor systems, because D_2 receptor antagonists (e.g., raclopride), as well as serotonin 5-HT_2 and 5-HT_3 receptor antagonists (ritanserin and ondansetron, respectively), are capable of blocking the induction of cocaine-induced behavioral sensitization. The purpose of the present study was to determine whether antagonism of the same dopamine and serotonin receptor systems would block the induction of behavioral sensitization in preweanling rats. On the pretreatment day [i.e., postnatal day (PD) 19], preweanling rats were injected with saline, ritanserin (0.3, 1, or 3 mg/kg), ondansetron (0.03, 0.1, or 0.3 mg/kg), raclopride (0.1, 0.5, 1, or 5 mg/kg), or a combined administration of SCH23390 (0.5 mg/kg) and raclopride (0.5 mg/kg) 15 min before a single injection of cocaine (30 mg/kg). Locomotor activity was measured for 30 min. One or two days later (i.e., on PD 20 or PD 21), rats were challenged with cocaine (20 mg/kg) and locomotor sensitization was assessed across a 120 min testing session. Results showed that raclopride, but not ritanserin or ondansetron, acutely blocked cocaine-induced locomotor activity on the pretreatment day. On the test day, however, cocaine-induced locomotor sensitization was evident regardless of whether rats had been pretreated with ritanserin, ondansetron, raclopride, or SCH23390 + raclopride. These results suggest that the neural mechanisms mediating behavioral sensitization vary across ontogeny. This conclusion is based on the finding that 5-HT_2, 5-HT_3, and D_2 receptor antagonism blocks the induction of behavioral sensitization in adult rats, while leaving the sensitized responding of preweanling rats unaffected.

Disclosures: **M.J. Stone:** None. **S.A. McDougall:** None. **A. Mohd-Yusof:** None. **A.E. Gonzalez:** None. **M.L. Becker:** None. **J.A. Mortola:** None. **A. Veliz:** None.

Poster

054. Cocaine Reinforcement I

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 54.15/W6

Topic: C.17. Drugs of Abuse and Addiction

Support: NIDA Grant K01DA031745

NINDS Grant T32NS007433

Pennsylvania Department of Health

Title: Role of amygdalar CaMKII in cocaine-associated memory reconsolidation and extinction

Authors: *M. T. RICH[1], T. ABBOTT[2], K. STONE[2], L. CHUNG[2], C. COLANGELO[2], A. NAIRN[2], J. R. TAYLOR[2], M. M. TORREGROSSA[1];
[1]Univ. of Pittsburgh, Pittsburgh, PA; [2]Yale Univ., New Haven, CT

Abstract: Memories of environmental cues associated with drug use contribute to relapse to drug-taking, which is prevalent in addiction. Evidence suggests that protein kinase activity within the amygdala regulates both the reconsolidation and extinction of drug-induced memories and manipulating this activity may serve as a potential treatment for addictive disorders. One such protein, calcium/calmodulin-dependent protein kinase II (CaMKII), has been shown to directly phosphorylate synaptic proteins, contributing to memory storage via modulation of synaptic activity. Here, we show that phosphorylation of CaMKIIα, specifically at Ser331 may be differentially involved in the extinction and reconsolidation of a drug-induced memory. Additionally, intra-basolateral amygdala (BLA) infusion of KN-62, a specific CaMKII inhibitor, seems to impact these same memory processes, leading to an alteration in drug-seeking behavior. Rats trained to self-administer cocaine paired with an audiovisual cue underwent a test session in which the drug-cue memory was either reactivated by brief presentation to induce reconsolidation, or extinguished by multiple unreinforced presentations. Proteomic analysis of tissue from the BLA revealed a decreased expression of pSer331 CamKIIα following memory reconsolidation and an increased expression following cue extinction. Furthermore, inhibition of CaMKII in the BLA via KN-62 resulted in decreased responding on a cue-induced reinstatement test both in rats that had undergone cue reactivation and in those in which the memory had been extinguished. Therefore, CaMKII activity may be critical for the maintenance of drug-associated memories and inhibitors of CaMKII might be useful adjuncts to extinction training in the prevention of relapse in addiction.

Disclosures: **M.T. Rich:** None. **T. Abbott:** None. **K. Stone:** None. **L. Chung:** None. **C. Colangelo:** None. **A. Nairn:** None. **J.R. Taylor:** None. **M.M. Torregrossa:** None.

Poster

054. Cocaine Reinforcement I

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 54.16/W7

Topic: C.17. Drugs of Abuse and Addiction

Support: TACTICS FP7/2007-2013 278948

Title: Glutamatergic transmission during adolescence is critically involved in the rewarding effects of cocaine in rats: Effects of early life stress

Authors: *R. M. O' CONNOR[1], R. D. MOLONEY[2], S. VLACHOU[4], J. F. CRYAN[3];
[1]Dept. of Anat. and Neurosci., [2]Alimentary Pharmabiotic Ctr., [3]Dept. of Anat. and Neurosci. and Alimentary Pharmabiotic Ctr., Univ. Col. Cork, Cork, Ireland; [4]Dublin City Univ., Dublin, Ireland

Abstract: Adolescence marks a critical time when the brain is highly susceptible to pathological insult yet also uniquely amenable to therapeutic intervention. Furthermore, it is during adolescence that the onset of the majority of psychiatric disorders, including substance use disorder (SUDs), occurs. Individuals affected by a SUD experience compulsions to seek and consume drugs, a loss of control over drug intake and the emergence of a negative emotional state. Risk factors present during adolescent development include high levels of impulsivity and immature reward circuitry. Furthermore, it has been well established that stress, particularly during early development, can contribute to the pathological changes which contribute to the development of SUDs. Glutamate is the main excitatory neurotransmitter in the mammalian CNS and plays a key role in regulating reward; glutamatergic innervations can converge on the nucleus accumbens increasing salience to drug-related stimuli. As such, reducing glutamatergic signalling in circuits associated with reward processing and impulsive control may serve to attenuate the rewarding and physiological effects of drugs of abuse in adolescents potentially attenuating stress-induced vulnerability to SUD development. To test this hypothesis we assessed the effects of reducing glutamatergic signalling using the glutamate transporter activator riluzole and the NMDA receptor antagonist memantine on intravenous self-administration of cocaine in rats. We induced early-life stress in male Sprague Dawley rats by separating pups from dams from PND 2-12 for 3 hours/day. During the adolescence (3 - 6 weeks) rats received 21 days treatment with riluzole (1, 3, 10 mg/kg or vehicle; i.p.) or memantine (3, 10, 30 mg/kg or vehicle; i.p.). Intravenous catheters were implanted and following recovery animals were allowed to self-administer cocaine in one hour daily sessions for 5 days/week with escalating dose being provided each week (0.125, 0.25 and 0.5 mg/kg/infusion respectively). Interestingly, maternally separated (MS) rats self-administered less cocaine in all three doses tested while riluzole at 3 and 10 mg/kg and memantine at all three doses tested attenuated this. Potentially, MS animals possess a reward deficit and as such rewarding stimuli do not engage the reward circuitry to the same degree as in non-separated animals (NS). These data suggests reducing glutamatergic signalling may be a viable therapeutic strategy for treating vulnerable individuals at risk of developing SUDs including certain adolescent populations, particularly those which may have experienced early-life stress.

Disclosures: **R.M. O' Connor:** None. **R.D. Moloney:** None. **S. Vlachou:** None. **J.F. Cryan:** None.

Poster

054. Cocaine Reinforcement I

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 54.17/W8

Topic: C.17. Drugs of Abuse and Addiction

Support: FM Kirby Foundation

BRODERICK BRAIN FOUNDATION

McKenzie Fdn

Title: Caffeine's neuroprotective properties against the reinforcing effects of cocaine

Authors: *L. B. MALAVE[1,2], P. A. BRODERICK[1,2,3];
[1]Dept. of Physiology, Pharmacol. and Neurosci., Sophie Davis Sch. CCNY, New York, NY;
[2]Biol., CUNY Grad. Ctr., New York, NY; [3]Neurol., NYU Langone Med. Ctr., New York, NY

Abstract: Caffeine has biphasic effects that are highly dose-dependent. The exact mechanism caffeine has on the adenosine system is still being extensively studied. Caffeine increases dopamine (DA) release within the brain acting on adenosine 1 receptors and adenosine A2 receptors and causes effects similar to cocaine at specific doses. However, although cocaine and caffeine are both stimulants, when combined together, caffeine has neuroprotective abilities on the reinforcing effects of cocaine that are dose dependent. These results are consistent over a number of behavioral and biochemical paradigms, including DA release *in vivo*, locomotor activity, conditioned place preference, pre-pulse inhibition, and estrus cycle changes. We propose important findings that provide insight into cocaine abuse. We suggest that inhibition of the adenosine system at specific doses and involvement of specific receptors may aid in the adverse effects of cocaine. Furthermore, we link our results to possible therapeutic options in mental disorders, such as schizophrenia, with malfunction of the DA circuit.

Disclosures: **L.B. Malave:** None. **P.A. Broderick:** None.

Poster

054. Cocaine Reinforcement I

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

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Topic: C.17. Drugs of Abuse and Addiction

Support: Neurobiology of Addiction Research Center at the Medical University of South Carolina P50 DAP50DA015369

Center for Behavioral Neuroscience

Brains & Behavior Program at Georgia State University

Title: Arc and BDNF expression after cocaine self-administration or cue-induced reinstatement of cocaine-seeking in adolescent and adult male rats

Authors: A. WHITE[1], C. LI[2], J. F. MCGINTY[3], *K. J. FRANTZ[1];
[1]Georgia State Univ., Atlanta, GA; [2]Anat. and Cell Biol., Temple Univ., Philadelphia, PA; [3]Dept. of Neurosciences, Med. Univ. of South Carolina, Charleston, SC

Abstract: In our laboratory, male rats that self-administer cocaine as adolescents exhibit lower levels of cue-induced reinstatement of cocaine-seeking, as compared with adults. Activity-regulated cytoskeletal-associated gene (arc) and brain-derived neurotrophic factor (bdnf) are two important neuroplasticity-related genes that influence drug-seeking behavior, change over development, and may play a role in age differences we observe. For the present study, we predicted that levels of cocaine-seeking correlate negatively with differential expression of Arc and BDNF in reward and reinforcement-related brain regions. Adolescent and adult rats were allowed to acquire lever-pressing maintained by i.v. infusions of cocaine in daily two-hour sessions over 13 days. A subset of rats in both age groups received only saline infusions. At three experimental time points (immediately after the last self-administration session, after extinction and reinstatement at 1 day abstinence from cocaine, and after extinction and reinstatement at 60 days abstinence), rats were sacrificed and brain tissue was collected. Arc and BDNF mRNA levels were analyzed by *in situ* hybridization and densitometry in the prelimbic and infralimbic cortex, nucleus accumbens core and shell, claustrum, caudate putamen, and motor cortex. Although Arc expression varied by drug treatment and time in region-dependent ways, Arc expression was similar across age groups in almost all cases. In contrast, BDNF expression was higher in adolescent compared to adult rats. Additionally, BDNF expression was higher in cocaine-experienced rats and rats sacrificed at the first and last experimental time points. These data generally support the hypothesis that higher levels of BDNF mRNA in reward and reinforcement-related brain regions during adolescence could attenuate some long-term effects of cocaine. Future studies should entail mechanistic analysis of BDNF mRNA and protein, as well as the role of BDNF receptors in age-dependent cocaine-related behaviors.

Disclosures: **A. White:** None. **C. Li:** None. **J.F. McGinty:** None. **K.J. Frantz:** None.

Poster

054. Cocaine Reinforcement I

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIH/NIDA DA08467 (FW)

NIH/NIDA DA07348 (FW)

NIH/NIDA DA033344 (RMF)

Title: Orexin/hypocretin in the paraventricular nucleus of the thalamus induces cocaine-seeking behavior in rats: reversal with specific orexin/hypocretin receptor antagonists

Authors: *A. MATZEU, F. WEISS, R. MARTIN-FARDON;
The Scripps Res. Inst., La Jolla, CA

Abstract: Growing evidence implicates a role for orexin/hypocretin (Orx/Hcrt) neurons that originate in the lateral hypothalamus (LH) and project to the paraventricular nucleus of the thalamus (PVT) in drug addiction. Although this thalamic region has not traditionally been thought of as part of the drug addiction circuitry, recent evidence indicates that the PVT modulates reward function in general and drug-directed behavior in particular. Our working hypothesis was that cocaine-induced dysregulation of the Orx/Hcrt system modifies Orx/Hcrt-PVT transmission, an effect that is long-lasting. We previously reported that administration of orexin-A/hypocretin-1 (Orx-A/Hcrt-1) in the PVT reinstates extinguished cocaine-seeking behavior in animals with short access (ShA, 2 h/day) or long access (LgA, 6 h/day) to cocaine and sweetened condensed milk (SCM) seeking but with different dose-response profiles. Knowing that Orx-A/Hcrt-1 interacts with both orexin receptor 1 (Hcrt-r1) and receptor 2 (Hcrt-r2), this study sought to investigate the extent to which Hcrt-r1 and Hcrt-r2 are involved in Orx/Hcrt-induced cocaine or SCM seeking. This was accomplished by co-administering Orx-A/Hcrt-1 (0.5 μg) with a specific Hcrt-r1 antagonist (SB334867) or Hcrt-r2 antagonist (TCSOX229) into the PVT. The 0.5 μg Orx-A/Hcrt-1 dose was selected based on earlier findings that showed that it was the minimal dose that produced comparable and significant reinstatement in all of the groups (i.e., SCM, ShA, and LgA). Male Wistar rats were trained to self-administer cocaine for 2 h/day (ShA) or 6 h/day (LgA) or SCM (30 min/day) for 21 days. Immediately following self-administration training, the animals were subjected to daily extinction training for

14 days. The following day, the animals were tested for the ability of SB334867 (0-15 μg) and TCSOX229 (0-15 μg) to reverse Orx-A/Hcrt-1-induced reinstatement. Unexpectedly, SB334867 did not have any effects on Orx-A/Hcrt-1-induced reinstatement in any of the groups (i.e., SCM, ShA, and LgA) at any dose. In contrast, co-administration of TCSOX229 with Orx-A/Hcrt-1 decreased cocaine seeking only, an effect that was more pronounced in the LgA group. This suggests that Hcrt-r1 in the PVT is not directly implicated in the Orx-A/Hcrt-1 priming effect and that a history of cocaine self-administration dysregulates Orx/Hcrt-PVT neurotransmission at the level of Hcrt-r2. These results shed light on molecular targets (i.e., Hcrt receptors) that are dysregulated by a history of cocaine dependence and further identify a possible therapeutic approach to preventing drug seeking without affecting normal motivated behavior toward natural reward.

Disclosures: **A. Matzeu:** None. **F. Weiss:** None. **R. Martin-Fardon:** None.

Poster

054. Cocaine Reinforcement I

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIH/NIDA DA08467 (FW)

NIH/NIDA DA07348 (FW)

NIH/NIDA DA033344 (RMF)

Title: Temporary inactivation of the paraventricular nucleus of the thalamus specifically blocks cocaine-seeking behavior: Comparison with natural reward seeking

Authors: *R. MARTIN-FARDON, F. WEISS, A. MATZEU;
MCND, The Scripps Res. Inst., LA JOLLA, CA

Abstract: Drug addiction is a chronic relapsing disorder characterized by compulsive drug seeking and use. However, it is unclear what differentiates neural signaling related to normal appetitive behavior vs. compulsive behavior that results from long-term drug exposure. One hypothesis concerning the control of drug-seeking behavior may be that the neural circuits that mediate these effects are common motivational circuits that are not specific to addiction-related events that are more robustly activated by drug-related stimuli. Although not included the “drug

addiction circuitry," recent evidence indicates that the paraventricular nucleus of the thalamus (PVT) is involved in the modulation of drug-directed behavior. The PVT is known to play a key role in energy homeostasis, arousal, endocrine regulation, and reward. Data from this laboratory have shown that the PVT is recruited during the conditioned reinstatement of cocaine seeking but not sweetened condensed milk (SCM) seeking. This activation was specifically correlated with cocaine-seeking behavior but not SCM-seeking behavior, suggesting that a history of cocaine intake alters the neural systems that regulate motivation that is normally directed toward natural rewards toward a preferential role in mediating the effects of stimuli that are conditioned to drugs vs. natural rewards. To test whether the PVT plays a pivotal role during cocaine seeking, the aim of this study was to investigate whether temporary inactivation of the PVT selectively prevents the cue-induced reinstatement of cocaine seeking but not SCM seeking. Male Wistar rats were trained to associate a discriminative stimulus (SD) with the availability of cocaine or SCM (S+) vs. saline or non-reward (S-) for 10 consecutive days. Following the extinction of cocaine- and SCM-reinforced responding, the rats were presented with the respective S+ or S- alone. The cocaine S+ and SCM S+ elicited identical levels of reinstatement, whereas the non-reward S- did not produce any reinstatement. Intra-PVT administration of the GABAA/GABAB agonists baclofen/muscimol (0.6/0.06 mM) prior to the presentation of the cocaine or SCM S+ completely prevented the conditioned reinstatement of cocaine seeking, with no effect on SCM-seeking behavior. These data show that the PVT plays an important role during cocaine-seeking behavior but not during natural reward-seeking behavior, further supporting the hypotheses that (i) the PVT is involved in drug addiction circuitry and (ii) a history of cocaine self-administration produces dysregulation within this thalamic structure and may tilt its function toward drug-directed behavior.

Disclosures: **R. Martin-Fardon:** None. **F. Weiss:** None. **A. Matzeu:** None.

Poster

054. Cocaine Reinforcement I

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIDA Grant DA037523

Title: Cocaine history sensitizes postsynaptic GABA receptors on dorsal raphe serotonin neurons in a stress-induced relapse model in rats

Authors: *C. LI, L. KIRBY;
Ctr. for Substance Abuse Res., Temple Univ. Sch. of Med., Philadelphia, PA

Abstract: The serotonin (5-hydroxytryptamine, 5-HT) system plays an important role in stress-related psychiatric disorders and substance abuse. Stressors can inhibit the dorsal raphe nucleus (DR)-5-HT system, which composes the majority of forebrain-projecting 5-HT. This inhibition is mediated via stimulation of GABA synaptic activity at 5-HT DR neurons by the stress neurohormone corticotrophin-releasing factor. Recent data from our laboratory indicate that morphine history sensitizes 5-HT DR neurons to GABAergic inhibitory effects of stress. Moreover, GABAA receptor-mediated inhibition of the serotonergic DR contributes to stress-induced reinstatement of morphine conditioned place-preference. In our current experiment, we tested the hypothesis that GABAergic sensitization of 5-HT DR neurons is a neuroadaptation elicited by multiple classes of abused drugs across multiple models of stress-induced relapse. Whole-cell patch-clamp recordings of GABA synaptic activity in 5-HT DR neurons were conducted in brain slices from Sprague-Dawley rats that underwent yohimbine stress-induced reinstatement of previously extinguished cocaine self-administration. Behavioral data indicate that the chemical stressor yohimbine triggered reinstatement of cocaine self-administration. Electrophysiology data indicate that 5-HT neurons in the cocaine group exposed to stress had increased amplitude of inhibitory postsynaptic currents compared to yoked-saline controls exposed to stress or unstressed animals in both drug groups. These data, together with previous findings, indicate that interaction between psychostimulant or opioid history and stress may increase postsynaptic GABA receptor density and/or sensitivity in 5-HT DR neurons to GABAergic inhibition. Such mechanisms may result in serotonergic hypofunction and consequent dysphoric mood states which confer vulnerability to stress-induced drug reinstatement.

Disclosures: **C. Li:** None. **L. Kirby:** None.

Poster

054. Cocaine Reinforcement I

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

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Topic: C.17. Drugs of Abuse and Addiction

Support: DA033206

DA033882

Title: Excitability of mPFC pyramidal neurons was abnormally increased in Fisher F344 rats after low doses of cocaine self-administration

Authors: *W. N. WAYMAN[1,2], L. CHEN[1,2], X.-T. HU[1,2], T. C. NAPIER[1,2,3];
[1]Pharmacol. Department,, [2]Ctr. for Compulsive Behavior and Addiction, [3]Psychiatry, Rush Univ., Chicago, IL

Abstract: The medial prefrontal cortex (mPFC) regulates reward-motivated behavior. Chronic exposure to cocaine disrupts the mPFC which contributes to drug-craving, drug-seeking and relapse to drug-taking during protracted abstinence. Our laboratory has shown that mPFC activity is upregulated in adult male Sprague Dawley rats 2-3 weeks after 14 days of cocaine self-administration wherein cocaine was given as 1.0mg/kg/infusion and resulted in a 2hr daily session total of ~20mg/kg. Adult male Fisher rats self-titrate much lower doses of cocaine, and it is not known if this also is sufficient to alter mPFC activity. We reasoned that if changes in mPFC activity reflect neuronal plasticity induced by cocaine-mediated reward rather than the dose of cocaine per se, then enhanced mPFC neuronal excitability would occur in the F344 rats, despite the strain-specific differences in the amount of cocaine self-administered. This hypothesis was tested using Fisher (F344) rats that self-administered cocaine ~1mg/kg/2hr session/day for 14 days (COC-SA), or saline (SAL)-Yoked controls. Fourteen days after the last self-administration session, forebrain slices were prepared and whole-cell patch-clamp recordings of mPFC pyramidal neurons were performed. We determined that pyramidal neurons from cocaine-exposed rats fired more frequently than those from SAL-Yoked rats. This increased firing was associated with a membrane depolarization, and reductions in action potential amplitude and the amplitude of the afterhyperpolarization. These physiological alterations were similar to those we previously observed in Sprague Dawley rats, which self-administer much higher quantities of cocaine. Thus, the positive value of cocaine may differ between the rat strains but the enduring consequence of self-titrated cocaine (wherein the rats can balance positive reinforcing value by the amount self-administered) on the mPFC is similar. Further testing with, e.g., Sprague Dawley rats cocaine-yoked to self-administering F344 rats would help to validate this interpretation.

Disclosures: **W.N. Wayman:** None. **L. Chen:** None. **X. Hu:** None. **T.C. Napier:** None.

Poster

054. Cocaine Reinforcement I

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 54.23/W14

Topic: C.17. Drugs of Abuse and Addiction

Support: NIH R01 DA033436

Title: Using a rodent model of simultaneous cocaine and alcohol use to screen medications to prevent cocaine relapse

Authors: *B. STENNETT, L. KNACKSTEDT;
Univ. of Florida, Gainesville, FL

Abstract: Cocaine addiction is a significant public health problem in the United States today. One of the difficulties in successful treatment of cocaine addiction is reducing the high risk of relapse that exists even after long periods of abstinence. Relapse can be modeled in animals using the extinction-reinstatement paradigm. This paradigm involves training animals to lever-press for cocaine reinforcement in an operant chamber. The operant response is then extinguished and reinstated either with cues previously paired with the response made to attain cocaine delivery, or an IP injection of cocaine. Previous research has established the role of nucleus accumbens glutamate transmission in the reinstatement of cocaine-seeking and has shown that the antibiotic ceftriaxone prevents relapse to cocaine seeking in rats. However, it is estimated that 60% to 90% of cocaine addicts use alcohol with cocaine. The combination of alcohol and cocaine potentially produces unique neuroadaptations that differ from those produced by either drug alone. Therefore, we used a model of poly-drug addiction in which rats self-administered cocaine for two hours in an operant chamber and subsequently drank alcohol (20% v/v) from bottles in the home cage for 6 hours. Following two weeks of drug consumption, animals then underwent extinction training for a minimum of two weeks. Animals were treated with IP ceftriaxone (100 or 200 mg/kg) or vehicle for 6 days prior to being tested for cue- and cocaine-primed reinstatement. Preliminary data indicates that ceftriaxone was not effective in preventing relapse in animals which consumed alcohol in addition to cocaine. These findings indicate that the neurobiological underpinnings of relapse to cocaine are altered when animals consume alcohol with cocaine.

Disclosures: **B. Stennett:** None. **L. Knackstedt:** None.

Poster

054. Cocaine Reinforcement I

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIH R01 DA033436

Title: The role of nucleus accumbens glutamate in the context-primed relapse of cocaine-seeking after abstinence

Authors: *L. A. KNACKSTEDT, M. SCHWENDT;
Psychology, Univ. of Florida, Gainesville, FL

Abstract: Cocaine addiction is a chronic disorder and the risk of relapse remains high even after long periods of abstinence. Animal models of relapse have been developed to screen pharmacological treatments for addiction. The extinction-reinstatement model has been extensively used to identify the neural circuitry involved in relapse, with glutamate release in the ventral striatum being identified as a key mediator of reinstatement. A second animal model is the abstinent-relapse model in which animals do not undergo extinction training following self-administration but instead experience abstinence in the home cage. Animals are then re-exposed to the drug-taking environment (operant chamber) for a context-induced relapse test. MTEP and ceftriaxone modulate glutamatergic transmission and have previously been shown to attenuate reinstatement of cocaine-seeking following extinction training. Here we screened them for their ability to attenuate cocaine relapse after abstinence. Rats were trained to self-administer cocaine for 2 hr/day for 12 days in a standard operant chamber. Following three weeks of abstinence in the home cage, animals were placed back into the operant chamber for a context-induced relapse test. A subset of animals received one of 3 doses of MTEP (0.5, 1, or 5.0 mg/kg) or vehicle immediately prior to the relapse test. A second group of animals received either chronic ceftriaxone (100 or 200 mg/kg) or vehicle once daily for 5 days prior to the relapse test. We found that both MTEP and ceftriaxone attenuated context-primed relapse following abstinence. Ceftriaxone significantly increased GLT-1 expression and reduced GluR1 expression in the nucleus accumbens. Microdialysis conducted during the context-primed relapse test showed a significant increase in glutamate release in the nucleus accumbens. These results implicate glutamatergic transmission in context-primed relapse following abstinence.

Disclosures: **L.A. Knackstedt:** None. **M. Schwendt:** None.

Poster

054. Cocaine Reinforcement I

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Title: Glucagon-like peptide-1 receptor activation in the VTA or accumbens core attenuates cocaine taking and seeking in rats

Authors: *E. G. MIETLICKI-BAASE, K. Y. IGE, D. R. OLIVOS, R. C. PIERCE, M. R. HAYES, H. D. SCHMIDT;
Psychiatry, Univ. of Pennsylvania, Philadelphia, PA

Abstract: Glucagon-like peptide-1 receptor (GLP-1R) signaling in the CNS is pharmacologically and physiologically relevant for energy balance control. The GLP-1R agonist exendin-4 (Ex-4) decreases intake of palatable food when administered into mesolimbic nuclei including the ventral tegmental area (VTA) and the nucleus accumbens (NAc) core. Since the VTA and NAc mediate the reinforcing effects of food and drugs of abuse, we hypothesized that GLP-1R activation in these two brain regions would suppress cocaine taking and seeking. Here, we show that administration of Ex-4 (0, 0.005, and 0.05 μg/100nl) directly into the VTA dose-dependently attenuated cocaine self-administration when rats were maintained on a progressive ratio schedule of reinforcement, as well as attenuated cocaine priming-induced reinstatement of drug seeking. Intra-VTA Ex-4 at these doses had no effect on sucrose reinstatement, suggesting that the cocaine effects are not due to locomotor impairment. To further investigate the role of mesolimbic GLP-1Rs in cocaine seeking, we assessed the ability of Ex-4 microinfusions into the NAc core to suppress the reinstatement of cocaine seeking. Ex-4 (0, 0.005, and 0.05 μg/100nl) microinjected directly into the NAc core attenuated the ability of an acute priming injection of cocaine (10 mg/kg, i.p.) to reinstate cocaine seeking. No effects of intra-NAc core Ex-4 on the reinstatement of sucrose seeking were observed. Previous and current research demonstrates that cocaine self-administration increases plasma levels of corticosterone during drug taking. Furthermore, the expression of endogenous CNS GLP-1 in the nucleus tractus solitarius (NTS) is increased by corticosterone, and these NTS GLP-1-producing neurons project monosynaptically to the VTA and NAc core. Taken together, these findings indicate that, during cocaine taking, increased corticosterone may increase mesolimbic GLP-1 signaling to stop or reduce the ongoing drug-taking behavior. Here, we show that 4th i.c.v. injection of corticosterone (0, 0.05, and 0.5 μg/1μl) decreases cocaine taking on a progressive ratio schedule of reinforcement. This finding

is consistent with our working hypothesis that cocaine-induced increases in plasma corticosterone increase NTS GLP-1 expression and subsequent GLP-1 signaling in the VTA and NAc core. Increased mesolimbic GLP-1R signaling may therefore represent a homeostatic response to cocaine taking that functions as an “emergency brake” mechanism to decrease further cocaine self-administration.

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Poster

054. Cocaine Reinforcement I

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 54.26/W17

Topic: C.17. Drugs of Abuse and Addiction

Support: DA034684

Title: The dorsal agranular insular cortex bidirectionally regulates drug-prime vs. cue-induced reinstatement of cocaine-seeking behavior

Authors: *C. V. COSME, R. T. LALUMIERE;
Univ. of Iowa, Iowa City, IA

Abstract: Previous findings have demonstrated the medial prefrontal cortex plays a critical role in mediating drug seeking. However, fewer studies have examined how regions of the lateral prefrontal cortex (lPFC) influence such behavior, despite evidence based on anatomical connections and human studies suggesting this area plays a potential role in regulating drug-seeking behavior. In particular, limited animal work has suggested that a subregion of the lPFC known as the dorsal agranular insular cortex (AId) is involved in the reinstatement of drug-seeking behavior. However, studies have only investigated the AId in contextual renewal of cocaine seeking and have yet to clarify the mechanisms by which this influence may be occurring. Thus the present study examined how the AId regulates different types of reinstatement of cocaine-seeking behavior. Male Sprague-Dawley rats underwent surgery for implantation of bilateral guide cannulae aimed at the AId or the posterior insular cortex (PIc) and implantation of an intravenous jugular catheter. After undergoing cocaine self-administration for at least 12 days (2 h daily), rats underwent extinction training prior to reinstatement testing. Reinstatement tests consisted of cue-induced, cocaine-prime, and cue-induced + cocaine-prime

(cue +cocaine) reinstatement. AId inactivation, via microinjections of the GABA receptor agonists baclofen and muscimol, attenuated drug seeking during cued reinstatement but, surprisingly, potentiated cocaine-prime reinstatement. AId inactivation had no effect on cue + cocaine reinstatement. PIc inactivation had no effect on any of the reinstatement types that were tested. Due to the relatively dense expression of corticotropin-releasing factor (CRF)-1 receptors in the AId and the potential involvement of CRF in mediating the negative interoceptive cues that are processed in the AId, we investigated the role of these receptors in the AId during reinstatement. CRF1 blockade, via microinjections of the CRF1 antagonist antalarmin into the AId, significantly reduced cue-induced reinstatement and potentiated cocaine primed reinstatement, similar to what was observed during AId inactivation. These findings suggest the AId has opposing influences over cued and cocaine-prime reinstatement, resulting in a bidirectional regulation of cocaine-seeking behavior depending on the type of reinstatement trigger. Moreover, the present study indicates that this influence is specific to the AId, and not the PIc, and that activation of CRF1 receptors within the AId appears to mediate this influence.

Disclosures: **C.V. Cosme:** None. **R.T. LaLumiere:** None.

Poster

054. Cocaine Reinforcement I

Location: Halls A-C

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIDA R01 DA027664

NIDA R01 DA03270

NIDA F31 DA035073

Title: The role of nuclear histone deacetylase 5 in cocaine addiction behavior

Authors: *M. B. CARREIRA[1,2], M. TANIGUCHI[3], D. GUZMAN[2], E. B. LARSON[4], D. W. SELF[2], C. W. COWAN[3];
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Abstract: The transition from cocaine use to abuse is a poorly understood phenomenon. Extensive evidence in the literature suggests that this transition to abuse may be mediated by changes in nervous system structure and function, including chromatin remodeling and epigenetic mechanisms. Recently, we reported that cocaine administration induces transient nuclear accumulation of the class IIa histone deacetylase, HDAC5, which functions to limit the development of cocaine reward behavior. Furthermore, the nuclear accumulation of HDAC5 in a key reward region, the nucleus accumbens (NAc), was sufficient to attenuate cocaine reward. We sought to explore the role of nuclear HDAC5 on the NAc in a model with high face validity for cocaine addiction, the self-administration model. For this purpose, we have developed adeno-associated virus (AAV) that express either the wild type (WT) form of the protein or a nuclear mutant full-length HDAC5. Targeted over-expression into the NAc revealed an attenuating role for nuclear HDAC5 in cocaine prime-dependent reinstatement behavior. This effect appears to be unique to primed-reinstatement as cue-dependent and stress-dependent reinstatement remains unchanged from controls. Furthermore, we observe no differences across groups during extinction of lever pressing in the absence of cocaine. These findings suggest that nuclear HDAC5 acts by reducing the rewarding properties of cocaine, and ongoing studies are testing whether the nuclear, dephosphorylated HDAC5 reduces sensitivity to cocaine. The downstream mechanisms by which HDAC5 suppresses cocaine reward and prime reinstatement is not yet clear, but we observe that nuclear, dephosphorylated HDAC5 dramatically suppresses MEF2-dependent gene expression, and our HDAC5 ChIP-seq findings indicate that HDAC5 associates with genomic DNA predominantly in regions containing MEF2 consensus binding sites. Together, our findings begin to elucidate the key roles and regulation of HDAC5 in cocaine addiction-relevant behaviors.

Disclosures: **M.B. Carreira:** None. **M. Taniguchi:** None. **D. Guzman:** None. **E.B. Larson:** None. **D.W. Self:** None. **C.W. Cowan:** None.

Poster

054. Cocaine Reinforcement I

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIH/NIDA DA14328 (MBG)

NIH/NIDA DA15758 (JRM)

Title: The selective and intake-dependent plasticity in group 1 metabotropic glutamate receptor signaling following cocaine self-administration

Authors: *M. GHASEMZADEH, M. VUONCINO, P. HEASLIP, D. FABRIS, R. DIDOMINICIS, C. SZEWCZYK, C. GWINN, G. RIES, J. R. MANTSCH;
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Abstract: A major obstacle in the treatment of addiction has been the propensity to relapse, often mediated by drug-associated cues, even after prolonged period of abstinence from drug use. Repeated exposure to cocaine leads to enduring alterations in glutamatergic signaling in the brain reward circuitry that play an important role in long-lasting molecular, cellular and behavioral neuroadaptations. Therefore, glutamate signaling has been investigated as a target for the development of treatment for addiction. Recent studies have suggested that the group I metabotropic glutamate receptors (mGluR1/5) play important roles in drug reinforcement and seeking and, therefore, have been pursued as promising targets for drug development. Here, we examined the role of mGluR1/5 receptors in abstinence drug seeking using animal models of cocaine self-administration. Sprague-Dawley rats were trained to self-administer cocaine (FR1; 1.0 mg/kg/200 µl/inf) during either 2-hr (ShA) or 6-hr sessions (LgA) for 14 days. Subsequently, animals were left undisturbed in home cage for 3, 10, or 60 days. Following abstinence period, rats were tested under extinction condition for cocaine seeking after either saline or an mGluR1/5 receptor antagonist administration (MTEP or JNJ16259685). Following a short abstinence period (3 or 10 days), the blockade of mGluR5 receptor reduced drug seeking only in ShA subjects without affecting the LgA animals, while mGluR1 receptor blockade were equally effective in reducing drug seeking in both groups. However, after a long abstinence period (60 days), the blockade of either of receptors significantly reduced drug seeking in ShA and LgA rats. Furthermore, mGluR5 blockade was effective in reducing drug taking (cocaine self-administration) in a dose dependent manner by ShA subjects but not by LgA animals. The results suggest that exposure to cocaine produced a transient intake dependent plasticity in mGluR5 signaling in the brain. The observed plasticity is specific to mGluR5 signaling since blockade of mGluR1 receptors reduced drug seeking similarly in both ShA and LgA animals. In order to identify the anatomical substrates contributing to the selective modulation of mGluR5 signaling, site-specific blockade of mGluR5 receptors in the nuclei of motive circuit will be performed. The selective, intake dependent, and transient plasticity in brain mGluR5 signaling mediated by exposure to cocaine suggest an important role for mGluR5 in cocaine mediated neuroadaptations and addiction behaviors. Understanding the mechanism of cocaine mediated effects may reveal new molecular targets for therapeutic development for the treatment of cocaine addiction.

Disclosures: **M. Ghasemzadeh:** None. **M. Vuoncino:** None. **P. Heaslip:** None. **D. Fabris:** None. **R. DiDominicis:** None. **J.R. Mantsch:** None. **C. Szewczyk:** None. **C. Gwinn:** None. **G. Ries:** None.

Poster

054. Cocaine Reinforcement I

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIH DA016511

CO6 RR015455

Title: Sex differences in attenuation of cocaine conditioned cue reinstatement by the central oxytocin receptor agonist FE-202739

Authors: *L. ZHOU, S. M. GHEE, J. PETERS, R. E. SEE, C. M. REICHEL;
Dept. of Neurosciences, Med. Univ. of South Carolina, Charleston, SC

Abstract: Sex differences clearly exist in psychostimulant addiction patterns. However, potential therapies and the underlying neurobiology have typically only been studied in males. In both males and females, systemic oxytocin treatment attenuated reinstatement of cocaine seeking in a self-administration/reinstatement model of relapse. However, as with all neuroactive peptides, the question remains as to whether enough systemic oxytocin crosses the blood brain barrier to an extent that will exert a central effect. Oxytocin also binds to vasopressin receptors, so the involvement of this system also remains unclear. Here, we compared the impact of systemic and centrally administered oxytocin and a novel oxytocin receptor agonist, FE-202739, on cocaine seeking in male and female rats. FE-202739 specifically binds to oxytocin receptors and does not have a central effect when administered systemically. Rats underwent 10 days of 2 hr cocaine self-administration followed by at least seven days of extinction. In the first experiment, FE-202739 was intraperitoneally injected before reinstatement of cocaine seeking induced by cocaine prime or conditioned cues. Unlike systemic oxytocin, systemic FE-202739 had no effect on reinstatement to cocaine seeking. In the second experiment, oxytocin and FE-202739 were infused intracerebroventricular (ICV). When given centrally, both compounds significantly attenuated cocaine-primed reinstatement in males and females. However, cue-induced reinstatement was only reduced in males, but not females. Based on these findings, a central action on the oxytocin receptor is necessary for the attenuation of cocaine seeking in males and females in response to a cocaine prime. However, in response to conditioned cues, central actions on the oxytocin receptor were only relevant in males because in females, both ICV oxytocin and FE-202739 were without effect. This sex difference for cue-induced cocaine

seeking may relate to the well-known sex differences in the role of oxytocin on peripheral organ sites of actions in females relative to males.

Disclosures: **L. Zhou:** None. **S.M. Ghee:** None. **J. Peters:** None. **R.E. See:** None. **C.M. Reichel:** None.

Poster

054. Cocaine Reinforcement I

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Program#/Poster#: 54.30/W21

Topic: C.17. Drugs of Abuse and Addiction

Title: Withdrawal periods following extended access cocaine self-administration do not alter drug intake during binge or resistance to punishment

Authors: *D. M. DIETZ, D. N. ADANK, A. GANCARZ-KAUSCH;
Dept. of Pharm and Tox; Res. Inst. on Addictions; Program in Neurosci., State Univ. of New York At Buffalo, Buffalo, NY

Abstract: Several of the key characteristics of drug addiction include: (i) a progression from recreational to compulsive drug-taking behavior, (ii) difficulty in limiting intake, and (iii) a high propensity to relapse following drug cessation. Changes in subjective drug craving have been reported to increase with periods of abstinence. Another facet of addiction is the continued use of drugs despite negative consequences (e.g., health complications, risk of incarceration, etc.). While there have been many studies correlating the length of the withdrawal period with drug craving and seeking, this phenomenon does not seem to apply to traditional drug-induced relapse models. Here, we investigated the effects of various withdrawal periods on relapse to cocaine self-administration (SA) “binge” and resistance-to-punishment models. Following 1, 14 or 30 d of withdrawal from extended access to cocaine SA, rats were tested on a cocaine binge (12 hr unlimited access). Surprisingly, there was no effect of withdrawal time on drug-taking performance during the binge. The second experiment evaluated the effect of withdrawal (1 or 14 d) on the persistence to responding for cocaine when the drug delivery was associated with a punishment. During this test, responding resulted in the delivery of both the drug and an iv injection of histamine. Similarly, rats exposed to a 14 d withdrawal self-administered the same amount of drug as rats exposed to a 1 d withdrawal. However, SA during the extended access was positively correlated with the degree of SA during the resistance-to-punishment test. These findings demonstrate the stability of drug-taking behavior despite long periods of drug

withdrawal and suggest that forced abstinence in clinical interventions is insufficient to decrease future drug relapse. Forthcoming studies will examine the transcriptional mechanisms that mediate such behavioral stability in an attempt to identify novel molecular targets that may provide an additional therapeutic intervention

Disclosures: **D.M. Dietz:** None. **D.N. Adank:** None. **A. Gancarz-Kausch:** None.

Poster

055. Methamphetamine and MDMA

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIH R01 DA026867

iM2CS-GEIRE

IUPUI RSFG

Title: In a warm environment hyperthermia induced by 3,4-methylenedioxy-N-methylamphetamine (MDMA) is dependent on locomotion

Authors: *M. V. ZARETSKAIA, D. V. ZARETSKY, P. J. DURANT, D. E. RUSYNIAK; Dept.Emergency Med., IU Sch. of Med., INDIANAPOLIS, IN

Abstract: The central mechanisms through which MDMA causes life-threatening hyperthermia are not well described. Recently, we have demonstrated that inhibiting the dorsomedial hypothalamus (DMH) or the medullary raphe pallidus in rats given MDMA in warm environment, did not prevent non-shivering thermogenesis or cutaneous vasoconstriction. Despite this, inhibiting the DMH, but not the raphe pallidus, decreased hyperthermia and prevented mortality. Unlike inhibition of the raphe, suppression of neuronal activity in the DMH suppresses locomotion produced by MDMA. We hypothesized that hyperthermia after administration of MDMA in warm environment is dependent on locomotion. To separate the role of locomotion from other DMH-mediated effects that might contribute to hyperthermia from MDMA (e.g., blood pressure, metabolic rate) we conducted the following experiment. Male Sprague-Dawley rats were implanted with telemetric transmitters measuring core temperature and microinjection cannulas targeting the DMH bilaterally. After recovering from surgery, rats were familiarized to running on a treadmill for 5 days. On the day of the experiment they were

randomized to microinjections of either muscimol (80 pmol/100nl) or artificial CSF and to i.v. injections of either MDMA (7.5 mg/kg) or equal volume of saline. Rats were adapted to warm environment for at least 60 min and microinjected with muscimol or aCSF. Five min later MDMA or saline were administered i.v., rats were immediately placed on the treadmill at a fixed speed (10 m/min at zero incline) in an environmental chamber at an ambient temperature of 32°C. Under these conditions, the temperature responses in animals given MDMA and microinjected with muscimol were identical to control animals (CSF/MDMA). We conclude that locomotion, mediated through the DMH, is a key contributor to MDMA-evoked hyperthermia in warm environment. These results are important as they represent a significant change in our understanding of how MDMA causes hyperthermia. Based on our results we suggest life-threatening hyperthermia from MDMA is similar to exertional heat stroke.

Disclosures: **M.V. Zaretskaia:** None. **D.V. Zaretsky:** None. **P.J. Durant:** None. **D.E. Rusyniak:** None.

Poster

055. Methamphetamine and MDMA

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIH grant DA026867

iM2CS-GEIRE

IUPUI RSFG

Title: Modeling orexinergic neurotransmission in temperature responses to methamphetamine

Authors: *A. BEHROUZVAZIRI[1], D. FU[3], P. TAN[4], Y. YOO[2], M. ZARETSKAIA[5], D. RUSYNIAK[5], D. V.ZARETSKY[5], Y. MOLKOV[2];
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Abstract: Amphetamines are widely abused. Deaths from hyperthermia have been reported after intentional overdose but there have also been deaths with low doses. Unfortunately, complex pharmacology of amphetamines is far from being understood. At room temperature, temperature

responses to methamphetamine are multiphasic, include both hypothermic and hyperthermic phases, and have non-trivial dose-dependence. Recently, we demonstrated the involvement of orexinergic neurotransmission in Meth-induced temperature responses. Low dose of SB-334867 (SB, 10 mg/kg), an antagonist of orexin receptors (ORX1), was injected 30 min prior to various doses of Meth. While this dose of antagonist clearly suppressed the response to low (1 mg/kg) and intermediate (5 mg/kg) doses of Meth, the effect was statistically significant only during the late phase ($t > 60$ min) of the response to intermediate dose. In the early phase ($t < 60$ min) any drug-related changes were marred by stress-induced temperature fluctuations resulting from two intraperitoneal injections. In a separate set of experiments a high dose of the same antagonist (30 mg/kg), suppressed the effect of low doses of Meth even more, but in contrast, it significantly amplified the responses to the higher doses (5 and 10 mg/kg) of Meth. To interpret the data, we advanced a model we recently described. The dose-dependent temperature responses to Meth could be modeled by interaction of two excitatory drives (low dose [Exc] and high dose[HD]) and one inhibitory drive, which are all activated by Meth. The extended model suggested that activation of excitatory (Exc) and inhibitory nodes, by Meth and stress, are mediated by activation of ORX1 receptors. In turn, amplification by SB of immediate responses to high dose of Meth cannot be explained by suppression of inhibitory drive, as we initially hypothesized. Instead, disinhibition of HD, with inhibitory tone coming directly from inhibitory node, perfectly fits. Presence of inhibition can explain why HD node requires high doses of Meth to be activated. However, disinhibition of HD required higher dose of SB. This points to non-specific effects of the ORX1 antagonist. Our modeling studies detail involvement of orexin receptors into temperature responses to Meth. Also, our data caution that administration of orexin antagonists may increase a risk of life-threatening hyperthermia from amphetamine-like stimulants.

Disclosures: **A. Behrouzvaziri:** None. **D. Fu:** None. **P. Tan:** None. **Y. Yoo:** None. **M. Zaretskaia:** None. **D. Rusyniak:** None. **D. V.Zaretsky:** None. **Y. Molkov:** None.

Poster

055. Methamphetamine and MDMA

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIH grant DA026867

iM2CS-GEIRE

IUPUI RSFG

Title: Locomotion-thermoregulatory coupling in responses to methamphetamine (Meth)

Authors: *D. V. ZARETSKY[1], M. V. ZARETSKAIA[1], Y. YOO[2], A. BEHROUZVAZIRI[2], D. E. RUSYNIAK[1], Y. MOLKOV[2];
[1]Dept Emergency Med., Indiana Univ. Sch. of Med., Indianapolis, IN; [2]Dept.Mathematical Sci., IUPUI, Indianapolis, IN

Abstract: Administration of Meth increases temperature and locomotion. Recently, we successfully designed a firing-rate based neural network model that accurately predicts the temperature responses to various doses of Meth. The temporal dynamics of one of the network nodes was surprisingly similar to patterns of locomotor responses observed in the same experiment. When we replaced the predicted time series of this node with the data of locomotor responses, we found that our neural network accurately reproduced experimental data from a low and high dose of Meth with one exception. Within 60 min of injection, the temperature prediction of the modified model with locomotion term significantly overestimated experimental values in responses to high doses. This means that initial locomotor responses to high doses of Meth are not accompanied by corresponding temperature response unlike late responses or responses to low doses. We sought to compare locomotion-thermoregulatory coupling in various paradigms. First, we estimated locomotion-induced changes in body temperature in rats running on a treadmill at various speeds (up to18 m/min at zero incline) and ambient temperatures. To our surprise, rate of heat accumulation at room temperature was not different between rats running and rats sitting on the treadmill. When the ambient temperature was increased to 32°C, rate of heat accumulation correlated with running speed. We conclude that running evokes compensatory thermoregulatory changes to offset heat generated by physical exercise. Furthermore, we conclude that these compensatory changes are no longer effective in a warm environment. Therefore, initial locomotor response to high doses of Meth is similar to running on the treadmill in terms of locomotor-thermoregulatory coupling. In contrast to running, chemical stimulation of the dorsomedial hypothalamus (DMH) causes locomotor responses that are correlated with hyperthermia at room temperatures. Locomotion coupled with hyperthermia is typical for mild stress, such as cage switch, and can be suppressed by inhibition of the neuronal activity in the DMH. Increases of locomotion and body temperature induced by Meth are also mediated by activation of neurons in the region of the DMH. This activation appears to be a common feature in various paradigms characterized by both locomotor activation and hyperthermia. We conclude that Meth evokes two types of locomotor responses in relation to thermoregulation - one which is accompanied by thermoregulatory response (early high dose, similar to running on treadmill) and another one which is not (low dose or late high dose, similar to responses mediated by the DMH).

Disclosures: **D.V. Zaretsky:** None. **M.V. Zaretskaia:** None. **Y. Yoo:** None. **A. Behrouzvaziri:** None. **D.E. Rusyniak:** None. **Y. Molkov:** None.

Poster

055. Methamphetamine and MDMA

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Topic: C.17. Drugs of Abuse and Addiction

Support: DA13367

DA19447

DA11389

DA031883

Title: Prior nicotine self-administration attenuates dopaminergic deficits induced by subsequent high-dose methamphetamine administration

Authors: *M. G. BALADI[1], S. M. NIELSEN[1], G. R. HANSON[1,2], A. E. FLECKENSTEIN[1]; [1]Dept. of Pharmacol. and Toxicology, Univ. of Utah, Salt Lake City, UT; [2]Sch. of Dent., Univ. of Utah, Salt Lake city, UT

Abstract: Methamphetamine (METH) use and abuse is a serious public health problem and can produce long-term changes in dopaminergic (DAergic) neurons. Of relevance are findings that METH abusers have an increased likelihood of developing Parkinson's disease (PD). Furthermore, METH abusers report high rates of cigarette smoking and interestingly, preclinical studies indicate that non-contingent nicotine exposure attenuates METH-induced DAergic deficits. However, it is unclear whether nicotine exposure that closely models tobacco use in humans (i.e. extended access to nicotine self-administration) attenuates these effects of METH. To address this issue, male, Sprague Dawley rats were allowed access to intravenous nicotine (0.032 mg/kg/infusion, fixed ratio 5) or saline for 23 h/day for 14 days and subsequently (i.e. 1 h after the final self-administration session) treated with methamphetamine (4 x 7.5 mg/kg/injection, s.c.) or saline (4 x 1 ml/kg/injection, s.c.). Results indicate that prior nicotine self-administration attenuated METH-induced decreases in striatal dopamine transporter activity as assessed 24 h after treatment. In addition, this ability of nicotine to attenuate the effects of METH was not due to prevention of METH-induced hyperthermia. Elucidating mechanisms underlying the interaction between nicotine and METH may contribute to the development of

selective therapeutic strategies to attenuate DAergic neuronal degeneration as well as understanding the etiology of disorders involving alterations in DAergic systems, such as PD.

Disclosures: **M.G. Baladi:** None. **S.M. Nielsen:** None. **A.E. Fleckenstein:** None. **G.R. Hanson:** None.

Poster

055. Methamphetamine and MDMA

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Topic: C.17. Drugs of Abuse and Addiction

Support: James D. Kennedy III Faculty Fellowship from The University of the South.

Title: Effects of early adolescent methamphetamine and nicotine exposure on behavior and cognition in adolescent mice

Authors: J. M. BUCK, *J. A. SIEGEL;
Psychology, Sewanee: The Univ. of the South, Sewanee, TN

Abstract: The neurotoxic effects of methamphetamine (MA) can lead to deficits in behavior and cognition. The rising rates of adolescent MA use necessitate that we understand effects of MA exposure on the adolescent brain. Adolescents in treatment for MA abuse show higher levels of depression and suicide ideation compared to those being treated for other substances. Adolescents using MA also show high rates of nicotine use. Previous research has shown that nicotine can mediate the effects of MA in the brain. However, the interaction between MA and nicotine in the adolescent brain, and the effects of these two substances, has not been examined. This research assesses the effects of early adolescent MA and nicotine exposure on cognition and behavior in male C57BL/6J mice later in adolescence. Current experiments are ongoing to study the effects of early adolescent MA and nicotine exposure on behavior in the open field test, the novel object recognition test, the Porsolt forced swim test, and Morris water maze to evaluate locomotor activity and anxiety-like behavior, object memory, depression-like behavior, and spatial memory, respectively. Based on previous data in late adolescent mice, we expect early adolescent MA exposure to impair cognition and increase depression-like behavior. We also predict that concurrent MA and nicotine exposure during early adolescence will attenuate the effects of MA exposure on behavior and cognition in late adolescence. These findings will

contribute to a greater understanding of how MA and concurrent nicotine exposure alters behavior and cognition in an age group that has been relatively understudied.

Disclosures: **J.M. Buck:** None. **J.A. Siegel:** None.

Poster

055. Methamphetamine and MDMA

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Support: Scientific Research on Innovative Areas of the Ministry of Education, Culture, Sports, Science and Technology, JAPAN

The Japan China Medical Association

Title: Role of BDNF-TrkB signaling in depression-like behaviors in mice after withdrawal from repeated administration of methamphetamine

Authors: ***Q. REN**, M. MA, C. YANG, W. YAO, J.-C. ZHANG, K. HASHIMOTO;
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Abstract: Depression is highly comorbid with methamphetamine (METH) abuse. METH can cause depression and psychosis during active use and withdrawal, and these symptoms persist in early abstinence. Several lines of evidence suggest the key role of brain-derived neruotrophic factor (BDNF) and its specific receptor, tropomyosin-related kinase (TrkB), signaling in the pathophysiology of depression. In this study, we examined whether BDNF-TrkB signaling plays a role in the depression-like behaviors in mice after withdrawal from repeated administration of METH. In the tail-suspension test, forced swimming test, 1% sucrose preference test, repeated administration of METH (3 mg/kg/day for 5 days) caused depression-like behaviors in mice, and depression-like behavior persisted more than 2-weeks after the final administration of METH. Western blot analysis showed that BDNF levels in the nucleus accumbens (NAc) of METH treated mice were significantly higher than those of control mice although BDNF levels in the other regions, including prefrontal cortex, hippocampus, were no different. Interestingly, METH-induced depression-like behavior could be improved after subsequent repeated administration of TrkB antagonist ANA-12. These findings suggest that BDNF-TrkB signaling plays a role in the depression-like behavior after withdrawal from repeated METH administration, and that TrkB

antagonists would be potential therapeutic drugs for depression associated with METH withdrawal in humans.

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Poster

055. Methamphetamine and MDMA

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Topic: C.17. Drugs of Abuse and Addiction

Support: VIEP-BUAP

Title: Clobenzorex-treatment induces amphetamine-like neurotoxicity

Authors: *G. S. ROJAS, V. PALAFOX-SÁNCHEZ, G. RAMÍREZ-GARCÍA, A. PATRICIO, D. LIMÓN;
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Abstract: Clobenzorex (Clx) is a derivative of amphetamine which is prescribed as anorectic in Mexico and its treatment has been correlated with the induction of abnormal movements. Also, Clx has been illegally used to increase motor activity, attention and arousal by drug abusers. This study was carried out in order to examine the physiological, behavioral and cellular effects of a sub-chronic Clx-treatment in rats. Wistar male rats were used and divided in groups: vehicle (n=4, v.o and s.c), amphetamine (n=4, 3mg/kg s.c) and Clx (n=4: 30, 50, 70 and 110 mg/kg, v.o), all treatments were given once daily during fifteen days. Motor activity was evaluated in the open field after drug administration in the 1st, 5th, 10th and 15th days of the experiment, we evaluated the number of squares crossed; also, rats were monitored every 30min during 3h after drug administration and were recorder in order to detect if there was a change in their behavior. Rectal temperature was measured to evaluate if Clx could raise it as an amphetamine treatment does. Brains were extracted and an immunostaining was performed to tyrosine hydroxylase (TH) in the substantia nigra pars compacta (SNpc). In this work we demonstrate that after a sub-chronic Clx-treatment motor activity decreased over time and stereotypes are induced as an amphetamine treatment. Hyperthermia and the loss of immunoreactivity to TH in the SNpc could be related to stereotypes because dopamine deregulation in the SNpc modifies the basal ganglia

crosstalk. The mechanism of neurotoxicity of Clx is poorly understood and because of that is necessary to study it furthermore.

Disclosures: **G.S. Rojas:** None. **V. Palafox-Sánchez:** None. **G. Ramírez-García:** None. **A. Patricio:** None. **D. Limón:** None.

Poster

055. Methamphetamine and MDMA

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DA019447

DA13367

Title: Age-dependent effects of nicotine pretreatment on methamphetamine-induced dopaminergic deficits: role of alpha4* and alpha6* nicotinic receptor subtypes

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Abstract: Epidemiological studies have demonstrated an inverse correlation between cigarette smoking and risk of Parkinson's disease (PD). Clinical and preclinical studies have indicated that nicotine (NIC) might be neuroprotective against toxicities to dopamine systems. The neuroprotective mechanisms involving NIC are not fully understood, although a role for alpha4* and alpha6* subtypes of nicotinic acetylcholine receptors (nAChRs) has been suggested. Of relevance to this study, high-dose methamphetamine (METH) administration causes persistent dopaminergic (DAergic) deficits resembling some aspects of PD. Accordingly, the current study assessed the impact of chronic NIC pre-administration on the expression of alpha4* and alpha6* nAChRs and on DAergic neuronal function in adolescent and adult METH-treated rats. Results revealed that METH alone caused persistent deficits in both striatal dopamine transporter (DAT) function and expression and alpha6* nAChR expression. In addition, METH alone did not decrease alpha4* nAChRs expression, indicating that alpha6* nAChRs are differentially sensitive to METH. In contrast, in NIC-pre-treated adolescent or adult rats, the ability of METH

to disrupt DAT function and expression was reduced. However, despite protecting against persistent METH-induced DAT function and expression deficits, NIC pre-treatment did not attenuate METH-induced alpha6* nAChRs expression deficits. In summary, these data indicate that NIC is neuroprotective against DAergic deficits caused by METH and NIC neuroprotection might occur via mechanisms that are not limited to the involvement of alpha4* or alpha6* nAChRs. (Acknowledgement: DA 031883, DA019447, DA13367)

Disclosures: **P.L. Vieira-Brock:** None. **L.M. McFadden:** None. **S.M. Nielsen:** None. **G.R. Hanson:** None. **A.E. Fleckenstein:** None.

Poster

055. Methamphetamine and MDMA

Location: Halls A-C

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Program#/Poster#: 55.09/W30

Topic: F.03. Motivation and Emotion

Support: NIDA R01 DA020036 (Minority Supplement)

Title: Multidimensional assessment of responses to a drug-associated cue in healthy humans

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Abstract: Drug use is strongly influenced by associations made between psychoactive drug effects and environmental stimuli (cues) present during the drug experience. Conditioning, the process by which a cue becomes associated with drug through repeated pairings, is the focal point of many theories of addiction, and is believed to contribute to the acquisition, maintenance, and relapse to problematic drug use. Drug-related cues can promote drug craving, seeking, and consumption, even after long periods of drug abstinence. Although cues are known to play a crucial role in the cycle of addiction, few studies have examined the acquisition process and the behavioral features of conditioned responses in humans. Therefore, we have developed a novel human drug conditioning paradigm to determine whether a cue paired with a typical drug of abuse (i.e. methamphetamine, MA) will acquire conditioned properties in humans. We have employed a multidimensional approach to determine the various ways in which the responses manifest and examined individual differences in conditioning. Healthy, non-dependent humans came in for 6 sessions, including a pre-test, 4 conditioning sessions, and a post-test. At the pre-test, we assessed how participants responded to two visual cues using the following measures:

behavioral preference, subjective ratings of "liking", emotional reactivity (as indicated by facial electromyography of zygomatic and corrugator facial muscle activity), and attention. Subjects then came in for the 4 conditioning sessions; 2 with drug (20mg MA) and 2 with placebo, administered under double-blind conditions in alternating order. For each subject, one study cue was always presented during drug sessions, while the other was presented during placebo sessions. Participants then came in for the post-test sessions where we again measured responses to study cues, with an interest in how the responses have changed as a result of experiencing one cue in the presence of drug, and the other in the absence. We found that participants demonstrated conditioned responses towards the drug-paired cue, including enhanced behavioral preference, enhanced positive emotional reactivity, and a bias in attention. Interesting, changes in emotional reactivity and attention were negatively correlated. However, subjective drug response (from conditioning sessions) predicted the increase in attentional bias. This research is critical to address the current knowledge gaps in drug conditioning research, identify individuals especially at risk for cue-elicited drug seeking and consumption, and inform future efforts to prevent or attenuate cue-facilitated drug seeking and relapse.

Disclosures: **L.M. Mayo:** None. **H. de Wit:** None.

Poster

055. Methamphetamine and MDMA

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Support: A grant (No. 110113-3) form the Korea Institute of Planning and Evaluation for Technology in Food, Agriculture, Forestry, and Fisheries (IPET), Korea

TTL Nguyen, DK Dang, HQ Tran, and Y Nam are involved in BK21 PLUS program, National Research Foundation of Korea

Title: Ginsenoside Re attenuates methamphetamine-induced neurotoxicity by microglial inactivation via genetic inhibition of PKCδ

Authors: ***M.-B. WIE**[1], T.-T. L. NGUYEN[2], D.-K. DANG[2], H.-Q. TRAN[2], Y. NAM[2], E.-J. SHIN[2], S. K. KO[3], H.-C. KIM[2];
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Abstract: Ginsenoside Re (Re), a protopanaxatriol-type saponin, is one of the main constituent of Panax ginseng and known to have antioxidant and anti-inflammatory properties. Recently, we reported that Re significantly attenuates methamphetamine (MA)-induced neurotoxicity by inhibiting impaired enzymatic antioxidant systems, mitochondrial oxidative stress, and mitochondrial translocation of protein kinase C (PKC) δ. To extend our understanding, we examined the effect of Re on the pro-inflammatory changes induced by MA. First, we evaluated the spatial pattern of microglial and astroglial activation in the dopaminergic system after MA treatment. Significant and robust microglial activation was observed in the striatum, but not in the substantia nigra, at 1 day and 2 days after the final MA injection. However, significant astroglial activation was shown in both striatum and substantia nigra, indicating that MA-induced neuroinflammatory changes seem to be specific in the striatum. To clarify the phenotype of activated microglia, we examine the mRNA expression of M1 (e.g. CD16, CD32, and CD86) and M2 (e.g. arginase 1 and CD206) phenotype markers. MA treatment significantly increased the mRNA expression of M1 microglia phenotype markers, but did not affect that of M2 phenotype markers. These pro-inflammatory changes were significantly attenuated by Re. Interestingly, PKCδ gene depletion also significantly attenuated MA-induced microglial activation and increases in the mRNA expression of M1 phenotype markers, and Re did not provide any further attenuation on the pro-inflammatory changes induced by MA in PKCδ gene knockout mice. Consistently, MA-induced dopaminergic toxicity and behavioral impairment were significantly attenuated by Re. Our results suggest that Re provides neuroprotection in response to MA-induced dopaminergic degeneration via inhibition of pro-inflammatory changes [supported by a grant (No. 110113-3) form the Korea Institute of Planning and Evaluation for Technology in Food, Agriculture, Forestry, and Fisheries (IPET), Korea. TTL Nguyen, DK Dang, HQ Tran, and Y Nam are involved in BK21 PLUS program, National Research Foundation of Korea].

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Poster

055. Methamphetamine and MDMA

Location: Halls A-C

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Topic: C.17. Drugs of Abuse and Addiction

Support: FDA protocol E7519.01

Title: The relationship between amphetamine-induced cerebrovascular and neurotoxicity to changes in the expression of genes related to the immune system present in circulating blood

Authors: *J. F. BOWYER[1], N. M. CRABTREE[1], K. M. TRANTER[2], N. I. GEORGE[3], J. P. HANIG[4], R. P. SCHLEIMER[5];
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Abstract: The magnitude of cerebrovascular damage and neurotoxicity correlates with the degree of hyperthermia produced during exposure to either amphetamine (AMPH) or methamphetamine (METH) and may be exacerbated by damage to muscle and organ systems outside the CNS. AMPH- and METH-induced damage to peripheral tissues may trigger immune signals in the circulating blood which could influence the CNS immune response and neurotoxicity. The present studies in Sprague-Dawley rats examined the gene expression (mRNA) and cytokine changes in whole blood under conditions where AMPH produced hyperthermia, cerebrovascular damage and striatal neurotoxicity (75% dopamine depletions). Four injections of D-AMPH (5, 7.5, 10 and 10 mg/kg) were given with 2 hr between each injection. Our objectives were to identify biomarkers of AMPH neurotoxicity and determine the immune-related responses in circulating blood. The AMPH hyperthermic group was compared to: a saline control group; a group given the same AMPH exposure but remained normothermic due to a cool environment (16°C); and environmentally-induced hyperthermia (EIH, AMPH-free in an ambient environment of 39°C) group, which is similar to heat stroke. EIH has adverse effects on peripheral organs and brain vasculature but does not produce damage to dopamine terminals in the striatum. The AMPH normothermic group remained normothermic and did not exhibit striatal neurotoxicity; however, their cerebrovasculature damage has not yet been determined. Relative to controls, all three treatments showed significant increases in mRNA expression in blood (*Cd14, Il1b, IL17ra and Ccr2*) as well as increases in the cytokines IL-6 and IL-10, indicating they elicited an innate immune response to varying degrees. Hundreds of genes (≈500) were differentially expressed between the EIH and AMPH hyperthermic groups but most were not directly related to the immune system. The mRNA expression of *Cd52, Cd82* and *Cd97*, which code for immune-related cell surface proteins, were significantly different between these two groups, and the interleukin-related genes *Il1rap*, *Il22ra2* and *Il6st* had higher expressions in the AMPH hyperthermic group. Unexpectedly, the AMPH normothermic group had almost 300 genes with significant expression differences from all other groups. Using bioinformatics approaches, we are now identifying the most prominent or characteristic genes, as well as the fewest necessary, needed to classify samples in the different treatment groups.

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Poster

055. Methamphetamine and MDMA

Location: Halls A-C

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Program#/Poster#: 55.12/W33

Topic: C.17. Drugs of Abuse and Addiction

Support: NIH DA023085

Title: Neurotoxic administration of methamphetamine alters microtubules within rat striatal dopaminergic axons

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Abstract: Methamphetamine (METH) is a commonly abused psychostimulant, which can induce neurotoxicity to dopaminergic (DAergic) terminals in the striatum without affecting DA cell bodies in the substantia nigra pars compacta (SNc). METH neurotoxicity is primarily characterized by reductions in striatal DAergic markers such as dopamine transporter (DAT) and tyrosine hydroxylase (TH) that indicate loss of DAergic axons. Multiple studies have demonstrated that striatal DAergic markers partially recover in experimental animals and humans, given a sufficient period of abstinence from METH. Most striatal DAergic markers require axonal transport to be replenished; therefore,we have hypothesized that the deficits in striatal DAergic markers are, in part, due to dysfunction of axonal transport in surviving DAergic axons. Currently, it is unknown whether METH alters axonal transport in striatal DAergic neurons. To test our hypothesis, we assessed post translational modifications (PTMs) of α-tubulin and neuron specific tubulin isoform ßIII tubulin in lysates from the whole striatum and within rat striatal DAergic axons at 3 days following neurotoxic METH administration (4 x 10mg/kg, every 2h, i.p.). In lysates, there was a statistically significant loss of detyrosinated α-tubulin (-14%, $p<0.05$) whereas the levels of acetylated α-tubulin, tyrosylated α-tubulin and ßIII tubulin remained unchanged. The decrease in detyrosinated α-tubulin was concurrent with significant reductions in striatal tissue levels of both TH and DAT (-43% and -68%, $p<0.05$).

None of the assessed indices were affected in the SNc. DAergic axons constitute less than 1% of striatal components. To determine whether alterations in tubulin PTMs occur in DAergic axons, we employed immunofluorescence confocal microscopy. Double labeling of striatal slices for TH and PTMs revealed a loss of acetylated α-tubulin (R = 0.24, Saline vs. R = 0.075, METH, $p<0.05$, Pearson's correlation) and overall α-tubulin (R = 0.28, Saline vs. R = 0.19, METH, $p<0.05$) in METH-treated rats as compared to saline controls. Furthermore, we observed a selective increase in cololocalization of ßIII tubulin with TH (R = 0.14, Saline vs. R = 0.07, METH, $p<0.05$) in the striatum following METH. No such differences were found in the SNc. Our results suggest that neurotoxic METH causes persistent changes to structure and stability of microtubules within surviving DAergic axons. This data supports the hypothesis of axonal transport impairment in striatal DAergic axons following neurotoxic METH.

Disclosures: B.A. Killinger: None. **A. Moszczynska:** None.

Poster

055. Methamphetamine and MDMA

Location: Halls A-C

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Topic: C.17. Drugs of Abuse and Addiction

Support: NS34696

NS084735

JPB Foundation

Title: Chronic methamphetamine accelerates pacemaking activity in the substantia nigra pars compacta

Authors: *S. M. GRAVES[1], J. N. GUZMAN[1], E. ZAMPESE[1], E. ILIJIC[1], J. H. KORDOWER[2], B. K. YAMAMOTO[3], D. J. SURMEIER[1];
[1]Physiol., Northwestern Univ., Chicago, IL; [2]Neurolog. Sciences, Neurosurg., Rush Univ., Chicago, IL; [3]Neurosciences, Univ. of Toledo, Toledo, OH

Abstract: Methamphetamine (METH) is a potent psychostimulant used to treat ADHD and exogenous obesity but is also abused by 13 million people in the US (NSDUH). Recent evidence indicates that METH use increases the risk of developing Parkinson's disease (PD) by nearly two-fold (Callaghan RC et al., Drug Alcohol Depend 120:35 2012). PD is a common

neurodegenerative movement disorder that results from the progressive degeneration of dopamine neurons in the substantia nigra pars compacta (SNc). Recent evidence indicates that Cav1.3 L-type Ca2+ channels that participate in pacemaking of SNc dopamine neurons are involved, as Ca2+ entry through Cav1.3 channels leads to mitochondrial oxidant stress (Sulzer D & Surmeier DJ, Mov Disord 28:715 2013). One-way in which METH might increase PD risk would be to increase the pacemaking of SNc dopamine neurons, which might increase Ca2+ entry and mitochondrial oxidant stress. To test this hypothesis, cell attached recordings of SNc dopamine neurons were performed. Acute METH (10μM bath application) in *ex vivo* brain slices from naïve mice significantly reduced SNc pacemaking (control median: 1.4hz; 10μM METH median: 0.4). In contrast, chronic METH (2.5mg/kg/day for 14d) led to increased pacemaking (control median: 1.9hz; METH median: 2.2hz) after withdrawal. These results suggest that chronic METH-induced inhibition of SNc pacemaking led to rebound acceleration of pacemaking. Experiments are underway to determine whether the METH-induced increase in pacemaking frequency elevates Ca2+ entry and mitochondrial oxidant stress.

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Poster

055. Methamphetamine and MDMA

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIH NS083410

Title: Methamphetamine induces direct effects on microglia

Authors: ***J. AREDO**[1], K. CONANT[2], A. KASSARDJIAN[2], K. MAGUIRE-ZEISS[2];
[2]Neurosci., [1]Georgetown Univ. Med. Ctr., Washington, DC

Abstract: The use of methamphetamine (MA) has become an increasing societal problem as it is the second most widely abused illegal substance worldwide. MA targets dopamine neurons via transporter-specific entry, and results in extensive vesicular dopamine release, oxidative stress, and eventual neuronal death. Interestingly, studies in animal models demonstrate that microglial activation in the brain of MA-treated animals precedes neuronal death. The mechanism of this glial activation and if microglia contribute to MA-induced pathology remain unknown. In this

study, we sought to determine whether MA directly alters microglial function. We employed two models: primary microglia (PMG) exposed to MA and mice injected with MA. Here we show that MA-treated PMG exhibit a change in morphology that is reminiscent of classically activated microglia. MA also induces a rapid increase in the nuclear translocation of p65 NF-κB. This transcription factor is known to regulate the expression of proinflammatory cytokines, reactive oxygen species, and anti-oxidant response proteins. PMG do not produce significant levels of prototypical proinflammatory molecules (i.e., TNF-α, nitric oxide) following MA treatment. However, PMG do exhibit robust expression of the anti-oxidant enzyme, heme oxygenase-1. Finally, striatal tissue harvested from mice following a single intraperitoneal dose of MA (40 mg/kg) show an increase in the microglial marker, Iba1. We have previously demonstrated that this dose of MA results in an increase in striatal MMP-9; also known to be regulated by NF-κB. Overall, we demonstrate that MA directly incites a morphofunctional change in PMG and increases markers of glial activation *in vivo*.

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Poster

055. Methamphetamine and MDMA

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Topic: C.17. Drugs of Abuse and Addiction

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Title: Super-resolution assessment of methamphetamine altered VMAT2 presynaptic terminal distribution

Authors: *C. L. GERMAN[1], M. V. GUDHETI[4,2], G. R. HANSON[1,3], E. M. JORGENSEN[2,5], A. E. FLECKENSTEIN[1];
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Abstract: The vesicular monoamine transporter-2 (VMAT2) is responsible for packaging cytoplasmic monoamines into synaptic vesicles throughout the central nervous system - a function that is altered by numerous psychostimulants, including methamphetamine (METH). METH reduces the dopamine (DA) uptake capacity of VMAT2 within presynaptic dopaminergic terminals of the striatum, which has been tied to a shift in presynaptic VMAT2 localization. Analysis of presynaptic vesicle fractions isolated from striatal synaptosomes by differential centrifugation indicates METH reduces VMAT2 content within a non-plasma membrane-associated fraction. Importantly, these acute METH-induced changes in VMAT2 localization and function are linked to persistent striatal dopaminergic deficits. How altered VMAT2 localization within *ex vivo* subcellular fractions translates into changes in physical distribution within striatal DA neurons is unknown, but likely a key component in understanding the process by which METH disrupts presynaptic DA regulation. Resolving the size, shape and orientation of VMAT2 synaptic vesicle distribution within presynaptic terminals using light microscopy is difficult given that the limits of confocal microscopy resolution (~200 nm) exceed the predicted size of synaptic vesicles (~50 - 100 nm). To overcome this barrier, biplane single molecule localization-based super-resolution microscopy was employed and METH-induced alterations in VMAT2 synaptic vesicles were assessed. Using this method, the size, shape and orientation of VMAT2 vesicle distribution was evaluated in three dimensions (3D), with resolutions reaching 20 nm in the XY plane and 50 nm in the Z plane, and given cellular context by co-staining presynaptic active zone and axonal proteins. Findings indicate that METH exposure causes widespread loss of organization in striatal presynaptic VMAT2 distribution that may be tied to a disruption of the presynaptic active zone.

Disclosures: **C.L. German:** None. **M.V. Gudheti:** A. Employment/Salary (full or part-time):; Vutara. **G.R. Hanson:** None. **E.M. Jorgensen:** None. **A.E. Fleckenstein:** None.

Poster

055. Methamphetamine and MDMA

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Program#/Poster#: 55.16/X1

Topic: C.17. Drugs of Abuse and Addiction

Support: NIDA-IRP, NIH

Title: Pathological brain hyperthermia induced by MDMA (ecstasy) in rats under conditions associated with human ‘rave parties’: critical role of peripheral vasoconstriction

Authors: *E. A. KIYATKIN, A. H. KIM, K. T. WAKABAYASHI, M. H. BAUMANN, Y. SHAHAM;
Behavioral Neurosci Br., NIDA-IRP, NIH, DHHS, Baltimore, MD

Abstract: MDMA (Ecstasy) is an illicit drug used by young adults at hot, crowed “rave” parties, yet the data on potential health hazards of its abuse remain controversial. Here, we examined the effect of MDMA on temperature homeostasis in male rats under standard laboratory conditions and under conditions that simulate human drug use. We chronically implanted thermocouple microsensors in the nucleus accumbens (a brain reward area), temporal muscle, and skin to measure temperature continuously from freely moving rats. While focusing on brain hyperthermia, temperature monitoring from the two peripheral locations allowed us to evaluate the physiological mechanisms (i.e., intra-cerebral heat production and heat loss via cutaneous vasoconstriction) that underlie MDMA-induced brain temperature responses. Our data confirm previous reports on high individual variability and relatively weak brain hyperthermic effects of MDMA under standard control conditions (quiet rest, 22-23°C), but demonstrate dramatic enhancements of drug-induced brain hyperthermia during social interaction (exposure to male conspecific) and in warm environments (29°C). Importantly, we identified peripheral vasoconstriction as a critical mechanism underlying the activity- and state-dependent potentiation of MDMA-induced brain hyperthermia. Through this mechanism, which prevents proper heat dissipation to the external environment, MDMA at a moderate non-toxic dose (9 mg/kg or ~1/5 of LD50 in rats) can cause fatal hyperthermia under environmental conditions commonly encountered by humans. Our results demonstrate that doses of MDMA that are non-toxic under cool, quiet conditions can become highly dangerous under conditions that mimic recreational use of MDMA at rave parties or other hot, crowded venues.

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Poster

055. Methamphetamine and MDMA

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Topic: C.17. Drugs of Abuse and Addiction

Support: DA023085

Title: The role of CDCrel-1 in methamphetamine-induced impairment of VMAT2 vesicle trafficking

Authors: *H. D. CHAUHAN, A. MOSZCZYNSKA;
Pharmaceut. Sci., Eugene Applebaum Col. of Pharm. and Hlth. Sci., Detroit, MI

Abstract: Methamphetamine (METH) is a widely abused psychostimulant. In experimental animals and humans, METH is toxic to dopaminergic (DAergic) nerve terminals in the striatum when administered at high doses. An early event in METH neurotoxicity is a release of dopamine (DA) from VMAT2 storage vesicles and its subsequent autoxidation, followed by an oxidative stress within the terminals. The vesicular monoamine transporter 2 (VMAT2) plays a neurprotective role by transporting cytoplasmic DA back into vesicles for storage and protection from oxidation. It has previously been shown that METH neurotoxicity is associated with impaired VMAT2 trafficking and oxidative damage to the ubiquitin proteasome system (UPS). The UPS regulates the levels of CDCrel-1, a protein involved in inhibition of exocytosis. The objective of our study was to determine whether CDCrel-1 and VMAT2 interact with each other and how these interactions affect trafficking of VMAT2 vesicles after METH administration. We have hypothesized that METH-induced inhibition of the UPS increases the levels of CDCrel-1, which leads to entrapment of VMAT2 vesicles at the plasma membrane, preventing their recycling and sequestration of cytosolic DA. To test this hypothesis, we examined all synaptosomal populations and DAergic synaptosomes in rat striatum for the levels of and interactions between VMAT2 and CDCrel-1. Adult male Sprague Dawley rats were treated with binge METH (4 x 10 mg/kg, every 2 h, i.p) or saline and sacrificed 1 h or 24 h after the last injection. Striatal synaptosomal fractions were subjected to SDS-PAGE and western blotting with CDCrel-1 and VMAT2 antibodies. As compared to saline controls, CDCrel-1 levels increased in DAergic as well as overall population of striatal synaptosomes in METH-treated rats at 1 hour after the last METH dose (+6.3%; +20%, $p<0.05$). VMAT2 immunoreactivity didn't travel from the plasma membrane to the cytosol in METH-exposed striatum, suggesting that the vesicles might be trapped at the plasma membrane by CDCrel-1. In support of this notion, co-immunoprecipitation detected an increased protein-protein interaction between CDCrel-1 and VMAT2 in rat striatal synaptosomes. Furthermore, plasma membrane-associated striatal vesicles from METH-treated rats had lower DA content than those from saline controls. In summary, our findings suggest that METH-induced accumulation of CDCrel-1 inhibits VMAT2 vesicles trafficking, thus impairing proper sequestration of DA to storage vesicles. Keywords: Methamphetamine, VMAT2, CDCrel-1

Disclosures: **H.D. Chauhan:** None. **A. Moszczynska:** None.

Poster

055. Methamphetamine and MDMA

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIDA/NIH R01 DA020142

Title: Neurotensin modulates the METH-induced striatal apoptosis through darpp32 phosphorylation, nitric oxide accumulation and glial cell activation

Authors: *Q. LIU[1,2], A. HAZAN[1], E. GRINMAN[1], J. A. ANGULO[1];
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Abstract: Methamphetamine (METH) is a widely abused psychostimulant second only to cannabis in popularity. METH abuse can impair cognitive functions and induces neurodegeneration in the striatum. Dysfunction of dopamine neurotransmission is found to play a critical role in METH-induced neurotoxicity. Acute METH (30mg/kg, i.p.) can induce striatal neurotoxicity characterized by striatal neuron apoptosis and dopamine terminal degeneration. We used this animal model of METH-induced toxicity to investigate the role of neurotensin. We observed that the neurotensin receptor 1 (NTR1) agonist PD149163, injected intraperitoneally, attenuated the METH-induced striatal apoptosis in a dosage-dependent manner. Also, infusion of 20 uM of the NTR1 antagonist, SR48692, into the striatum, augmented the METN-induced apoptosis. These data demonstrate that neurotensin modulates METH-induced striatal apoptosis through NTR1 in the striatum. To further investigate the corresponding mechanism, we assessed its effect on glial cell activation, nitric oxide accumulation and DARPP32 phosphorylation in the striatum, which are all believed to aggravate such neurodegeneration. We found that the NTR1 agonist attenuated the effects of METH on all three of these markers. The results also show that NTR1 agonist alone has no effect on glial cell activation and nitric oxide accumulation, but leads to a decrease in phosphorylation of DARPP32 at Thr34 by 54%. Since the DARPP32 phosphorylation pathway participates in all neurotransmission into the striatal projection neurons, neurotensin possibly modulates its phosphorylation through regulation of dopamine and glutamate neurotransmission. We conclude that neurotenisn modulates METH-induced striatal apoptosis through diverse mechanisms involving glial cell activation, nitric oxide accumulation and DARPP32 phosphorylation in the striatum. Additionally, the agonist of neurotensin, PD149163, may be considered a potential drug for METH toxicity treatment. (Supported by R01 DA020142 from NIDA/NIH)

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Poster

055. Methamphetamine and MDMA

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Title: Cannabinoid CB1 receptor mediates dopaminergic neurotoxicity induced by methamphetamine

Authors: *H.-C. KIM, D.-K. DANG, T.-T. NGUYEN, H.-Q. TRAN, Y. NAM, E.-J. SHIN; Col. of Pharm., Kangwon Natl. Univ., Chunchon, Korea, Republic of

Abstract: Accumulating evidences indicated that Cannabinoid CB1 receptor (CB1R) plays a modulatory role in dopaminergic system. However, the pharmacological mechanism mediated by CB1R remains to be elucidated. In the present study, we examined the role of CB1R in the MA-induced dopaminergic toxicity. We observed that treatment with MA (7 mg/kg, i.p., 4 times with a 2 hours' interval) resulted in a significant increase in the striatal CB1R mRNA expression of CB1R wild-type (WT) mice. MA-induced hyperthermia, behavioral impairment and dopaminergic toxicity (i.e., decrease in dopamine level and tyrosine hydroxylase expression) were significantly abolished in CB1R (-/-) mice. In addition, MA-induced pro-apoptotic factors expression (i.e., Bax and caspase 3), oxidative stress (i.e., ROS, lipid peroxidation and protein oxidation), and cleaved-PKCδ expression were significantly attenuated in CB1R (-/-) mice. These attenuations were also observed in WT mice treated with AM251 or Rimonabant, CB1R antagonists. Consistently, we observed that multiple high doses of CB1R agonist (WIN 55,212-2 or ACEA) produce dopaminergic toxicity (as shown by decreases in dopamine level and tyrosine hydroxylase expression), behavioral impairments (as shown by hypolocomotor activity and impairment in rota-rod performance), oxidative stress and pro-apoptotic changes in the striatum of mice. In addition, WIN (36 mg/kg) or ACEA (16 mg/kg) significantly increased cleaved-PKCδ expression in the striatum after the final injection of WIN or ACEA. Our results suggest that CB1R mediates MA-induced dopaminergic toxicity, and that CB1R activation requires proteolysis of PKCδ gene for induction of pro-apoptosis. Thus, inhibition of CB1R may be a useful target for protection against MA-induced dopaminergic toxicity [DK Dang, TTL Nguyen,

HQ Tran, and Y Nam are involved in BK21 PLUS program, National Research Foundation of Korea. This research was supported by a grant (14182MFDS979) from Ministry of Food and Drug Safety in 2014, Republic of Korea].

Disclosures: **H. Kim:** None. **D. Dang:** None. **T. Nguyen:** None. **H. Tran:** None. **Y. Nam:** None. **E. Shin:** None.

Poster

056. Addiction Treatment: Translational and Clinical Studies

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 56.01/X5

Topic: C.17. Drugs of Abuse and Addiction

Title: Intravenous administration of nicotinamide adenine dinucleotide significantly reduces self report craving ratings associated with opiate and alcohol withdrawal

Authors: ***S. L. BROOM**[1], R. F. MESTAYER[2], E. STULLER[3], D. W. COOKE[4], J. M. CARSON[2], K. R. SIMONE[2], P. NORRIS[2], P. HOTARD[2];
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Abstract: Introduction: Treatment of substance abuse disorders continues to challenge clinicians and "cravings" for the abused substance are often impediments to sobriety. Nicotinamide Adenine Dinucleotide (NAD) has been used in the past with claims of having anti-craving properties. Previous data from this clinic using a similar formulation of NAD support the use of NAD as a valid treatment for drug cravings. Methods: *This pilot study retrospectively examined the anti-craving properties of NAD in a group of 60 patients*. The patients were adult males and females with addictions to primarily opiates and alcohol. The treatment comprised IV infusions of NAD as well as vitamins, oral amino acids and variable prescription medications for an average of 10 consecutive days ranging from 5 to 10 hours daily. Self-reported craving ratings (1-10 Scale) were collected on Day 1 (before starting treatment), Day 5, and on Day 10 (after completion of treatment). Findings: 1) All patients were able to achieve an abrupt cessation of their use of abused drug by the end of Day 1 of the 10-day treatment. 2) Self-reported ratings showed that patients experienced a continually increasing alleviation of the adverse consequences associated with drug withdrawal throughout the treatment. *Specifically, patients reported significantly reduced craving ratings at both Day 5 and Day 10.* 3) Respondent interviews at 3 and 6 months post treatment suggest that NAD was effective in reducing the

number and magnitude of relapse episodes, as well as severity of drug cravings. Discussion: These data suggest that NAD is an effective detox treatment for alcohol and opiate addicts. Furthermore, NAD shows potential as a long-term therapy in maintaining sobriety through minimizing drug cravings and preventing relapse.

Disclosures: **S.L. Broom:** F. Consulting Fees (e.g., advisory boards); Springfield Wellness Center. **R.F. Mestayer:** None. **E. Stuller:** None. **D.W. Cooke:** None. **J.M. Carson:** A. Employment/Salary (full or part-time):; Springfield Wellness Center. **K.R. Simone:** A. Employment/Salary (full or part-time):; Springfield Wellness Center. **P. Norris:** A. Employment/Salary (full or part-time):; Springfield Wellness Center. **P. Hotard:** A. Employment/Salary (full or part-time):; Springfield Wellness Center.

Poster

056. Addiction Treatment: Translational and Clinical Studies

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 56.02/X6

Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grant H26088

Title: The neural correlates of reorganizing attachment in mothers with unresolved trauma

Authors: *U. IYENGAR[1,2], S. KIM[1,3], L. STRATHEARN[1,2,3];
[1]Pediatrics, Baylor Col. of Med., Houston, TX; [2]Clinical, Educational, and Hlth. Psychology, Univ. Col. London, London, United Kingdom; [3]Psychiatry and Behavioral Sci., Menninger, Houston, TX

Abstract: Attachment theory emphasizes the quality of early relationships and its impact on adult functioning, especially with regard to the development of interpersonal relationships. Mothers classified as having unresolved trauma (Utr) based on the Adult Attachment Interview (AAI) tend to be compromised in their ability to respond sensitively to emotional cues of their own infant, reflected in their dampened amygdala responses when viewing their own infant's sad face (Kim et al, 2014). One newly defined aspect of attachment is "reorganization", which refers to the process by which individuals are changing their understanding of past and present experiences, in the direction of secure attachment. While still not "secure" in attachment, the process of reorganization indicates positive adaptation to new circumstances and life events. No study to date has explored the neural correlates of reorganizing attachment. Our fMRI study

examines patterns of brain response in mothers with and without reorganizing attachment in a clinical sample of substance-abusing mothers (with a high prevalence of Utr). Twenty-three substance-abusing mothers completed AAIs, and their 3- to 12-month old infants were videotaped to obtain images for use in a subsequent scanning session. All mothers who had Utr were dichotomized into those reorganizing towards secure attachment (N=6) and those who were not (N=17). Mothers returned for a scanning session, in which they viewed unique stimuli of own and unknown infant faces randomly presented for 2 seconds as part of an event-related design. The mothers' brain response to happy images of their own infants was contrasted with that of unknown infants. The between-group effects for the own vs. unknown happy contrast were evaluated in a 2 (infant identity) x 2 (reorganizing status) random effects ANOVA. Preliminary analysis revealed that reorganizing mothers demonstrated bilateral activation of the striatum (putamen; q(FDR) <.05, p<.008) when viewing images of their own versus unknown infant, while striatal activation was absent in non-reorganizing mothers. This differential brain response between reorganizing and non-reorganizing mothers in the striatum, a key reward-processing region of the brain, suggests that the process of reorganization toward secure attachment may involve increased reward processing of salient infant cues. This may translate into improved infant-directed behavior in these mothers, and improved developmental and attachment-related outcomes in the child. Reference: Kim, S., Fonagy, P., Allen, J., and Strathearn, L. (2014). Mothers' Unresolved Trauma Blunts Amygdala Response to Infant Distress. *Social Neuroscience*.

Disclosures: **U. Iyengar:** None. **S. Kim:** None. **L. Strathearn:** None.

Poster

056. Addiction Treatment: Translational and Clinical Studies

Location: Halls A-C

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Program#/Poster#: 56.03/X7

Topic: C.17. Drugs of Abuse and Addiction

Support: NCCR SYNAPSY operating grant

Title: Optogenetically inspired deep brain stimulation reverses drug-evoked plasticity

Authors: ***M. CREED**[1], V. PASCOLI[1], C. LÜSCHER[1,2];
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Abstract: Exposure to drugs of abuse, such as cocaine, induces characteristic forms of synaptic plasticity, for example a potentiation of glutamatergic inputs onto medium spiny neurons of the nucleus accumbens (NAc). One behavioral correlate of this plasticity is locomotor sensitization, a phenomenon in which repeated exposure to equivalent doses of cocaine induces progressive increases in locomotor activity. Deep brain stimulation (DBS) is a surgical therapy used primarily for movement disorders, in which electric current is passed through electrodes implanted into specific brain nuclei. We sought to reverse cocaine-evoked plasticity and behaviour using DBS applied to the fiber bundle of excitatory projections originating in the mPFC and targeting the NAc shell. We found that classical DBS protocols (high frequency: 130Hz, 100μA) effectively decreased the locomotor response to cocaine. However, this effect was transient and lasted less than four hours after the cessation of DBS. In line with these behavioral observations, the cocaine-induced enhancement of excitatory inputs onto MSNs was unchanged, as inferred by measuring the AMPA:NMDA ratio *ex vivo* 24 hours following the cessation of DBS. We then demonstrated that depotentiating the projection from the medial prefrontal cortex (mPFC) to the NAc using optogenetic stimulation delivered at 12 Hz *in vivo* restores normal synaptic transmission and abolished locomotor sensitization to cocaine, which we then attempted to mimic with DBS. However, given that the 12Hz protocol is dependent on mGluR activation, and that signaling through dopamine D1-receptors in MSNs opposes mGluR signaling, we hypothesized that D1 antagonism may be necessary to unmask a DBS-induced depotentiation onto MSNs. Indeed, when we administered the D1 antagonist SCH23390 (0.3mg/kg, i.p.) in conjunction with DBS, cocaine sensitization and cocaine-evoked plasticity were abolished. We have further confirmed that this combination of DBS and D1-antagonism reverses plasticity via an mGluR-dependent mechanism. Taken together, our results provide a proof of principle that combined with pharmacology, DBS may be used to reverse to cocaine-induced synaptic plasticity and drug-adaptive behavior, which may have important implications for the clinical application of DBS for the treatment of addictive disorders.

Disclosures: **M. Creed:** None. **V. Pascoli:** None. **C. Lüscher:** None.

Poster

056. Addiction Treatment: Translational and Clinical Studies

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Topic: C.17. Drugs of Abuse and Addiction

Support: P30 DA028821

K05 DA020087

R21 MH093844

Welch Foundation

T32 GM089657-03

John Sealy Memorial Endowment Fund

Title: Design, synthesis, and pharmacological characterization of selective serotonin (5-HT) 5-HT2C receptor positive allosteric modulators as potential small molecule therapeutics for psychostimulant use disorders

Authors: *C. WILD, C. DING, G. ZHANG, N. ANASTASIO, R. FOX, S. STUTZ, R. HARTLEY, K. CUNNINGHAM, J. ZHOU;
Pharmacol. and Toxicology, Univ. of Texas Med. Br., Galveston, TX

Abstract: Preclinical evidence suggests that dampened serotonin (5-HT) 5-HT2C receptor (5-HT2CR) signaling during withdrawal from psychostimulant use promotes relapse, a major hurdle of overcoming addiction. Therefore, restoration of 5-HT2CR signaling capacity via positive modulation of the receptor during withdrawal provides the conceptual framework for the development of pharmacotherapies that promote abstinence maintenance. While developing selective ligands for the 5-HT2CR remains a challenge, targeting the topologically distinct allosteric site - as opposed to the conserved orthosteric site - represents a promising drug design strategy. A series of new small molecules have been rationally designed, chemically synthesized, and pharmacologically characterized by using privileged fragments as templates, homology modeling and molecular docking techniques, as well as a battery of in-house *in vitro* (functional and radioligand binding studies) and *in vivo* (behavioral studies) assays to assess allosteric modulation of the 5-HT2CR. To date, several compounds have been identified to enhance 5-HT2CR-mediated Cai++ release and ERK1/2 activation induced by the endogenous ligand 5-HT or the selective 5-HT2CR agonist WAY 163909. Our drug development efforts open new avenues in probing 5-HT2CR function and the discovery of novel pharmacotherapeutics for cocaine addiction and other central nervous system disorders.

Disclosures: **C. Wild:** None. **C. Ding:** None. **G. Zhang:** None. **N. Anastasio:** None. **R. Fox:** None. **S. Stutz:** None. **R. Hartley:** None. **K. Cunningham:** None. **J. Zhou:** None.

Poster

056. Addiction Treatment: Translational and Clinical Studies

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Program#/Poster#: 56.05/X9

Topic: C.17. Drugs of Abuse and Addiction

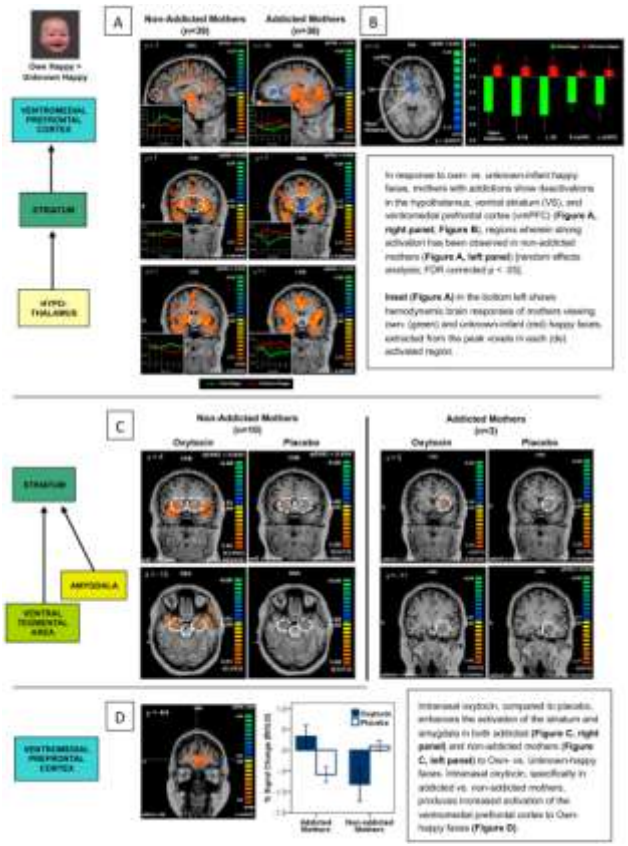
Support: NIH Grant R01 DA026437

NIH Grant R01 HD065819

Title: Mothers with addictions show reduced reward response to infant cues: Can oxytocin reverse this pattern?

Authors: *S. KIM[1,2], U. IYENGAR[1,3], L. C. MAYES[4], M. N. POTENZA[4], H. J. V. RUTHERFORD[4], L. STRATHEARN[1,2];
[1]Dept. of Pediatrics, [2]Menninger Dept. of Psychiatry and Behavioral Sci., Baylor Col. of Med., Houston, TX; [3]Res. Dept. of Clinical, Educational, and Hlth. Psychology, Univ. Col. London, London, United Kingdom; [4]Child Study Ctr., Yale Univ. Sch. of Med., New Haven, CT

Abstract: Maternal drug addiction constitutes a major public health problem affecting children, with high rates of abuse, neglect, and foster care placement. However, little is known about the ways in which drug addiction alters brain function underlying maternal behavior. Prior studies have shown that infant cues and drugs of abuse similarly activate dopaminergically innervated brain reward circuits. Here, we report on an fMRI study documenting that mothers with addictions demonstrate reduced activation of reward regions when shown cues of their own infants. We further report on preliminary data suggesting that intranasal oxytocin may be effective in reversing the disrupted maternal brain responses in these mothers. Thirty-six mothers seeking treatment at an inpatient drug abuse facility underwent fMRI scanning at 6 months postpartum, while viewing happy and sad face images of their own infant, along with those of a matched unknown infant. Effects of intranasal oxytocin were examined as part of a randomized double-blinded crossover study, involving a subsample of 3 addicted mothers and an independent sample of 10 non-addicted mothers, who were given a nasal spray of oxytocin or placebo prior to one of two fMRI scanning sessions. When viewing happy face images of their own infant compared to those of an unknown infant, mothers with addictions showed a striking pattern of decreased activation in dopamine- and oxytocin-innervated brain reward regions, including the hypothalamus, ventral striatum, and ventromedial prefrontal cortex--regions in which increased activation has been observed in non-addicted mothers. Intranasal oxytocin, compared to placebo, was found to enhance activation in these brain reward regions, especially in mothers with addictions. Our results are the first to demonstrate that mothers with addictions show reduced activation in key reward regions of the brain in response to their own infant's face cues. Intranasal oxytocin may have the potential to improve altered brain reward responses in these mothers, and may prove useful as a novel treatment in this population.



Disclosures: **S. Kim:** None. **U. Iyengar:** None. **L.C. Mayes:** None. **M.N. Potenza:** None. **H.J.V. Rutherford:** None. **L. Strathearn:** None.

Poster

056. Addiction Treatment: Translational and Clinical Studies

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 56.06/X10

Topic: C.17. Drugs of Abuse and Addiction

Title: Transcranial direct current stimulation for the reduction of alcohol cravings

Authors: *D. RUDDER, C. TESCHE, P. COULOMBE;
Dept. of Psychology, Univ. of New Mexico, Albuquerque, NM

Abstract: Background: Craving is implicated the maintenance of alcohol abuse and dependence as well as relapse during attempts at recovery. A 2008 investigation by Boggio et al. (Drug &

Alcohol Dependence, 92, 55-60) demonstrated that transcranial direct current stimulation (tDCS) applied over dorsolateral prefrontal cortex (DLPFC) was effective in reducing craving among individuals with alcohol dependence. The present study is the first to continue to explore the potential of tDCS to manipulate craving in the context of alcohol use and abuse. Methods: The study design was a randomized, controlled crossover experiment utilizing repeated measures. Eleven adult participants completed assessments of alcohol abuse severity [Alcohol Use Disorders Identification Test (AUDIT)] as well as pre- and post-stimulation assessments of alcohol craving [Alcohol Urge Questionnaire (AUQ)] and mood [Quick Mood Scale (QMS)]. Subjects received active and sham stimulation delivered in two separate experimental sessions spaced 3 to 7 days apart. During active stimulation, 2mA was applied for 20 minutes through an anodal electrode placed over the left DLPFC and a cathodal electrode placed over the right DLPFC. Sham stimulation entailed applying 2mA of stimulation for only the first 15 seconds of the session. Results: Active stimulation produced a significant reduction in craving as measured by change in AUQ scores ($p = .03$), whereas sham did not ($p = .42$). A multilevel model for repeated measures demonstrated that, controlling for alcohol abuse severity (as measured by AUDIT scores), active stimulation produced a greater reduction in craving than did sham stimulation ($p = .03$). In addition, higher AUDIT scores were associated with a greater reduction in craving ($p < .001$), and this effect did not differ between the active and sham conditions. Overall, regardless of whether participants scored high or low on alcohol abuse severity, providing them with active stimulation led to greater reductions in craving relative to sham stimulation. There were no significant changes in mood as measured by the QMS for either condition. Conclusions: Our results lend further support for the potential of tDCS to modulate craving in individuals with alcohol use disorders who experience frequent or intense cravings. These results motivate additional studies on the utility of tDCS for alcohol abuse in clinical treatment settings. Future research utilizing larger sample sizes and targeting participants who meet the diagnostic criteria for alcohol abuse or dependence is recommended.

Disclosures: **D. Rudder:** None. **C. Tesche:** None. **P. Coulombe:** None.

Poster

056. Addiction Treatment: Translational and Clinical Studies

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Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 56.07/X11

Topic: C.17. Drugs of Abuse and Addiction

Support: Conselho Nacional de Desenvolvimento Cientifico e Tecnologico (CNPQ)

Fundacao de Amparo a Pesquisa do Estado de Sao Paulo (FAPESP – Auxilio Pesquisa Regular (#2012/06731-4)

Associação Fundo de Incentivo a Pesquisa (AFIP)

Coordenação de Aperfeiçoamento Pessoal de Nível Superior (CAPES)

Title: "Analyzing the concentration and the effects of omega 3 on nicotine dependence"

Authors: *J. ZAPAROLI, E. K. SUGAWARA, A. A. L. SOUZA, S. TUFIK, J. F. GALDURÓZ;
UNIFESP - Univ. Federal De São Paulo, São Paulo, Brazil

Abstract: Abstract: Background: Free radicals present in cigarette play a role in several diseases associated with smoking. The omega 3 series present changes in concentration *in situ*ations of high oxidative stress, like contact with cigarette smoke. Since omega-3 play a role on dopaminergic neurotransmission and this is related to development and perpetuation of dependence. Therefore it is important to understand the levels and the effects of omega 3 on nicotine dependence. **Methods:** The first study consisted of a cross-sectional to compare levels of omega 3 between smokers and non-smokers in a sample of 171 individuals, matched by age, gender, height, weight, and BMI. The second was a clinical-trial, double-blind, randomized, placebo controlled, in which 60 smokers received daily treatment with capsules of fish (source of omega 3) or mineral oil (placebo), taken three times a day for 90 days. The outcome was evaluated by means of psychometric (Beck Anxiety and Depression Inventory; Fagerström Test for Nicotine Dependence; Questionnaire of Smoking Urges; and Richmond Test) and biological measures (percentage of carbon monoxide in the exhale breath and cotinine concentrations) as well as self-reports of tobacco use (smoking dairy). **Results:** The evaluation of omega-3 lipid profile showed that smokers present lower concentrations of docosahexaenoic acid (DHA). At the end of the clinical intervention we observed that the group treated with omega 3 showed a significant reduction in the levels of dependence, but no difference was observed on the other outcomes. **Interpretation:** Smokers showed lower peripheral levels of omega 3, which might interfere in the normal function of different systems, including the dopaminergic system. On the other hand, the treatment with omega-3/day brought about reduction in nicotine dependence without, however, resulting in quit cigarette consumption. Although this study presents limitations it is possible that higher dosage administered and prolonging the duration of the treatment may be helpful achieving better rates of success on the treatment of nicotine dependence.

Support: Conselho Nacional de Desenvolvimento Cientifico e Tecnologico (CNPQ), Fundacao de Amparo a Pesquisa do Estado de Sao Paulo (FAPESP – Auxilio Pesquisa Regular (#2012/06731-4), Associação Fundo de Incentivo a Pesquisa (AFIP), Coordenação de Aperfeiçoamento Pessoal de Nível Superior (CAPES). **Ethical approval:** The study was approved by the Ethics Committee in Research of the Universidade Federal de São Paulo

(CAAE#03850412.2.0000.5505) and registered in clinical trial (NCT01735279).
Conflicts of interest: The authors declare no conflicts of interest.

Disclosures: **J. Zaparoli:** None. **E.K. Sugawara:** None. **A.A.L. Souza:** None. **S. Tufik:** None. **J.F. Galduróz:** None.

Poster

056. Addiction Treatment: Translational and Clinical Studies

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Program#/Poster#: 56.08/X12

Topic: C.17. Drugs of Abuse and Addiction

Support: Sponsored by Demerx Inc

Title: Results of a double-blind, placebo-controlled study of the safety and pharmacokinetics of noribogaine administered to healthy volunteers

Authors: L. FRIEDHOFF[1], M. LOCKHART[2], F. LAM[3], N. HUNG[3], C. T. HUNG[3], *P. GLUE[4];
[1]Demerx Inc, Fort Lauderdale, FL; [2]Univ. of Auckland, Auckland, New Zealand; [3]Zenith Technol. Ltd, Dunedin, New Zealand; [4]Dunedin Sch. of Med., Dunedin, New Zealand

Abstract: Background: The iboga alkaloikds have been reported to have striking efficacy for treatment of various addictions in case reports and case series reports. In spite of this long history of uncontrolled results, no blinded, placebo-controlled clinical trials of an iboga alkaloid have ever been conducted. Here we report the first placebo-controlled clinical trial of an iboga alkaloid, noribogaine hydrochloride. Aims: The objectives of this Phase I study were to assess the safety, tolerability, pharmacokinetic and pharmacodynamic profile of noribogaine. Methods: This was an ascending single-dose, placebo-controlled, randomised, double-blind, parallel-group study in 36 healthy drug-free male volunteers. Four cohorts (n=9) received oral doses of 3, 10, 30 or 60mg or matching placebo, with intensive safety and pharmacokinetic assessments out to 216h following dosing, along with pharmacodynamic assessments sensitive to the effects of mu-opioid agonists. Safety monitoring included: physical examinations, vital signs, serum chemistry, hematology, urinalysis, ECGs, oximetry and capnography. Results: Noribogaine was rapidly absorbed, with peak concentrations occurring 2-3 hours after oral dosing, and showed dose-linear increases of AUC and Cmax between 3-60mg. The drug was slowly eliminated, with mean half-life estimates of 27-40 hours across dose groups. The apparent volume of distribution was high.

No safety or tolerability issues were identified in any cohort. No mu-opioid agonist effects were noted in pupillometry or cold-pressor testing. Conclusions: Single oral doses of noribogaine 3-60mg were safe and well tolerated in healthy volunteers. There was no evidence of mu-agonist activity on pharmacodynamic testing up to 60mg.

Disclosures: **L. Friedhoff:** 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.; Employee of Demerx Inc. **M. Lockhart:** 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.; Sponsored by Demerx Inc. **F. Lam:** 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.; Employee of Zenith Technology Ltd. **N. Hung:** 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.; Employee of Zenith Technology Ltd. **C.T. Hung:** 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.; Employee of Zenith Technology Ltd. **P. Glue:** 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.; Sponsored by Demerx Inc.

Poster

056. Addiction Treatment: Translational and Clinical Studies

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Program#/Poster#: 56.09/X13

Topic: C.17. Drugs of Abuse and Addiction

Support: Korea Institute of Science and Technology Intramural Funding (No. 2E24480)

Korea government (MEST) (No. 2N37373)

Title: Serum exosomal microRNA-137 as biomarker for repeated cocaine exposure in mice

Authors: E. NAM, *H.-I. IM;
Ctr. for Neurosci., Korea Inst. of Sci. & Technol., Seoul, Korea, Republic of

Abstract: Diagnosis, prognosis and therapy in neuropsychiatric disorders including drug addiction have been very difficult as of now. The measurement of circulating microRNAs (miRNAs) is most recent effort to identify novel biomarkers in preclinical safety. An important fraction of miRNAs are circulated in plasma or serum either in free forms or within exosomes. Exosomes are small (40~100 nm) vesicles originating from within multi-vesicular bodies, which are secreted into the extracellular space and reflects the physiological and pathological status of the source cells. In this study, we examined whether exosomal miRNAs in the serum might reflect the changes of the brain in repeated cocaine exposure in mice. Firstly, we observed the expression of various miRNAs (miRNA-137, -9, and -132) in the whole serum and serum exosomes. The levels of miRNA-137 are robustly increased in serum and exosomes at 2hrs and 24hrs after the last exposure. Secondly, we examined whether exosomal miRNAs in serum are originated from brain tissue so that the changes of exosomal miRNAs are relevant to those of brain regions. To end this, we used the lenti-WPRE (Woodchuck Hepatitis Virus Posttranscriptional Regulatory Element) fragments injection, which are not existed in physiological conditions of mammalian, into striatum. We confirmed that not only the expressions of WPRE could be detected in serum exosomes but also those are getting decreased in the striatum over time (6hrs vs 6wks). Interestingly, the levels of miRNA-137 are significantly decreased in ventral striatum and lateral habenula in repeated cocaine exposure in mice, which is along with 'WPRE' experiments. Finally, we confirmed that the exosomal miRNAs including miRNA-137 would be a quite stable in various conditions such as extreme temperatures and chemical treatment compared to the free forms of miRNAs in serum. Taken together, the serum exosomal miRNA-137 may serve as diagnostically sensitive biomarker for drug addiction.

Disclosures: **E. Nam:** None. **H. Im:** None.

Poster

056. Addiction Treatment: Translational and Clinical Studies

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Program#/Poster#: 56.10/X14

Topic: C.17. Drugs of Abuse and Addiction

Support: Arkansas Biosciences Institute

College of Education and Behavioral Science

Title: Chronic oral nicotine consumption does not alter circulating estradiol levels but reduces cotinine in sera and urine of female rats

Authors: S. HALDER[1], J. M. LYNCH[2], *A. R. PEARCE[3];
[1]Mol. Biosci., Arkansas State Univ., State University, AR; [2]Psychology, [3]Arkansas State Univ., STATE UNIV, AR

Abstract: With the increasing awareness of complications in pregnancies or reduced fertility, many women who smoke turn to nicotine replacement products. Nicotine delivered orally is a route for nicotine replacement therapy, however, the risk and safety of oral nicotine have not been adequately assessed. Nicotine inhibits aromatase, an enzyme responsible for conversion of testosterone to estradiol, one of the principal female reproductive hormones; hence, it is important to investigate changes in estradiol levels linked to chronic oral nicotine consumption. Using an established multiple bottle approach and adult female Sprague-Dawley rats, animals were provided with 4 bottles of nicotine solution (30 µg/ml) and water, or water only. Nicotine dosages were determined across 14 days and sera and urine were collected following the first 24 hours, 7, or 14 days of nicotine intake. Estrous cycle stages were evaluated and sera from animals in proestrus were analyzed for estradiol by ELISA. Sera and urine were also analyzed by ELISA for cotinine, a major nicotine metabolite. Despite notable individual differences, overall daily nicotine intake patterns were comparable across exposure days. Circulating estradiol levels did not differ significantly between nicotine and control groups (6.30±3.96 pg/ml vs. 5.81±2.88 pg/ml, $p>0.05$). Consistent with previous pilot studies, there was intra-individual variation, yet trends showed a substantial amount of cotinine in serum following the first 24 hours of nicotine intake; however, cotinine concentrations decreased to negligible levels after 7 and 14 days. Cotinine in urine followed a similar pattern: concentrations diminished from approximately 93.55±33.85 ng/ml after 24 hours of nicotine intake, to 11.13±9.49 ng/ml on day 7, and 16.23±19.76 ng/ml on day 14. Findings suggest decreases in serum cotinine at 7 and 14 days were not due to a reduction in nicotine intake across exposure days, nor to increased cotinine clearance through urine. We contend that oral nicotine intake for the given concentration or duration may not alter circulating estradiol and the initial elevated yet later reduced cotinine levels in serum and urine may indicate an inhibition in the nicotine metabolic pathway. Considering cytochrome P4502B1 is the main nicotine metabolizing enzyme in rats, assessing its gene and protein expression as well as enzyme activity will likely elucidate cotinine's systemic reduction, and also provide information about the risk and safety of oral nicotine use in females.

Disclosures: **S. Halder:** None. **J.M. Lynch:** None. **A.R. Pearce:** None.

Poster

056. Addiction Treatment: Translational and Clinical Studies

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 56.11/X15

Topic: C.17. Drugs of Abuse and Addiction

Title: A preclinical electrophysiological biomarker of D3-receptor antagonism using the selective D3-receptor antagonist PF-04363467

Authors: *C. B. PURYEAR[1], D. P. NGUYEN[2], T. KISS[1], D. L. BUHL[1], A. MEAD[3]; [1]Neurosci. Res. Unit, [2]Dept. of Pharmacokinetics, Pharmacodynamics and Metabolism, Pfizer, Inc, Cambridge, MA; [3]Global Safety Pharmacol. Drug Safety R&D Statistics, Drug Safety Res. and Develop., Pfizer, Inc, Groton, CT

Abstract: Drug dependence is associated with marked changes in mesocorticolimbic dopaminergic circuitry of the brain. In addition to the extensive characterization of dopamine D1 and D2 receptors in the neurocircuitry of addiction, recent work suggests an important role of dopamine D3 receptors (D3Rs) in mediating drug-dependent states, especially given their unique spatial distribution in pathways involved in reward processing. In particular, upregulation of D3 receptors has been shown in key mesolimbic areas in rodent models of drug seeking. Furthermore, increased D3R binding potential has been demonstrated in a variety of human populations with experience of drug use, suggesting the role of D3Rs may overlap across different types of addiction. This body of evidence has led to a number of studies showing that selective D3R antagonists reduce drug seeking, intake, and relapse in preclinical rodent models. Given the efficacy of D3R antagonists in drug addiction models, we sought to develop a robust biomarker of D3 antagonism by utilizing electroencephalography (EEG) in freely behaving rats. Although effects of D1 and D2 antagonists on EEG activity have been described, the effects of D3 antagonists on neuronal oscillations remain unknown. We therefore investigated the effects of the selective D3 antagonist, PF-04363467, on EEG patterns in freely behaving rodents in order to determine whether a D3-mediated biomarker could be established. Male Sprague-Dawley rats (N=9) implanted with 3-channel telemeters (DSI, Int.) were used to enable continuous home-cage wireless recording of EEG and EMG for 24-48 hours. Rats received 3 doses of PF-04363467 (3.2, 10.0, 32.0 mg/kg) or vehicle using a crossover Latin square design, with 3 days between each dose. To detect any potential pharmacodynamic responses, we calculated broadband changes in EEG power for 4 hours post-dose relative to a 1-hour pre-dose baseline period. A dose-dependent decrease in theta (7-12 Hz) and alpha (12-14 Hz) power was observed concomitant with an increase in gamma (30-80 Hz) power that persisted for ~1 hour post-dose. In addition to quantitative EEG, we present a pharmacokinetic/pharmacodynamic model that describes the relationship between spectral power changes and unbound brain concentration of PF-04363467. The combination of the selective action at the D3 receptor and a dose-dependent pharmacodynamic response suggests that the spectral changes we observe may

serve as a translatable, non-invasive biomarker of D3R antagonism in human studies of addiction or other psychiatric disorders.

Disclosures: **C.B. Puryear:** None. **D.P. Nguyen:** None. **T. Kiss:** None. **D.L. Buhl:** None. **A. Mead:** None.

Poster

057. Learning, Memory, Dependence, and Addiction

Location: Halls A-C

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Program#/Poster#: 57.01/X16

Topic: C.17. Drugs of Abuse and Addiction

Support: Academy of Finland

Sigrid Juselius foundation

Finnish Foundation for Alcohol Studies

Foundations professor pool

Jane and Aatos Erkko foundation

Title: Expression of c-Fos after GABA-A receptor agonist gaboxadol in mouse brain regions: link to aversion?

Authors: *E. R. KORPI[1,2], O. VEKOVISCHEVA[1], E. KANKURI[1], E. VASHCHINKINA[1]; [1]Univ. of Helsinki, Helsinki, Finland; [2]Dept. of Pharmacol., National University of Singapore, Singapore

Abstract: Previous studies have demonstrated that THIP (gaboxadol), a selective agonist of extrasynaptic GABA-A receptors, induces aversion in place conditioning in mice (Vashchinkina et al., J Neurosci 32: 5310-20, 2012), although it as a single dose induces neuroplasticity in glutamate synapses of VTA dopamine neurons similar to rewarding drugs of abuse. Here, we used c-Fos immunohistochemistry to measure neuronal activation in C57BL/6J, GABA-A receptor δ subunit-knockout (δ-KO) mice and wild-type controls 2 h after THIP (6 mg/kg, IP). Possible anxiety behavior as well as plasma corticosterone levels were also assessed. In wild-type mice, THIP treatment triggered an increase in the number of c-Fos-positive neurons in the oval part of the Bed Nuclei of Stria Terminalis (BNST). This effect was abolished in δ-KO mice.

Behavioral changes suggested negative withdrawal-type, anxiety-mimicking effects, such as increased latency in entering the center of an open field, in contacting with a new object, and in entering the lit compartment in light-dark box at 2 h after the THIP treatment. At 45 min after THIP treatment, a transient increase in plasma corticosterone level was observed in wild-type mice, but not in δ-KO mice. In summary, these data suggest that THIP treatment shifts the pattern of response of the oval BNST to an anxiogenic/aversive mode.

Disclosures: **E.R. Korpi:** None. **O. Vekovischeva:** None. **E. Kankuri:** None. **E. Vashchinkina:** None.

Poster

057. Learning, Memory, Dependence, and Addiction

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIDA Grant DA029127

Title: Cocaine-related deficits in cognitive flexibility and inhibitory response control are attenuated by the alpha-2A adrenergic agonist guanfacine in a novel nose-poke based set-shifting task in rats

Authors: *P. M. CALLAHAN[1,2], L. VANDENHUERK[2], A. V. TERRY, Jr.[1,2];
[1]Dept. of Pharmacol. and Toxicology, [2]Small Animal Behavioral Core, Georgia Regents Univ., Augusta, GA

Abstract: Cognitive flexibility (task or rule switching) and the ability to inhibit inappropriate responses in a changing environment are key components of executive function that are often impaired in substance abuse disorders. Accordingly, it has been argued that targeting these behavioral alterations in drug addicted patients is a rational therapeutic strategy. There were 3 objectives of the experiments described here 1) to introduce a new, computer-automated set shifting task for rats that utilizes nose-poke based spatial location and visual discrimination strategies and allows for the assessment of cognitive flexibility as well as inhibitory response control, 2) to evaluate the effects of cocaine on cognitive flexibility and inhibitory response control in this new task, and 3) to evaluate the ability of the alpha-2A selective adrenergic agonist, guanfacine to attenuate cocaine-related alterations in task performance. Male Wistar rats were trained on a spatial response location (nose-poke center aperture) to visual-cue (nose-poke

steady vs. blinking light any aperture) discrimination; switching from spatial response to visual-cue required the subject to complete 10 consecutive correct responses; session terminated after subjects completed 10 consecutive correct responses on the visual-cue strategy or 90 min lapsed. Cognitive flexibility was assessed by evaluating the number trials to criterion associated with the spatial location and visual discrimination strategies and inhibitory response control was assessed by measuring the number of premature, perseverative, and timeout responses. Under vehicle conditions, subjects required fewer trials to reach criterion for the spatial vs. the visual-cue discrimination. Cocaine (2.5-15 mg/kg, ip) dose-dependently increased the number of trials to criterion as well as the number of premature and timeout responses during both discriminations. Co-administration of guanfacine (0.1-0.6 mg/kg, ip) with cocaine (15 mg/kg, ip) attenuated the cocaine-related deficits in cognitive flexibility and inhibitory response control. These animal studies support the argument that our new computer-automated set shifting task is sensitive to alterations of executive function that are induced by acute cocaine exposure. The studies also suggest that the alpha-2A selective adrenergic agonist guanfacine may have therapeutic potential for treating impairments of executive function that are associated with the abuse of cocaine.

Disclosures: **P.M. Callahan:** None. **L. Vandenhuerk:** None. **A.V. Terry:** 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.; NIDA grant.

Poster

057. Learning, Memory, Dependence, and Addiction

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 57.03/X18

Topic: C.17. Drugs of Abuse and Addiction

Support: R01DA033049

Title: Methamphetamine-induced inhibition of perirhinal long-term depression underlies meth-induced deficits in novel object recognition memory

Authors: *M. D. SCOFIELD[1], H. L. TRANTHAM-DAVIDSON[2], M. SCHWENDT[3], J. PETERS[2], R. E. SEE[2], C. M. REICHEL[2];
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Abstract: Methamphetamine (meth) addiction is often characterized by cognitive impairment including episodic, verbal, and working memory deficits. In human meth addicts, the extent of memory dysfunction has been positively correlated with relapse probability. Consequently, the molecular mechanisms underlying meth-induced memory impairment are an important consideration for the development of pharmaceutical therapies designed to aid in the cessation of drug abuse. Recently, we demonstrated that 6-hour/day chronic meth self-administration (SA) results in novel object recognition (NOR) memory deficits in rats. Recognition of novelty is dependent upon intact perirhinal (pRh) cortex function, which is compromised by chronic meth SA. We have shown that pRh expression of GluN2B-containing NMDA receptors is downregulated following chronic meth SA. GluN2B-containing NMDA receptors mediate the induction of pRh long-term depression (LTD), which is one of the principle physiological processes underlying NOR memory. We hypothesized that the meth induced down regulation of pRh GluN2B receptors would contribute to a loss of pRh LTD. To test this hypothesis, male Sprague-Dawley rats self-administered meth (0.02 mg/infusion, i.v.) along an FR1 schedule of reinforcement or received yoked saline infusions. After 7 daily 1-hour sessions, rats were switched to 6-hour sessions for 14 days, followed by a period of abstinence. On day 7 of abstinence, rats were tested for NOR memory using a two-item object recognition task. Immediately after the test, rats were decapitated and tissue was used to measure LTD or surface expression of GluN2B in the pRh. Chronic meth SA decreased surface expression of the GluN2B NMDA receptor subunit and blocked the induction of pRh LTD. Further, pharmacological activation of pRh NMDA receptors with D-cycloserine (DCS) both reversed meth-induced NOR deficits and restored induction of LTD. Importantly, Ro 25-6981, a GluN2B-contiaing NMDA receptor antagonist, blocked restoration of pRh LTD by DCS. Taken together, our data indicate that GluN2B-containing NMDA receptors play a central role in the physiological processes that underlie novel object recognition memory, and may serve as a potential target for pharmacotherapies designed to treat meth-induced cognitive deficits.

Disclosures: **M.D. Scofield:** None. **H.L. Trantham-Davidson:** None. **M. Schwendt:** None. **J. Peters:** None. **R.E. See:** None. **C.M. Reichel:** None.

Poster

057. Learning, Memory, Dependence, and Addiction

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Program#/Poster#: 57.04/X19

Topic: C.17. Drugs of Abuse and Addiction

Support: R01DA033049

Title: Activation of perirhinal mglur5 receptors reverses recognition memory deficits resulting from long-access methamphetamine self-administration in rats

Authors: J. PETERS, M. D. SCOFIELD, H. TRANTHAM-DAVIDSON, S. M. GHEE, *R. E. SEE, C. M. REICHEL;
Dept. of Neurosciences, Med. Univ. of South Carolina, Charleston, SC

Abstract: Previous work from our laboratory has indicated that long-access (LA), but not short-access (SA), methamphetamine self-administration leads to cognitive deficits in rats. In particular, performance in a novel object recognition task, which relies upon the perirhinal cortex, is impaired in LA rats. This seems to result, at least in part, from a downregulation in perirhinal mGluR5 protein expression and impaired long-term depression (LTD). We have previously shown that systemic administration of the positive allosteric modulator (PAM) 3-Cyano-N-(1,3-diphenyl-1H-pyrazol-5-yl)benzamide (CDPPB) reverses this memory deficit in LA rats. However, intracranial infusion studies to identify the brain site whereby these therapeutic systemic effects are elicited have to date been untenable, given the poor solubility of mGluR5 PAMS. The novel mGluR5 PAM, 1-(4-(2,4-difluorophenyl) piperazin-1-yl)-2-((4-fluorobenzyl)oxy)- ethanone, or DPFE, demonstrates greater solubility and selectivity relative to CDPPB, thus allowing us to test the hypothesis that mGluR5 activation restores object recognition memory in LA rats via action in the perirhinal cortex. Male, Sprague-Dawley rats self-administered meth (0.02 mg/infusion, i.v.) along an FR1 schedule of reinforcement. After 7 daily 1-h sessions, rats were switched to 6-h daily access sessions for 14 days, and then underwent drug abstinence. On abstinence days 7 and 8 (90 min and 24 hr tests, respectively), rats were tested for object recognition memory using a two-item object recognition task. Immediately after object familiarization, LA-meth rats were bilaterally infused with DPFE (0.5 µg/side) or vehicle (20% 2-hydroxypropyl-β-cyclodextrin) into the perirhinal cortex. Ninety min and 24-h later, they underwent a short-term and long-term memory test, respectively. Perirhinal mGluR5 activation restored object recognition memory at both time points, suggesting that DPFE rescued the ability to consolidate the memory in perirhinal cortex. Current experiments are underway to assess the ability of DPFE to restore perirhinal LTD, which we have recently shown to be the electrophysiological substrate of the recognition memory deficits in LA rats. These findings implicate mGluR5 activation as a promising pharmacological therapy for restoring cognitive function in methamphetamine addicts. Activation of mGluR5 may have therapeutic implications for relapse as well, particularly *in situ*ations where environmental novelty can be protective against competing meth-conditioned cues.

Disclosures: J. Peters: None. **M.D. Scofield:** None. **H. Trantham-Davidson:** None. **S.M. Ghee:** None. **R.E. See:** None. **C.M. Reichel:** None.

Poster

057. Learning, Memory, Dependence, and Addiction

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 57.05/X20

Topic: C.17. Drugs of Abuse and Addiction

Support: R01DA033049

Title: Relapse involving choice between a novel cue and a methamphetamine-conditioned cue relies on perirhinal cortex

Authors: *J. PETERS, M. D. SCOFIELD, S. M. GHEE, C. M. REICHEL;
Dept. of Neurosciences, Med. Univ. of South Carolina, Charleston, SC

Abstract: Our lab has previously identified deficits in novel object recognition memory in rats that have self-administered methamphetamine under long-access (LA), but not short access (SA), conditions. This deficit results from neuroadaptations in perirhinal cortex, a brain region known to be critical for the detection of novelty. Novel cues are naturally rewarding, and animals will respond more to novel cues versus familiar ones. A methamphetamine-associated cue is a highly familiar cue to a LA meth rat and is also a conditioned cue that possesses the ability to drive expression of the conditioned response to seek meth. Here, we developed a novel test for relapse involving choice between a novel cue vs a conditioned-meth cue that reveals patterns of responding between SA and LA meth rats in cue preference. Male Sprague-Dawley rats self-administered meth (0.02 mg/infusion, i.v.) on an FR1 schedule of reinforcement. After 7 daily 1-h sessions, half the rats remained on 1 hr sessions and half were switched to 6-h daily access sessions for 14 days; then all rats underwent home cage abstinence. On abstinence day 7, rats were returned to the self-administration chamber and tested for a novel cue preference. During the test rats had access to the previously meth-associated cue and a novel cue. SA rats prefered novel cues, whereas LA rats prefered meth cues, measured by the number of lever presses for each cue. Furthermore, a priming injection of methamphetamine (1 mg/kg, i.p.) shifted this preference in SA rats to one resembling LA rats. Additionally, perirhinal microinfusion of 1-(4-(2,4-difluorophenyl) piperazin-1-yl)-2-((4-fluorobenzyl)oxy)- ethanone, or DPFE, an mGluR5 positive allosteric modulator, restored novelty preference in LA meth rats. Interestingly, this new type of relapse test can parse apart different components of a relapse episode: the effect of novelty competition was evident at the start of the session (within the first 15-min), and the effect of motivation to seek meth was evident on longer timescales (1-2 hours), the latter being more akin to traditional models of relapse. Importantly, the early-session novelty preference is consistent with meth-induced memory deficits found in a novel object recognition task. We

hypothesized that both tasks depend on perirhinal cortex function based on the ability of a memory-repairing compound (i.e. DPFE) to restore novelty preference in both of these tasks. These findings collectively underscore the importance of novelty as a protective agent against relapse.

Disclosures: **J. Peters:** None. **M.D. Scofield:** None. **S.M. Ghee:** None. **C.M. Reichel:** None.

Poster

057. Learning, Memory, Dependence, and Addiction

Location: Halls A-C

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Program#/Poster#: 57.06/X21

Topic: C.17. Drugs of Abuse and Addiction

Support: SIP-IPN

Fundación Miguel Alemán

Instituto Nacional de Psiquiatría Ramón de la Fuente

Title: Effects of environmental enrichment on memory impairment induced by toluene

Authors: ***N. PAEZ-MARTINEZ**[1,2], R. C. SOLIS-GUILLEN[1], S. MONTES[3];
[1]Escuela Superior de Medicina. Inst. Politécnico Nacional, Mexico. D.F., Mexico;
[2]Neurociencias, Inst. Nacional de Psiquiatría Ramón de la Fuente Muñiz, Mexico City, Mexico;
[3]Neuroquímica, Inst. Nacional de Neurología y Neurocirugía Manuel Velasco Suarez, Mexico City, Mexico

Abstract: Inhalant abuse is a health problem worldwide and toluene is an organic solvent found in in the main commercial products misused with intoxication purposes. Memory impairment has been described as a medical consequence among inhalant abusers and this effect has been confirmed in different preclinical protocols of toluene exposure; however therapeutic alternatives that can counteract this effect are limited. Environmental enrichment is a preclinical approximation that has demonstrated to produce positive effects on memory in different animal models of central nervous system diseases. Altogether the aims of the present work were to model memory alterations after repeated toluene exposure and subsequently to evaluate the influence of environmental enrichment on these effects. Adolescent mice were exposed to toluene vapors from 1 to 4 weeks. Effects on memory were tested every week using the object recognition test. In the second part of the experiment, control animals and mice exposed to

toluene were housed on standard conditions or environmental enrichment during four additional weeks. Memory evaluation was conducted again after completing the housing treatment. Results showed that chronic toluene exposure impair memory from the first up to the last week of evaluation. Memory impairment was independent of the toluene concentration and the time of evaluation. On the other hand the result of environmental enrichment showed that this treatment was able to counteract toluene-induced memory alteration; while animals with history of toluene exposure and housed on standard conditions maintained memory impairment. Overall, the present study showed that environmental enrichment positively impacts some effects produced by repeated exposure to toluene.

Disclosures: **N. Paez-Martinez:** None. **R.C. Solis-Guillen:** None. **S. Montes:** None.

Poster

057. Learning, Memory, Dependence, and Addiction

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Program#/Poster#: 57.07/X22

Topic: C.17. Drugs of Abuse and Addiction

Support: NIH GRANT R21DA030225

Title: Redox-based epigenetic changes via the cysteine transporter EAAT3: A novel unifying mechanism for the actions of drugs of abuse

Authors: *M. S. TRIVEDI;
Neuro-Pharmacology, Northeastern Univ., Boston, MA

Abstract: Canonically, drugs of abuse can activate intracellular signaling cascades, leading to epigenetic changes, inducing long lasting alterations in gene expression, affecting learning, memory and behavior. Additionally, these drugs can also alter glutathione (GSH) levels and induce oxidative stress; however, the consequences are unclear. Building upon substantial preliminary data, we investigated the acute and long-term effects of selected drugs of abuse and their mechanism of influence on pathways of redox metabolism and DNA methylation (DNAMe) status in cultured neuronal cells. Drugs of abuse alter EAAT3 activity and subsequent changes were observed in the GSH as well as S-adenosylmethionine levels, which define the redox and methylation status of neuronal cells, respectively. As a prototype, we characterized the effects of morphine-induced methylation changes at CpG sites in LINE-1 retrotransposons, measured via MBD-sequencing and bisulfite sequencing, along with genome-wide changes in

transcription. qRT-PCR analysis of mRNA for enzymes and transporters involved in antioxidant and methylation pathways was also carried out. Lastly, redox and methylation responses were also investigated upon removal of drugs of abuse after a prolonged exposure (i.e. *in vitro* washout). Changes in cysteine uptake, cellular levels of GSH and cysteine and DNAMe were observed both acutely and upon washout of morphine. Taken together, these findings provide an improved understanding of the mechanism by which drugs of abuse can influence neuronal cell redox and methylation status, including DNAMe. Since epigenetic changes are also implicated in withdrawal phenomenon, we suggest a novel mechanism for addiction and the action of drugs of abuse.

Disclosures: **M.S. Trivedi:** None.

Poster

057. Learning, Memory, Dependence, and Addiction

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIDA R03 DA033428

NIH UL1 TR000071

UTMB Center for Addiction Research - Pilot Research Award

UTMB Center for Addiction Research

Title: Amygdala-hippocampal phospholipase d (PLD) signaling: Identifying neural substrates for therapeutic disruption of cocaine-environment maladaptive long-term memories

Authors: *B. KRISHNAN;
Pharmacol. and Toxicology, Univ. of Texas Med. Br. At Galveston, Galveston, TX

Abstract: Long-term conditioned memory mechanisms are critical in recall of drug-environment associations and are key contributors in relapse to cocaine (and other drug) dependence. Recently, we reported an important role for rat amygdala phospholipase D (PLD) downstream to dopaminergic and glutamatergic systems in expression of cocaine-conditioned behavior. In the present study, we elucidate the role for PLD signaling downstream to serotonergic transmission in the expression of conditioned responses to cocaine in different drug-free states by studying the

limbic regions of the rat amygdala and hippocampus implicated in associative memory mechanisms. We observed conditioned hyperactivity in both early (Day-1) and (Day-14) withdrawal (drug-free states) following 7-day training. Increased phosphorylation of PLD isoforms in the amygdala crude synaptosomal fractions characterized the Day-1 drug-free state while overall increase of the total protein levels for PLD1 and PLD2 was additionally observed in the Day-14 drug-free state. Further, cellular studies confirmed that increased PLD phosphorylation states contribute to the signaling mechanism. Thus, the novel PLD signaling mechanisms associated with maladaptive long-term mechanisms can provide possible therapeutics targets in preventing relapse to cocaine use and other neuropsychiatric disorders.

Disclosures: **B. Krishnan:** None.

Poster

057. Learning, Memory, Dependence, and Addiction

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grant DA027870

University of Wisconsin-Milwaukee Research Growth Initiative

Title: Prelimbic beta-adrenergic receptor blockade during trace fear memory retrieval reduces fear and prevents reinstatement following extinction

Authors: *D. MUELLER[1], J. M. OTIS[2], M. K. FITZGERALD[2], J. L. BURKARD[2], M. A. DRAKE[2];
[1]Dept. of Psychology, [2]Univ. of Wisconsin-Milwaukee, Milwaukee, WI

Abstract: Retrieval of aversive memories can drive fear in fear/anxiety disorders, whereas retrieval of drug-associated memories can induce drug seeking in addiction. Disruption of memory retrieval would therefore be beneficial for treatment of these disorders. Inhibition of beta-adrenergic receptor (beta-AR) activity within prelimbic medial prefrontal cortex (PL-mPFC) causes long-lasting impairments in contextual drug-associated memory retrieval (Otis et al, 2013), and these impairments provide protection against drug-induced reinstatement (Otis et al, 2011, 2014). Thus, PL-mPFC beta-AR activation maintains drug-associated memory retrieval, but whether this activity is a fundamental mechanism that maintains retrieval of

memories across paradigms is unclear. Here, we examined the effects of PL-mPFC beta-AR blockade on retrieval of recent and remote contextual and trace fear memories. For contextual fear conditioning, rats were trained to associate a training context with 4 presentations of a shock unconditioned stimulus (UCS; 1s, 0.8mA). Rats returned to the training context 1 or 30 days later, during which PL-mPFC microinfusions of the beta-AR antagonist nadolol were given. Nadolol reduced context-induced freezing during these tests, but not during subsequent nadolol-free extinction tests or during UCS-induced reinstatement. In the next experiment, rats were underwent trace fear conditioning, which is known to require PL-mPFC (Gilmartin et al, 2013). Conditioning consisted of 6 paired presentations of a tone conditioned stimulus (CS, 10s) and a shock UCS (1s, 1.0mA) separated by a 20s trace interval. Rats were then tested within an alternative testing context 1 or 30 days later, during which a single presentation of a tone CS was presented followed by a 120s stimulus free period. PL-mPFC microinfusions of nadolol reduced freezing during the stimulus free period for rats tested at 30 days but not 1 day following conditioning, suggesting that remote trace fear memory retrieval requires beta-AR activation. Although freezing returned to control levels during subsequent extinction tests, nadolol-treated rats expressed significantly less freezing during a UCS-induced reinstatement test following extinction. These data suggest that inhibition of beta-AR activity during remote trace fear memory retrieval may weaken the CS-UCS contingency, such that extinction is capable of persistently suppressing retrieval.

Disclosures: **D. Mueller:** None. **J.M. Otis:** None. **M.K. Fitzgerald:** None. **J.L. Burkard:** None. **M.A. Drake:** None.

Poster

057. Learning, Memory, Dependence, and Addiction

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Support: NIH Grant DA027870

UWM Research Growth Initiative

Title: Inhibition of PKA signaling in the prelimbic cortex persistently disrupts retrieval of a cocaine-associated memory and prevents subsequent reinstatement

Authors: *M. FITZGERALD, J. M. OTIS, M. A. DRAKE, J. L. BURKARD, D. MUELLER; Univ. of Wisconsin - Milwaukee, Milwaukee, WI

Abstract: Drug-associated cues trigger craving and relapse in addicts, and preventing retrieval of cue-evoked memories would reduce relapse susceptibility. Previously we found that retrieval of drug-associated memories can be blocked by β-adrenergic receptor (β-AR) inhibition in the prelimbic medial prefrontal cortex (PL-mPFC; Otis et al., 2013), but the β-AR signaling cascades underlying retrieval remains uncertain. β-AR activation is known to stimulate adenylyl cyclase increasing intracellular cAMP and activating protein kinase A (PKA; Mueller et al., 2008). Additionally, β-AR has been shown to stimulate a receptor tyrosine kinase ultimately leading to the activation of a MAPK signaling pathway (Meitzen et al., 2011). Therefore, using a cocaine-induced conditioned place preference (CPP) paradigm, we first determined whether retrieval required activation of MAPK or PKA signaling pathways in the PL-mPFC. We found that a bilateral PL-mPFC microinfusion of the MAPK inhibitor UO126 prior to the initial CPP test had no effect on expression of a CPP. In contrast, a single bilateral PL-mPFC microinfusion of a PKA inhibitor, Rp-2'-O-Monobutyryl-cAMPS, persistently disrupted expression of a CPP across trials and prevented reinstatement to a cocaine priming injection following extinction. Thus, cocaine-associated memory retrieval requires PKA signaling, but not MAPK signaling, in PL-mPFC. Previous work has shown β-AR activation and PKA signaling enhances the intrinsic excitability of neurons, an effect mediated by a reduction in the slow afterhyperpolarization (sAHP; Zhang et al., 2013). PKA phosphorylates calcium-activated potassium channels and blocks their activity, thereby reducing the sAHP (Neylon et al., 2006). Thus, we next determined whether PKA mediates retrieval by reducing the sAHP using co-infusion of the PKA inhibitor and a sAHP blocker, UCL2077, into the PL-mPFC prior to the initial CPP. We found that the co-infusion rescued CPP expression. These results suggest β-AR activation results in PKA activity which reduces sAHP resulting in enhanced neuronal excitability necessary for cocaine-associated memory retrieval and reinstatement. Thus, enhancing sAHP function could impair cocaine-associated memory retrieval, thereby preventing cue-induced drug seeking and relapse long after treatment.

Disclosures: **M. Fitzgerald:** None. **J.M. Otis:** None. **M.A. Drake:** None. **J.L. Burkard:** None. **D. Mueller:** None.

Poster

057. Learning, Memory, Dependence, and Addiction

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 57.11/Y2

Topic: C.17. Drugs of Abuse and Addiction

Support: DA027870

University of Wisconsin-Milwaukee Research Growth Initiative

Title: Infralimbic NR2A-containing NMDA receptors are necessary for the reconsolidation of cocaine self-administration memory

Authors: *M. HAFENBREIDEL, C. RAFA TODD, J. M. OTIS, R. C. TWINING, D. MUELLER;
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Abstract: Addiction is characterized by high susceptibility to relapse, which can be triggered by drug-associated cues. Cue presentation results in retrieval of the original drug-cue memory that becomes labile and must be reconsolidated back into long-term storage. Repeated unpaired cue presentation, however, induces extinction. Thus, cue-reactivity can be reduced by either blocking reconsolidation or facilitating extinction. Previous research revealed that systemic blockade of NMDA receptors (NMDArs) can disrupt reconsolidation of drug-cue associations in a modified self-administration paradigm (Milton et al., 2008) or extinction in a standard self-administration paradigm (Hafenbreidel et al., 2014). To further characterize these processes, we examined the effects of post-extinction injections of an NMDAr antagonist on drug seeking following self-administration. Rats acquired cocaine self-administration (0.25 mg/inf, i.v., 90 min/day) followed by extinction. Extinction consisted of four 45-min extinction sessions in which rats were administered the NMDAr antagonist CPP (10 mg/kg, i.p.) immediately after each session. Extinction retention was then tested during a subsequent 90-min session. CPP treatment decreased lever pressing during subsequent extinction sessions, suggesting either disrupted reconsolidation or facilitated extinction consolidation. We next targeted the infralimbic medial prefrontal cortex (IL-mPFC), a structure implicated in extinction (Quirk & Mueller, 2008). Using the same procedure, CPP infusions (36 μg/ 0.3 μL) before or after four brief extinction sessions resulted in a similar reduction in lever pressing across subsequent days. To determine the NMDAr subtype involved, we infused either the NR2A-selective antagonist NVP (1 μg/ 0.3 μL) or the NR2B-selective antagonist Ro25 (2 μg/ 0.3 μL) after four 45-min extinction sessions. Similar to the effects of nonspecific NMDAr blockade, blocking NR2A- but not NR2B-containing NMDArs reduced lever pressing across subsequent days. Finally, to dissociate if blocking NR2A-containing NMDArs disrupts reconsolidation or facilitates extinction consolidation, NVP was infused into the IL-mPFC after four 10-min reactivation trials or in the absence of behavioral testing. Memory retention was then tested during a subsequent 90-min session, revealing that blocking NR2A-containing NMDArs after memory reactivation results in reduced lever pressing. Overall, these results indicate that blocking NR2A-containing NMDArs

in the IL-mPFC disrupts reconsolidation following reactivation of the original drug-cue memory rather than facilitates extinction consolidation.

Disclosures: **M. Hafenbreidel:** None. **C. Rafa Todd:** None. **J.M. Otis:** None. **R.C. Twining:** None. **D. Mueller:** None.

Poster

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University of Wisconsin-Milwaukee Research Growth Initiative

Title: Prelimbic neuronal excitability and synaptic potentiation underlie cocaine-associated memory retrieval and are reversible during retrieval

Authors: ***J. M. OTIS**[1], M. A. DRAKE[2], J. L. BURKARD[2], D. MUELLER[2]; [1]Psychology, Univ. Wisconsin-Milwaukee, Milwaukee, WI; [2]Univ. of Wisconsin-Milwaukee, Milwaukee, WI

Abstract: Retrieval of drug-associated memories promotes addictive behaviors, as presentation of drug-associated cues induces both drug seeking and relapse. The prelimbic medial prefrontal cortex (PL-mPFC) is critical for retrieval of cocaine-associated memories (Otis et al., 2013) and for reinstatement of drug seeking (see Peters et al., 2011). The plasticity mechanisms within PL-mPFC that maintain cocaine-associated memory retrieval and reinstatement, however, remain unclear. Thus, we examined intrinsic and synaptic changes in PL-mPFC using a model that requires contextual drug-associated memory retrieval, the conditioned place preference (CPP) paradigm. Following conditioning and two daily cocaine-free retrieval tests, rats were sacrificed for electrophysiological recordings. We first assessed the intrinsic neuronal excitability of layer V/VI PL-mPFC pyramidal neurons in cocaine-conditioned and naïve rats. Although conditioning did not modify overall intrinsic neuronal excitability, the excitability of PL-mPFC neurons was positively correlated with CPP scores ($r(5)=0.84$; $p=0.02$). Given that PL-mPFC excitability is regulated by beta-adrenergic receptors (beta-ARs; Otis et al., 2013), we examined the effects of propranolol, the beta-AR antagonist, on retrieval. Propranolol injections (10 mg/kg; i.p.) before

the first CPP test prevented retrieval during that test and during the following propranolol-free test (consistent with previous findings, Otis et al., 2011, 2013, 2014). Moreover, CPP scores from rats treated with propranolol did not correlate with intrinsic excitability of PL-mPFC neurons ($r(2)=-0.38$; $p=0.62$). Thus, intrinsic excitability of PL-mPFC neurons regulates CPP memory retrieval. Next, we characterized the effects of cocaine conditioning on the strength of synaptic inputs to layer V/VI PL-mPFC pyramidal neurons. Cocaine conditioning induced potentiation of PL-mPFC postsynaptic strength, as evidenced by an increase in AMPA-mediated currents as compared to NMDA-mediated currents (AMPA:NMDA ratio). Conditioning also modified presynaptic input, as evidenced by paired-pulse facilitation. Thus, synaptic plasticity in layer V/VI PL-mPFC pyramidal neurons may regulate cocaine CPP memory retrieval. In support of this, propranolol treatment reversed the increase in AMPA:NMDA ratio, indicating that beta-AR blockade induced synaptic depotentiation in PL-mPFC. Our data demonstrate that PL-mPFC intrinsic excitability and synaptic plasticity regulate drug-associated memory retrieval, and this plasticity is maintained by beta-AR activation during retrieval.

Disclosures: **J.M. Otis:** None. **M.A. Drake:** None. **J.L. Burkard:** None. **D. Mueller:** None.

Poster

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Topic: C.17. Drugs of Abuse and Addiction

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Title: Paternal cocaine exposure elicits transgenerational learning deficits

Authors: ***M. E. WIMMER**, L. A. BRIAND, C. P. CRAIGE, L. A. GUERCIO, A. C. ARREOLA, H. D. SCHMIDT, R. C. PIERCE;
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Abstract: Cocaine addiction is associated with profound cognitive impairments, including deficiencies in memory function. In some cases, these memory deficits predict poor treatment retention and outcome. Additionally, growing evidence indicates that drug abuse can influence behavior and neurophysiology not only in adults, but also in offspring. We previously showed that paternal cocaine self-administration reduces the reinforcing efficacy of cocaine in offspring. Here, behavioral and electrophysiological techniques were combined to examine the influence of paternal cocaine self-administration on memory formation and synaptic plasticity in offspring. Object-based paradigms are ideally suited to evaluate memory traces because they circumvent potentially confounding alterations in motor function or stress response, and the underlying neural circuits have been well defined. Male rats self-administered cocaine daily for 60 days and controls received yoked saline infusions. Cocaine-experienced and saline-experienced sires were then bred to drug-naïve females and memory formation was assessed in the resulting adult (60 days and older) offspring. Paternal cocaine self-administration elicited short-term (30 minutes) spatial memory deficits in male, but not female, offspring (F1 generation) and grand-offspring (F2 generation). F1 and F2 animals showed normal novel object recognition in a hippocampus-independent version of this task. These findings indicate that paternal cocaine self-administration produces transgenerational spatial learning deficits and disrupts hippocampal function. Theta bursts-induced LTP, a cellular model of memory formation, was evaluated to directly test hippocampal plasticity in the descendants of cocaine-exposed sires. LTP induction was impaired in adult male F1 offspring, suggesting that NMDA receptor signaling was reduced. Consistent with this hypothesis, bath application of the endogenous NMDA receptor co-agonist D-serine restored LTP induction in hippocampal slices from F1 rats. Taken together, these findings indicate that the offspring and grand-offspring of cocaine-experienced rats show spatial learning deficits, which may be caused by reduced NMDA receptor signaling in the hippocampus.

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Poster

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Support: NSFC Grant 81271472

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Title: The interaction between NMDA receptor and CaMKII in prelimbic cortex modulates extinction of cocaine self-administration

Authors: Z.-Y. ZHANG[1], Y.-Z. ZHANG[2], S.-Q. CHEN[2], *H. SHEN[1];
[1]Natl. Inst. On Drug Dependence, Peking Univ., Beijing, China; [2]Inst. of Neurosci., Guangzhou Med. Univ., Guangzhou, China

Abstract: Vulnerability to relapse to drug seeking is the most important characteristic in drug addiction, which linked to activating prefrontal cortex (PFc) projections to the nucleus accumbens during the cue exposure. Pervious study have demonstrated that inactivation of PFc inhibited cue-induced or drug-primed reinstatement in drug self-administered animals. Furthermore, PFc is involved in extinction training of drug seeking which is considered as a learning process for disassociation of cue and reinforcement. In present study, we investigated whether that the interaction between NMDA receptor (NMDAR) and CaMKII in PFc contributes to extinction of cocaine self-administration. To disrupt the NMDAR/CaMKII complex, CN class of peptides were packaged in a lentiviral construct (LV-CN21) that interfere NMDAR binding to CaMKII. During abstinence from cocaine self-administration, LV-CN21 or LV-GFP was infused in prelimbic cortex (PL). Three weeks after microinjection, reinstatements were triggered by context and drug-associated cue, and consequent extinction training were conducted for further 2 weeks. We found that LV-CN21 failed to prevent the reinstatements, but decelerate the extinction procedure in the second week of extinction training. These data implies that NMDAR-mediated signaling in PL might underlie the molecular mechanism for extinction learning and would be a pharmacological target for addiction treatment.

Disclosures: **Z. Zhang:** None. **Y. Zhang:** None. **S. Chen:** None. **H. Shen:** None.

Poster

057. Learning, Memory, Dependence, and Addiction

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Topic: C.17. Drugs of Abuse and Addiction

Title: Short-term exposure to nicotine sensitizes the anxiety-producing effects of nicotine when later encountered

Authors: *R. C. BARNET, A. HOGENMILLER;
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Abstract: We previously reported that chronic (14-day) nicotine exposure can produce long-term alterations in hippocampus dependent context conditioning (Spaeth et al., 2010). Chronic nicotine exposure has also been shown to produce long-lasting changes in anxiety measured in Light Enhanced Startle (LES; Barnet et al., 2013). Effects of acute nicotine are more variable with anxiolytic or anxiogenic properties reported depending on species, task, and age, among other factors (e.g., Faraday et al., 2003; File et al., 1998; Picciotto et al., 2002). In our own laboratory, acute nicotine has been shown to be anxiogenic in both adults and younger animals when measured in the LES paradigm. In the present experiment we sought to examine nicotine's anxiogenic profile following limited, short term, nicotine preexposure. Adult rats were exposed to saline, 0.15 mg/kg, or 0.40 mg/kg nicotine (i.p.) for 4 consecutive days. Following this nicotine preexposure phase, all animals were exposed to a single acute 0.40 mg/kg nicotine injection and the effect of this acute nicotine exposure on anxiety was measured in LES. Nicotine's anxiogenic effect on LES was found to be significantly greater in nicotine preexposure groups compared to saline preexposure groups. The results suggest that even limited exposure to nicotine can sensitize the brain's response to nicotine's stress-inducing effects when nicotine is later encountered.

Disclosures: **R.C. Barnet:** None. **A. Hogenmiller:** None.

Poster

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIDA-SPO, R01DA016765

NIDA-T32, DA07290

Title: The role of the dentate gyrus in morphine CPP: Adult-generated neurons regulate extinction of reward learning

Authors: *P. D. RIVERA, R. K. RAGHAVAN, S. YUN, M.-K. MCGOVERN, S. LATCHNEY, S. BIRNBAUM, A. J. EISCH;
Dept. of Psychiatry, UT Southwestern Med. Ctr., Dallas, TX

Abstract: The hippocampus, and dentate gyrus (DG) in particular, is involved in several aspects of addiction. For example, after conditioned place preference (CPP) for psychostimulants or opiates there is an increase in DG cellular activity (cFos+ cells). Interestingly, the adult DG gives rise to new neurons throughout life. These adult-generated neurons are thought to be important in hippocampal function and activated by spatial tasks, although prior work suggests they are not important in cocaine CPP acquisition or retention. Here we addressed two unanswered questions: Is DG activity actually induced by the retrieval of a morphine/context association? And are adult-generated neurons important in the retrieval, retention, and extinction of a morphine/context association? To determine cellular activity within the DG granule cell layer (GCL) after saline/saline and saline/morphine CPP training, respectively, C57BL/6J mice were sequestered into their non-drug paired context or drug paired context on test day. Quantification of cFos+ cells 90min post-test showed mice conditioned to a previously-learned drug context have ~30% more cFos+ GCL cells than controls. Thus, retrieval of a drug/context association reward memory appears to occur in the DG GCL. To examine if adult-generated neurons are important in the retrieval, retention, and extinction of a morphine/context association, C57BL/6J mice received sham irradiation (IRR) or image-guided X-Ray IRR (15Gy) 6 wks prior to morphine CPP training, and then assessed for acquisition, retention, and extinction. Similar to prior work with cocaine, IRR mice are able to retrieve and retain a morphine/context association similar to controls. However, while Sham mice extinguished preference ~8 days post-CPP, IRR mice surprisingly never extinguish, and by 20 days post-CPP still spend significantly more time (~200%) in the drug context compared to Sham. These data suggest cranial IRR abolishes the extinction of reward learning in morphine CPP. Taken together, these data clarify the role of the DG GCL in morphine CPP and the potential importance of adult-generated neurons in the extinction of reward learning. Ongoing studies are using DREADD technology to selectively activate/inactivate adult-generated neurons during extinction, and a conditional knock out strategy to ablate adult neurogenesis before and after morphine CPP training to dissect the role of adult-generated neurons in this hippocampal-dependent memory task.

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Poster

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Title: The effects of β-arrestin dependent signaling pathways on alcohol dependence and memory reconsolidation

Authors: *X. LIU, Y. TAO, L. MA, B. HUANG, L. MA;
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Abstract: β-Arrestins, known as key regulators of GPCR signaling, are highly expressed in the central nervous systems. But their roles in brain functions remain largely unknown. Here we report that β-arrestin 2 expression can regulate preference and sensitivity for alcohol in mice. mGluR5 antagonist MPEP inhibited alcohol dependence in the chronic alcohol drinking mice. The chronically free consumption of alcohol increased the phosphorylation levels of Akt, a β-arrestin 2 binding partner, and GSK3β, in the dorsal striatum of wild type mice, but not β-arrestin 2 knockout mice. These data reveal that β-arrestin 2 is a negative regulator of striatal Akt/GSK3β pathway and a protective molecule to inhibit alcohol preference and dependence. The increased activation of AKT/GSK3β in the dorsal striatum induced by stable alcohol consumption was greatly suppressed by MPEP, while no significant changes were detected in signaling pathways such as ERK/CREB and S6K1/S6 in dorsal striatum after the chronic alcohol intake. The intracerebroventricular administration of either MPEP or wortmannin, the PI3K inhibitor, markedly reduced the voluntary alcohol intake in mice. What's more, our data suggest that memory reconsolidation is mediated by a β-arrestin-dependent β-adrenergic signaling pathway. Our study implicates that the β-arrestin mediated mGluR5 cascade in the dorsal striatum and β-adrenergic signaling in hippocampus formation may be individually involved in the development of alcohol dependence and memory reconsolidation, and reveals the theraputic potential for β-arrestin regulated signaling processes in treatment of alcohlism and memory related disorders.

Disclosures: **X. Liu:** None. **Y. Tao:** None. **L. Ma:** None. **B. Huang:** None. **L. Ma:** None.

Poster

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIDA IRP

Title: Unique molecular alterations in synapses following acute cocaine and novelty exposure

Authors: *V. SELVAM, F. C. CRUZ, R. M. LEAO, B. T. HOPE;
NIDA, Natinal Inst. On Drug Abuse - NIH, Baltimore, MD

Abstract: Learned associations between drug effects and stimuli in the drug environment play an important role in drug addiction. These associations are thought to be encoded by sparsely distributed patterns of neurons called neuronal ensembles that are selected by the drug-related stimuli. Alterations in dendritic spines of neuronal ensembles are thought to play an important role in learning addiction-related behaviors. Our goal is to identify synaptic alterations uniquely within synapses activated during drug-related behavior. We will determine the time course for cocaine and novelty-induced Arc, which will set the time constraints for using this molecules as a marker of activated synapses in future behavioral studies. We developed a procedure that uses flow cytometry to purify behaviorally activated synaptosomes (which contain the presynaptic terminal and attached post-synaptic density) to identify unique molecular and cellular alterations that are distinct from those in non-activated synapses. We injected rats with 20 mg/kg cocaine or saline vehicle and immediately placed them in a novel environment (bowls). Rats were decapitated 0, 60, 90 or 120 min following injections and the accumbens was dissected from 2 mm coronal slices. Synaptosomes were prepared and immunolabeled for PSD95 (to label all synapses) and Arc (to label activated synapses). For flow cytometry, we identified unique forward and side light-scattering characteristics of synaptosomes and assessed alterations in ARC protein levels over time in the PSD95 positive synaptosomes. Cocaine increased total Arc protein levels in synaptosomes at 60 min, suggesting that Arc translocation to the PSD of synaptosomes can be used as a marker of cocaine-activated synapses in flow cytometry. We conclude that Arc can be used as a marker of cocaine-activated synapses that make up the neuronal ensembles that encode learned associations between cocaine and the drug-associated context and play a role in context-induced reinstatement of cocaine seeking.

Disclosures: **V. Selvam:** None. **F.C. Cruz:** None. **R.M. Leao:** None. **B.T. Hope:** None.

Poster

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grant RO1 DA034116

Title: Systemic inhibition of myosin II disrupts the storage of drug-associated memories

Authors: *E. J. YOUNG[1], A. M. BLOUIN[1], S. B. BRIGGS[1], G. RUMBAUGH[2], C. A. MILLER[1];
[1]Metabolism & Aging, Neurosci., [2]Neurosci., Scripps Res. Inst., Jupiter, FL

Abstract: Exposure to environmental cues associated with previous drug use can cause the involuntary retrieval of deeply engrained memories capable of redirecting behavior towards obtaining drugs. Our lab is focused on identifying mechanisms capable of disrupting these powerful, consolidated memories, with the goal of developing therapeutics. Regulators of synaptic dynamics are one particularly promising avenue, as emerging evidence indicates that memory formation depends on structural and functional plasticity of dendritic spines, for which actin polymerization is critical. Indeed we have found that, when performed days after training, home cage delivery of an actin depolymerizing agent to the amygdala (AMY) immediately and persistently prevents memory-induced drug seeking. This unexpected disruption of drug-associated memory storage had no impact on other types of amygdala-dependent memories, indicating that the actin dynamics supporting drug-associated memories remain uniquely active post-consolidation. While promising, the potential for actin polymerization inhibitors as therapeutics is limited, as they cannot be administered systemically because a multitude of peripheral processes rely on dynamic actin (e.g. cardiac function). For this reason, we have shifted our focus upstream of actin polymerization to identify mechanisms that could be safely manipulated from the periphery. We recently reported that myosin IIb, a non-muscle form of myosin, imparts a mechanical force that triggers spine actin polymerization in response to synaptic stimulation. To test the role of myosin II in the maintenance of drug-associated memories, we employed two models of context-induced drug seeking: conditioned place preference (CPP) and context-induced reinstatement of self-administration. Similar to an actin depolymerizing compound, pre-test inhibition of myosin IIb ATPase activity in the AMY produced a rapid and lasting disruption of drug-seeking behavior. We next determined the

potential therapeutic value of myosin II inhibition by establishing that systemic inhibition of myosin II successfully disrupted CPP to the same degree as direct intra-AMY delivery. Systemic inhibition of myosin II also reversed the drug-induced increase in AMY spine density but not in Area CA1 of the hippocampus. Consistent with this, inhibiting myosin II in Area CA1 failed to disrupt drug-associated memory, despite being a brain region necessary for this type of memory. Together, these findings indicate that myosin II represents a potential therapeutic target for disrupting these powerful, extinction-resistant memories capable of triggering relapse.

Disclosures: **E.J. Young:** None. **A.M. Blouin:** None. **S.B. Briggs:** None. **G. Rumbaugh:** None. **C.A. Miller:** None.

Poster

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Topic: C.17. Drugs of Abuse and Addiction

Support: NIH Grant DA029091

Title: Inoculation stress model of environmental enrichment

Authors: *E. J. CROFTON, Y. ZHANG, X. FAN, D. LI, T. A. GREEN;
Pharmacol. and Toxicology, Ctr. for Addiction Res., Univ. of Texas Med. Br., Galveston, TX

Abstract: Environmental enrichment is a non-drug, non-surgical manipulation that confers robust protective addiction and depression phenotypes in rats. In this paradigm, rats are housed for 30 days post-weaning in large cages with plastic children's toys that are changed and rearranged daily. Enriched rats are exposed to novelty, social contact and exercise and will self-administer less cocaine and amphetamine than isolated rats despite being more sensitive to the locomotor activating effects of the drugs. Additionally, enriched rats show decreased anhedonia-like behavior in the sucrose preference test, decreased social withdrawal shown by longer grooming time in the social contact test, and longer mobility time in the forced swim test. A major question in this field is how environmental enrichment can produce such robust behavioral differences in such a wide range of behavioral paradigms. Our hypothesis is that animals in the enriched environment undergo inoculation stress through living in a complex and dynamic environment, interacting non-aggressively with conspecifics, and exercising. Environment enrichment is a chronic mild stress environment that yields the animals less sensitive to future,

more severe perturbations, such as exposure to drugs of abuse or stress. Psychological stress is a major factor in the development and relapse to both addiction and depression in humans and determining the underlying molecular mechanism of the protection from addiction and depression could lead to efficacious treatments for both.

Disclosures: **E.J. Crofton:** None. **Y. Zhang:** None. **X. Fan:** None. **D. Li:** None. **T.A. Green:** None.

Poster

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CIC-UMSNH. 26.10

Title: Effect of chronic misused solvent exposure on adrenergic responses in isolated perfused rat heart

Authors: N. ALVARADO GÓMEZ[1,2], G. HERRERA LÓPEZ[3], L. ORTEGA VARELA[4], D. GODÍNEZ HERNÁNDEZ[2], *M. Y. GAUTHEREAU[1];
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Abstract: The intentional inhalation of misused solvents to achieve intoxicating states is an important public health issue in Mexico. It has been reported that chronic administration of abused drugs in animal experimental models can produce tolerance, physical dependence and sensitization. Several reports indicate that inhaling solvents can lead to the occurrence of cardiac arrhythmias and a phenomenon known as sudden sniffing death, but the mechanisms by which these effects are produced are not completely understood. However, there are some reports indicating that cardiac arrhythmias due to sensitization of the heart to epinephrine are probably the most common cause of death. Based on this evidence, the purpose of this study was to investigate the effect of chronic exposure to 3 representative misused solvents (toluene, xylene and benzene) on the reactivity of the heart to epinephrine and on protein expression of the α_1 and

β_1-adrenergic receptors in aorta, atrium and ventricles. Male Wistar rats (250-300 g) were placed in a static exposure chamber and exposed to 6000 ppm of solvent (toluene, xylene, benzene) or air (control) during 30 minutes, twice a day for 30 days. On 31st day rats were anesthetized with sodium pentobarbital (50 mg/kg), and the hearts were isolated and perfused according to Langendorff method. Concentration-response curves to epinephrine (adrenergic agonist: 1 x 10^{-9}- 1 x 10^{-4} M) were made and parameters such as perfusion pressure, heart rate and strength of ventricular contraction were measured. The results showed that chronic solvent exposure produced a decrease in perfusion pressure; on the other hand, it was observed that repeated exposure to toluene, xylene or benzene produced an increase in the strength of ventricular contraction of isolated rat heart, i.e., there was a positive inotropic effect. Furthermore, in hearts of animals exposed to solvents, it was shown a decrease in the density of β_1 adrenergic receptors in ventricle. In conclusion, the results suggest that chronic solvent exposure can produce desensitization or tolerance to epinephrine effects on perfusion pressure and sensitization to solvent action on the strength of ventricular contraction; however, additional studies are required to elucidate the mechanisms involved in these effects.

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Poster

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Topic: C.17. Drugs of Abuse and Addiction

Title: Comparison of conditioned place preference and progressive ratio operant conditioning to high fat/sugar foods: Correlation with nucleus accumbens c-Fos expression

Authors: *G. C. LOPEZ[1], M. L. NGBOKOLI[1], J. C. HONOHAN[1], R. H. MARKSON[1], L. CAMERON[1], K. S. BANTIS[1], E. LUNER[2], J. A. SCHROEDER[1];
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Abstract: Obesity is a rapidly expanding epidemic that has serious health consequences and is the leading cause of preventable death in the United States. Psychostimulant and opioid addiction is a separate serious societal issue stemming from an individual's inability to control reward-seeking behavior. Cravings for drugs of abuse as well as highly palatable foods can be triggered simply by exposure to a reward-paired environment. Stimulation of the nucleus accumbens by

addictive substances, including high fat/sugar foods triggers expression of immediate early genes, the measurement of which can be used as an indicator of cellular activation. The current study employed an 8-day biased CPP paradigm to compare the rewarding properties of a high fat/sugar food (Oreo cookies) that is highly palatable to humans and rats to the rewarding properties of cocaine and morphine. Reward behavior was correlated with immunohistochemical measurement of nucleus accumbens c-Fos expression. In a on-going follow-up study, a progressive ratio operant condition paradigm was used to compare the break points in responding to regular rat food vs. chocolate flavored high fat/sugar pellets with the same nutritional content as Oreos. Results indicate that the reward behavior associated with consuming Oreos is equivalent to cocaine or morphine reward behavior. The magnitude of conditioned place preference to all three substances was positively correlated with nucleus accumbens c-Fos expression. These findings suggest that high fat/sugar foods and drugs of abuse trigger brain addictive processes to the same degree and lend support to the hypothesis that maladaptive eating behaviors contributing to obesity can be compared to drug addiction.

Disclosures: **G.C. Lopez:** None. **M.L. Ngbokoli:** None. **J.C. Honohan:** None. **R.H. Markson:** None. **L. Cameron:** None. **K.S. Bantis:** None. **E. Luner:** None. **J.A. Schroeder:** None.

Poster

057. Learning, Memory, Dependence, and Addiction

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 57.23/Y14

Topic: C.17. Drugs of Abuse and Addiction

Support: NIH grant R01 DA019666

NIH grant K02 DA035459

Title: Synaptic depotentation via mGluR5 activation and AMPAR internalization in the nucleus accumbens drives cocaine-primed reinstatement

Authors: *M. A. BENNEYWORTH, A. J. ASP, M. C. HEARING, C. E. SCHMIDT, S. R. EBNER, A. E. INGEBRETSON, M. J. THOMAS;
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Abstract: Relapse after periods of drug abstinence is a major obstacle to long-lasting recovery for many addicts. Understanding the neurobiological responses to drug-associated stimuli that incite drug craving and drive relapse has the potential to help target our efforts to treat addiction. The nucleus accumbens (NAc) serves as a critical interface of mesolimbic dopamine circuitry with excitatory cortical afferents in the regulation of motivation and drug seeking. Repeated *in vivo* cocaine exposure potentiates synaptic strength in the NAc medium spiny neurons (MSN), which is thought to promote addiction-related behavior. However, the present studies test the hypothesis that reversal of that augmented synaptic strength, or depotentiation, in the NAc shell may be a necessary factor in relapse. Metabotropic glutamate receptor subtype 5 (mGluR5) activation is one cellular mechanism known to promote synaptic depression. Therefore, we investigated whether cocaine-primed reinstatement of place preference in C57BL/6J mice is mGluR5-dependent and involves reductions in AMPA-type glutamate receptor signaling using conditioned place preference (CPP) behavior and *ex vivo* whole-cell electrophysiology. In support of our hypothesis, we observed that cocaine-primed reinstatement of CPP was disrupted by either intra-NAc shell infusion of the tat-GluA23Y "interference" peptide (inhibitor of activity-dependent AMPAR internalization; Ahmadian et al., 2004) or the mGluR5 antagonist MTEP (tat-GluA23Y:91.1% reduction in preference; MTEP: 63.6% reduction in preference). Importantly, preliminary data suggests that MTEP blocks NAc shell AMPAR depotentiation that would otherwise be induced by a cocaine-primed injection. Additionally, intra-NAc shell infusion of the mGluR5 agonist CHPG produced a dose-responsive reinstatement of CPP in extinguished mice (339.6 vs -6.97 CPP score in CHPG and control animals, respectively). Further experiments are being done to explore whether CHPG-induced reinstatement is also blocked by tat-GluA23Y. These findings support a model in which mGluR5-mediated functional reduction in GluA2-containing AMPARs in the NAc shell MSNs is a critical factor in the reinstatement of cocaine-primed behavior

Disclosures: **M.A. Benneyworth:** None. **A.J. Asp:** None. **M.C. Hearing:** None. **C.E. Schmidt:** None. **S.R. Ebner:** None. **A.E. Ingebretson:** None. **M.J. Thomas:** None.

Poster

057. Learning, Memory, Dependence, and Addiction

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Support: CAPES

CNPq

UFCSPA

SENAD

Title: Increased BDNF in hippocampus of female rats after acute administration of methylphenidate and hormonal influence

Authors: *L. FREESE[1], Y. BOITA[2], N. D. COUTO-PEREIRA[4], G. AGNES[3], M. SOUZA[3], H. BARROS[5];
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Abstract: Methylphenidate (MPH), commonly prescribed for attention deficit hyperactivity disorder, is used recreationally by adolescents or adults. Women represent a risk group for abuse of psychostimulants because they respond more intensely, probably due to sex hormones. BDNF is a neurotrophic factor that modulates several neuronal functions in the CNS and with important implications in memory. Studies show that female hormones, specially estrogen, influences neuroplasticity of the hippocampus. Objective: to evaluate the modulatory effects of female sex hormones in the mRNA levels of BDNF mRNA in the hippocampus of MPH sensitized rats. Female adult Wistar rats were assigned to the ovariectomized or intact groups and submitted to a sensitization protocol in subgroups: acute MPH (ACT), repeated MPH (RPT) and control (CTR). Two weeks after ovariectomy, for 5 consecutive days, RTP rats received MPH 2,5mg/Kg i.p. while ACTs and CTRs received 1mg/Kg saline. After a 7day washout, ACT and RPT rats received MPH 2,5mg/Kg as challenge. The horizontal activity was scored for 60 minutes. After the test, rats were euthanized by decapitation and the hippocampus were dissected for BDNF real-time PCR. Data analysis: 2v-ANOVA/Bonferroni post-hoc. As expected (Freese et al., 2012) RPT showed sensitized behavior. The intact group had a greater expression of BDNF (2.45 +0.40) in respect ovariectomized (0.139 +0.347), ($F(1,17) = 6.112$; $p=0.024$). ACT treatments presented higher levels of BDNF ($F(2,17) = 6.982$; $p=0.006$). ACT treatment induces higher levels of BDNF in intact females ($Fint(2,17) = 6.982$; $p=0.001$). All animals of the intact group were evenly distributed through the estrous cycles and castration was confirmed. It was observed that acute exposure to MPH induces significant increment in the expression of BDNF in the hippocampus, specially in females under physiologic estrous cycling. As also seen in GAD65 and GAD67, RPT treatment with MPH induced tolerance to the BDNF increase, in animals that are behaviorally sensitization. The neuronal adaptations reflected by normalized levels of GABA synthesis and of BDNF after repeated exposure to MPH may be a consequence of a mal-adaptation that may be associated to increased risk of a psychostimulant abuse.

Disclosures: **L. Freese:** None. **Y. Boita:** None. **N.D. Couto-Pereira:** None. **G. Agnes:** None. **M. Souza:** None. **H. Barros:** None.

Poster

058. Olfaction: Central Circuits and Neurotransmitters

Location: Halls A-C

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Topic: D.01. Chemical Senses

Support: R01 DC006640

Title: Integration of olfactory bulb glomeruli by the anterior olfactory nucleus

Authors: *B. E. SANCHEZ, N. E. SCHOPPA;
Physiol., Ucdenver Anschutz Med. Campus, Aurora, CO

Abstract: There is now strong consensus that the piriform cortex is a major locus where information about different molecular features of an odor are integrated, leading to perception of an olfactory object (Stettler and Axel, 2009; Apicella et al., 2010; Davison and Ehlers, 2011; Miyamichi et al., 2011). However, very little is known about such integration in the anterior olfactory nucleus (AON), which also receives direct input from the olfactory bulb. In addition, AON differs from piriform cortex in that much of its input is derived from tufted cells, which show quite distinct odor-evoked responses as compared to mitral cells (Igarashi et al., 2012). To examine the integrative capacity of AON, we measured pyramidal cell calcium responses to electrical stimulation of one or two glomeruli in the bulb in fura-2,AM-loaded brain slices from mouse. The slices often included the bulb, AON, and aPC so that responses could be compared between AON and aPC. To ensure that each electrode activated only one glomerulus, calcium responses of bulbar cells that surrounded glomeruli were closely monitored. Following electrical stimulation of single glomeruli (4 pulses at 5 Hz; 40-150 uA), we found that many pyramidal cells were activated in each structure, with no spatial ordering. When two glomeruli (≥240 μm-separation) were stimulated separately, 25±4% of AON cells (930 cells across 8 slices) and 35±5% of aPC cells (808 cells across 8 slices; $p = 0.046$, unpaired t-test) responded to stimulation of both glomeruli. Hence, different glomeruli appear to be less effective at driving cell activation in AON versus aPC. The fraction of cells that required simultaneous stimulation of both glomeruli to be activated was similar (13±3% of AON cells, 18±5% of aPC cells; $p = 0.31$). At least part of the difference between AON and aPC in their integrative capacity appeared to be due to intracortical mechanisms. Upon addition of the GABAB receptor agonist

baclofen (40 μM) to inhibit recurrent excitatory synapses, the fraction of cells that responded to separate stimulation of both glomeruli increased by 29±13% in AON (418 cells in 5 slices) but decreased by 22±10% in aPC (400 cells in 5 slices; $p = 0.015$ in unpaired t-test between AON versus aPC). These results suggest that different glomeruli in the bulb send convergent inputs onto single pyramidal cells in both AON and aPC, but that intracortical mechanisms, most likely inhibition, limits the ability of AON cells to respond to activation of different glomeruli.

Disclosures: **B.E. Sanchez:** None. **N.E. Schoppa:** None.

Poster

058. Olfaction: Central Circuits and Neurotransmitters

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Topic: D.01. Chemical Senses

Support: NIH Grant DC004657

NIH Grant DC000566

Title: Role of basal forebrain cholinergic neurons in olfactory learning

Authors: A. NUNEZ-PARRA, *D. RESTREPO;
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Abstract: The ability of the olfactory system to represent sensory cues is strongly influenced by neuromodulators released in response to a challenging and constantly changing external environment. Different processing stations throughout the olfactory pathway receive massive afferents from several brain regions that alter neuronal excitability and modify olfactory processing. Of particular interest is the neuromodulator acetylcholine (ACh), which has been linked in several brain regions with attention and cue detection. The olfactory bulb (OB) and piriform cortex (PC) receive abundant cholinergic innervation from the basal forebrain, specifically from the horizontal diagonal band of Broca (HDB) and mangocellular preoptic area (MCPO). It has been suggested that in these regions ACh promotes contrast enhancement among similar odorants and network synchronization to generate a more efficient olfactory coding, ultimately affecting olfactory learning and memory. Great efforts has been made to study the cellular effects of ACh in the olfactory pathway, yet how basal forebrain neurons are activated during active olfactory learning remains unknown. Here, we performed electrophysiological

recordings from the HDB/MCPO of awake and freely moving animals exposed to the associative learning paradigm go-no go. This task studies the capacity of a water-deprived rodent to actively and continuously discriminate between two odorants (a rewarded and a non-rewarded odor). Importantly, the electrophysiological and neurochemical properties of neurons in the basal forebrain are very diverse. Thus, the complex neuronal heterogeneity of the HDB/MCPO presents a challenge to study the exclusive contribution of cholinergic release to olfactory learning. To bypass this constraint we used selective optogenetic stimulation to identify cholinergic neurons of the HDB/MCPO. Specifically, we stereotactically inserted an optetrode (composed of an optical fiber and four tetrodes) into the HDB/MCPO of animals expressing channelrhodopsin (ChR) under the control of the choline acetyltransferase (ChAT) promoter (ChAT-ChR mice), an enzyme that is exclusively expressed in cholinergic neurons. Cholinergic neuron identification was performed by applying blue light directly into this region and by performing off-line spike sorting analysis.

Disclosures: **A. Nunez-Parra:** None. **D. Restrepo:** None.

Poster

058. Olfaction: Central Circuits and Neurotransmitters

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Title: Structural basis for serotonergic regulation of neural circuits in the mouse olfactory bulb

Authors: Y. SUZUKI, *E. KIYOKAGE, K. TOIDA;
Kawasaki Med. Sch., Kurashiki, Japan

Abstract: Serotonin (5-HT), a monoamine neurotransmitter, originates from the dorsal and median raphe nucleus and distributes fibers throughout the forebrain. It also distributes the olfactory bulb (OB) and plays a crucial role in olfactory processing. Odor information processing

occurs in the OB and is regulated by bulbar interneurons and afferent neurons from other brain regions including 5-HT, noradrenalin, and acetylcholine. However, a detailed anatomical basis for the role of 5-HT in the OB remains to be understood. We thus focus on serotonergic regulation of OB function in the present study. We analyzed the projection pathway of 5-HT neurons from the dorsal raphe nucleus to the OB, and synapses between 5-HT axons and bulbar interneurons. We injected GFP-tagged sindbis viral vector into the dorsal raphe nucleus of mice and visualized the infected neurons. Infected neurons were identified as 5-HT immunoreactive by immunocytochemistry and reconstructed three dimensionally with Neurolucida. Confocal laser microscopy was performed to examine whether 5-HT axons contact with bulbar interneurons. Subsequently, pre-embedding immuno-electron microscopy for 5-HT and chemical markers of these interneurons was conducted to analyze the synapse. Moreover, we evaluated whether 5-HT neurons in the OB expressed vesicular glutamate transporter 3 (VGLUT3) or not. Our results demonstrate that 5-HT neurons project axons from dorsal raphe nucleus to the OB with bifurcations to other brain regions. 5-HT axons contacted several interneurons and formed asymmetrical synapses containing heterogeneous and dense-cored vesicles with these neurons in plural layers in the OB, suggesting that 5-HT neurons in the OB inhibit projection neurons in several ways and thus regulate the OB function at various steps. In addition, the 5-HT neurons in the OB were confirmed as VGLUT3 immunoreactive by co-localizing with VGLUT3 in electron microscopy. Our present study strongly indicates that afferent regulations from other brain regions contribute to elaborate mechanisms in olfactory processing and possibly coordinate or link olfactory encoding with other neural systems.

Disclosures: **Y. Suzuki:** None. **E. Kiyokage:** None. **K. Toida:** None.

Poster

058. Olfaction: Central Circuits and Neurotransmitters

Location: Halls A-C

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Title: Illuminating the role of inhibitory microcircuits in higher-order olfactory processing in zebrafish

Authors: *T. FRANK, R. W. FRIEDRICH;
Friedrich Miescher Inst., Basel, Switzerland

Abstract: The brain creates dynamic representations of the sensory environment by extracting stimulus features at early processing stages and synthesizing more abstract object representations in higher brain areas. We dissect the function of neuronal microcircuits in a higher olfactory brain area in order to identify elementary computations of basic cortical circuits and to analyze the underlying cellular mechanisms. To this end, we use a combination of genetic, electrophysiological and optical approaches to visualize and manipulate different types of interneurons (INs) in the posterior zone of the dorsal telencephalon (Dp) of adult zebrafish. This brain area is homologous to olfactory cortex in mammals and assumed to be involved in olfactory object representations and associative memory. We identified two types of INs that have similar electrophysiological properties but are differently connected to other neurons in Dp. Preliminary results suggest that optogenetic silencing of these IN types has different effects on odor-evoked activity patterns in Dp. Ongoing experiments further examine the influence of these interneurons on the processing of odor information in order to elucidate computations performed by different microcircuits in Dp. The results are expected to provide insights into canonical computations performed by basic cortical circuits.

Disclosures: **T. Frank:** None. **R.W. Friedrich:** None.

Poster

058. Olfaction: Central Circuits and Neurotransmitters

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Topic: D.01. Chemical Senses

Support: R01-DC005813

T32-DC000044

Title: Modulation of amygdalar circuits important for chemosensory signal processing

Authors: L. BIGGS[1], *M. MEREDITH[2];
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Abstract: The medial amygdala receives chemosensory information from the vomeronasal organ (VNO) and the main olfactory system and evaluates chemosignals used by many rodent species to convey social information. Medial amygdala (Me) responds differentially to conspecific and heterospecific chemosensory signals that convey different meanings and evoke different behavioral responses; and it may be responsible for routing information to hypothalamic and preoptic circuits involved in producing appropriate responses. The main intercalated nucleus (m-ICN), one of the GABA-ir intercalated cell groups dispersed throughout the amygdala, is located just lateral to posterior medial amygdala (MeP) and appears to be involved in the processing of chemosensory signals in Me. The role of m-ICN in chemosignal processing may be similar to the regulation of basolateral and central amygdala by adjacent paracapsular intercalated cell groups in the fear conditioning circuit. Previous research using immediate-early gene (IEG) expression has shown a negative correlation between m-ICN and MeP IEG responses to various types of chemosensory signals, however the circuitry has not been directly studied. Current research using whole cell patch-clamp electrophysiology in hamster coronal brain slices has shown a functional connection between m-ICN and MeP, e.g hyperpolarization of MeP neurons after localized field stimulation of m-ICN in a GABAa receptor dependent manner. Further, bath-applied dopamine significantly reduces the amplitude of the m-ICN induced IPSP seen in MeP neurons, with a return to baseline amplitude after DA washout. The effect of DA on m-ICN neurons directly has also been investigated using whole cell electrophysiology. Connections between anterior medial amygdala (MeA) and MeP, and potential connections between MeA and m-ICN are under study in horizontal brain slices including all 3 nuclei.

Disclosures: **L. Biggs:** None. **M. Meredith:** None.

Poster

058. Olfaction: Central Circuits and Neurotransmitters

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Topic: D.01. Chemical Senses

Support: NIH Grant DC011375

Title: Disinhibition mediated by VIP-interneurons in piriform cortex

Authors: A. M. LARGE, N. W. VOGLER, *A.-M. M. OSWALD;
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Abstract: Odor information is processed by the neural circuitry of the piriform cortex. Pyramidal cell (PC) responses to odor information are controlled by GABAergic inhibitory interneurons. Recent studies have characterized interneurons in piriform cortex (1) but little is known about the functions of distinct classes. Interneurons that express vasoactive intestinal peptide (VIP) are a major class of Layer 2 (L2) interneurons that have been proposed to inhibit L2/3 PCs. We used optogenetics to selectively activate VIP interneurons in brain slices from VIP-cre/lox-ChR2 mice while recording inhibitory postsynaptic currents (IPSCs) in VIP(-) interneurons and PCs in anterior piriform cortex (APC). Surprisingly, we found VIP cells inhibited only 20% of recorded PCs (n=2/10) but 75% of interneurons (n=14/19) and all neurons that received inhibition were in L3. Recipient interneurons could be divided into two classes based on inhibitory strength and input resistance (R_n) of the postsynaptic interneuron. Interneurons with low R_n (102 ± 5 MΩ) received significantly less inhibition from VIP cells (18 ± 3 pA) than high R_n interneurons (R_n: 183 ± 37 MΩ, IPSC: 94 ± 19 pA, $p<0.01$). Based on preliminary analysis of morphology, we putatively identified the low R_n interneurons as parvalbumin (PV) interneurons and high R_n neurons as somatostatin (SOM) interneurons. In other cortical areas, VIP interneurons mediate disinhibition of pyramidal cells through disynaptic circuits with PV or SOM interneurons (2,3). We have previously shown that the caudally-biased gradient of inhibition onto pyramidal cells in piriform cortex (4) may be due to disinhibition through are rostrally-biased gradient of inhibition onto interneurons. We investigated the spatial profile of VIP-mediated inhibition onto VIP(-) interneurons in APC slices from our VIP-cre/lox-ChR2 transgenic mice. We find a rostrally-biased gradient of inhibition ($p<0.05$) onto interneurons that is consistent with our previous findings using a VGAT-ChR2 mouse line. Taken together, our results suggest a potential mechanism by which VIP-interneuron to interneuron disinhibitory circuitry generates a rostro-caudal inhibitory gradient and regulates pyramidal cell activity. Ongoing studies are directed toward identifying the functional significance of VIP-mediated disinhibition for olfactory processing. 1) Suzuki and Bekkers (2010) Cerebral Cortex 20:2971-84 2) Pi et al. (2013) Nature 503:521-4 3) Lee et al. (2013) Nature Neuroscience 16:1662-70 4) Luna and Pettit. (2010) Nature Neuroscience 12:533-5

Disclosures: **A.M. Large:** None. **A.M. Oswald:** None. **N.W. Vogler:** None.

Poster

058. Olfaction: Central Circuits and Neurotransmitters

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Title: Characterization of postsynaptic targets of M72 odorant receptor expressing olfactory sensory neurons via local electroporation

Authors: ***A. LIU**[1], M. GERAMITA[1], N. N. URBAN[2];
[1]Dept. of Neurosci., Univ. of Pittsburgh, Pittsburgh, PA; [2]Dept. of Biol. Sci., Carnegie Mellon Univ., Pittsburgh, PA

Abstract: The organization of the olfactory system is distinct from that of other sensory systems that possess more intuitive topographic organization. Odorant molecules bind to receptors located on olfactory sensory neurons (OSNs), which send projections to the olfactory bulb. These projections are organized into glomeruli that each receive input from OSNs that express the same odorant receptor. Both local and global chemotopic patterns of glomerular activation exist, and these suggest that the glomerular circuitry also may show stereotyped spatial organization. Transgenic mice such as the M72-IRES line, in which all OSNs that express the odorant receptor M72 produce GFP, have allowed for detailed study of how axons of OSNs converge onto glomeruli. Alterations of receptor expression and of activity have been shown to modify olfactory sensory neuron targeting to the M72 glomerulus, suggesting that organization of the chemotopic map within the bulb is dependent upon or closely tied to receptor expression. Since M72 OSNs reliably converge onto glomeruli in the same spatial location across mice, we hypothesize that additional elements of the glomerular circuitry also will be stereotyped. Specifically, we aim to further the study of glomerular identity by analyzing the anatomical and morpohological properties of excitatory neurons, mitral and tufted cells, that are associated with the M72 glomerulus. Here, we use *in vivo* electroporation of the fluorescent dye Alexa 594 to label the mitral and tufted cells that project their dendrites to the M72 glomerulus in M72-IRES-GFP mice. Following a craniotomy created over the dorsolateral M72 glomerulus, glass electrodes containing dye were targeted to the M72 glomerulus using two-photon microscopy. Current pulses (750-1000 in number) were applied to perform the localized electroporation. Mice were subsequently perfused and sacrificed. The olfactory bulbs were sectioned, and confocal imaging was performed to visualize the labeled glomerulus and the cells having dendrites that entered the labeled glomerulus. The connected cells were quantified and described based on spatial location relative to the electroporated glomerulus. We demonstrate that local electroporation successfully labels postsynaptic targets of targeted glomeruli. These postsynaptic cells are located within 300 microns of the corresponding glomerulus. This analysis allows for a further description of M72 glomerulus characteristics, including the degree to which this glomerulus is stereotypical across animals, as well as future comparison of experience-dependent changes to the circuitry.

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Poster

058. Olfaction: Central Circuits and Neurotransmitters

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Topic: D.01. Chemical Senses

Support: NIH Grant DC012425

Title: BDNF increases apical spine density of olfactory bulb granule cells *in vivo*

Authors: B. MCDOLE, C. ISGOR, *K. M. GUTHRIE;
Dept Biomed Sci., Florida Atlantic Univ., BOCA RATON, FL

Abstract: The neurotrophin brain-derived neurotrophic factor (BDNF) promotes maturation and plasticity of dendritic spines, both during development and in adulthood. GABAergic granule cells in adult olfactory bulb mediate lateral inhibition of excitatory mitral/tufted cells via dendrodendritic synapses in the external plexiform layer (EPL). Granule cells express low levels of BDNF, and knockout of TrkB receptors in adult-born granule cells that migrate to the bulb from the subventricular zone alters their dendritic maturation. Apical dendritic spine density is reduced by this manipulation in cells that are 3 wks old and establishing synapses in the EPL. Suppression of BDNF/TrkB signaling therefore impairs normal spine formation and/or maintenance in this population of young neurons. To determine if increasing endogenous BDNF availability can promote dendritic spine formation/maintenance in the granule cell population overall, we examined bulbs from adult transgenic mice that chronically over-express BDNF throughout the granule cell layer. Elevations in bulbar BDNF mRNA and protein were quantified by *in situ* hybridization and Western blotting/ELISA, and Golgi-Cox staining was used to visualize olfactory granule cells in their entirety. Computer-based image analysis was performed using Neurolucida (Microbrightfield Inc.) to reconstruct dendritic arbors and quantify dendritic lengths, number of branches, and spine distribution, type and density. Comparison of measures from transgenic and wild-type mice showed that increased BDNF had no significant effect on total dendritic length or complexity of granule cell branching. However apical dendritic spine densities were significantly higher in the transgenic group (mean=0.37 spines/um +/- 0.03, SEM) compared to control mice (0.28 spines/um +/-0.02 SEM, p=0.012, unpaired t-test). This included an increased frequency of mushroom-type "headed" spines (0.25 spines/um +/- 0.02 SEM, vs 0.18 spines/um +/- 0.01 SEM, transgenic vs control respectively; p=0.018, t-test). Sholl analysis demonstrated that significant differences in spine density between genotypes emerged in distal

portions of apical dendrites beginning ~120-140um from the cell soma. These differences likely reflect autocrine/paracrine BDNF effects on granule cells and their synaptic partners, and provide insights into how endogenous BDNF may regulate dendritic morphology to shape bulb circuit organization.

Disclosures: **B. McDole:** None. **K.M. Guthrie:** None. **C. Isgor:** None.

Poster

058. Olfaction: Central Circuits and Neurotransmitters

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Topic: D.01. Chemical Senses

Support: Telethon GGP11116A

Title: Role of oligophrenin 1 in circuit formation in the olfactory bulb of a mouse model of X-linked intellectual disability

Authors: N. REDOLFI[1], E. SAVOIA[1], I. ZAMPARO[2], *C. LODOVICHI[1,3];
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Abstract: Among the X-linked forms of intellectual disability, oligophrenin 1 (OPHN1) encodes a Rho GTPase-activating protein. OPHN1 is thought to regulate several processes including dendritic morphology, axon outgrowth and cellular migration. To assess how this morphological alteration at the cellular level can affect circuit formation and function, we studied neural circuit in the olfactory bulb (OB), namely whether OPHN-1 can regulate the neurogenesis and the morphology of the newly generated granule cells (GCs). We quantified the number of newly generated GCs in the olfactory bulb in control and OPHN1 ko mice, using BrdU as a division marker. We found that 24 hours after BrdU injection (dpi), the number of new GCs in the subventricular zone (SVZ) was similar in control and OPHN1 mice. However at 15 dpi, the number of the new GCs was significantly lower in the OB of OPHN1 ko mice than in controls. At 50 dpi, new GCs were reduced by one-half in experimental animals as in controls. To examine the morphology of the new GCs, neuronal precursor were labelled in the SVZ with lentivirus expressing GFP. The morphology of the GCs was analyzed in the olfactory bulb, 30 days after the SVZ injection. We found that the length and the branching of dendrites was similar in control and experimental animals. However we found that the morphology of the spines was

significantly perturbed in OPHN1 ko mice. Our data indicate that in OPHN1 ko mice both the neurogenesis and the maturational process of the newly generated GCs in the OB are significantly perturbed.

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Poster

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Topic: D.01. Chemical Senses

Support: Harvard University

NARSAD

NIH grant R01 DC011291

Title: Differential projection patterns of principal neurons in the olfactory cortex

Authors: ***C. G. LAU**[1], C. MAZO[1], J. GRIMAUD[1], Y. SHIMA[3], S. NELSON[3], V. N. MURTHY[2];
[1]Mol. and Cell. Biol., Harvard Univ., CAMBRIDGE, MA; [2]Mol. and Cell. Biol., Harvard Univ., Cambridge, MA; [3]Biol., Brandeis Univ., Waltham, MA

Abstract: The anterior piriform cortex (APC) is the largest cortical area receiving direct inputs from the olfactory bulb (OB). Odorants are represented in APC by neural ensembles that are not organized in a topographic manner (horizontally). However, whether odor information is organized in a systematic manner across layers (vertically) is unclear. Recent data suggest that principal neurons in layer 2 are not homogeneous, but can be broadly classified into 2 subtypes: semilunar (SL) and superficial pyramidal (SP) cells. Here we investigated the axonal projections of these 2 subtypes using transgenic mice and viral-expression of fluorescent proteins. We found that CaMK2-Cre mouse can be used to label SP cells when paired with retrograde labeling using a Cre-dependent adeno-associated virus (AAV) from the OB. The axon collaterals of SP cells project to various olfactory cortical regions including the posterior piriform cortex, corroborating our previous results obtained by Retrobead labeling. We used a transgenic mouse line that allowed conditional expression of myristoylated-mCherry in SL cells to visualize their axonal projection. We found that SL cell axons project to similar olfactory cortical regions as SP cells,

but do not send collaterals to the OB. This suggests that feedback information received by the OB will be critically dependent on the signals represented by the SP cells. Ongoing efforts are aimed at mapping all axons emanating from these 2 cell types using CLARITY and whole brain imaging. Understanding the logic of axonal and synaptic connectivity of this first cortical relay has important implications for neural coding of olfactory information.

Disclosures: **C.G. Lau:** None. **C. Mazo:** None. **J. Grimaud:** None. **Y. Shima:** None. **S. Nelson:** None. **V.N. Murthy:** None.

Poster

058. Olfaction: Central Circuits and Neurotransmitters

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 58.11/Y26

Topic: D.01. Chemical Senses

Title: Genome-scale analysis of main and accessory olfactory bulb spatial heterogeneity

Authors: *J. B. CASTRO[1], T. NOTO[2], S. J. TRIPATHY[3];
[1]Psychology, [2]Bates Col., Lewiston, ME; [3]Ctr. for High-Throughput Biol., Univ. of British Columbia, Vancouver, BC, Canada

Abstract: It has long been known that peripheral inputs to the olfactory bulb are labeled, modular, and topographically organized. By contrast, we have comparatively little understanding of whether the bulb's intrinsic circuits show similar modularity. At one extreme, the bulb may consist of only a single canonical circuit providing uniform physiological 'readout' of segregated, parallel inputs. Alternatively, the bulb may be segregated into parallel channels that are physiologically and genetically distinct, allowing for input-specific readout. To investigate these and intermediate possibilities, have clustered spatially-registered genome-scale expression data from the Allen Brain Atlas (ABA; Lein et al, 2007), using non-negative matrix factorization (NMF). Applying NMF to ABA voxels corresponding to the accessory olfactory bulb (AOB), we observed a robust dichotomous clustering that recapitulated the well known division of the AOB into anterior and posterior divisions. Specifically, we found this division to be driven by genes [leading rank ordered genes for posterior and anterior: pcbp3 and fam108b, respectively] and gene categories corresponding to axonal guidance and dopaminergic synaptic transmission functional classes. That is, clustering expression data was sufficient to reveal genetically delineated, spatially contiguous regions of the bulb. In ongoing work, we are using this approach in an exploratory context to identify genetically defined subregions of the bulb that can then be

validated using targeted physiological recordings. In parallel, we are integrating axonal connectivity data from the Allen Mouse Brain Connectivity Atlas (Oh et al, 2014) to assess whether these genomically-defined bulbar subregions are recapitulated via afferent and efferent projections. Moreover, this suite of tools that we are developing can be applied to any brain structure to densely characterize neural heterogeneity. References: Lein et al (2007). Genome-wide atlas of gene expression in the adult mouse brain. Nature 445: 168-76. Oh et al (2014). A mesoscale connectome of the mouse brain. Nature 508: 207-14.

Disclosures: **J.B. Castro:** None. **T. Noto:** None. **S.J. Tripathy:** None.

Poster

058. Olfaction: Central Circuits and Neurotransmitters

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 58.12/Y27

Topic: D.01. Chemical Senses

Support: NIH Grant DC000338

Title: Neocortical markers in the olfactory cortex

Authors: *P. C. BRUNJES, S. K. OSTERBERG;
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Abstract: Olfactory cortical regions such as the anterior olfactory nucleus (AON), piriform cortex (PC) and olfactory tubercle (OT) share many features with the neocortex such as clear lamination, an outer plexiform layer, pyramidal output neurons and similar interneuron types. How do their 2-3 layers compare with the six found in the neocortex? Substantial research has demonstrated that neocortical laminae can be described by a variety of developmental markers (e.g., Molyneaux et al, *Nature Rev, Neurosci.* 8, 2007). The present work examines the expression of some of these markers in three olfactory cortices: the anterior olfactory nucleus (AON), the anterior piriform cortex (APC) an olfactory tubercle (OT). Both coronal and horizontal sections were immunostained and confocal images gathered for 5 markers that span the neocortex. **CART**, which has been reported to label neocortical layer 2 (NL 2), was found to be absent from the AON, but densely labeled cells in Layer 2 of the PC and OT. **CUX1,** reported in NL 2-4, was found in all portions of the pars principalis of the AON, but differentially distributed within the structure. All of layer 2 in pars ventroposterior contained immunoreactive cells, but only the deep portion of layer 2 in pars lateralis and dorsalis. Profiles were very sparse

in pars medialis. In the PC only Layer 3 was labeled and in the OT the marker was absent. **TRB1**, a marker for NL 2356, was found throughout Layer 2 in the AON and in Layers 2 and 3 in the PC. It was absent from the OT. **Foxp2** (NL 6) was observed only in a line of cells just below the lateral olfactory tract in the AON. It was sparse in the PC but labeled Layer 2 in the OT. **Nurr1** (subplate) was absent from all regions. The findings indicate that the expression of these markers varies both within and between olfactory cortices, suggesting a) that understanding the developmental histories of the areas may yield important information about their similarities and differences and b) that the markers may be useful to distinguish between these higher olfactory system areas.

Disclosures: **P.C. Brunjes:** None. **S.K. Osterberg:** None.

Poster

059. Retina: Circuits and Coding

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 59.01/Y28

Topic: D.04. Vision

Support: NIH Grant EY021855

NIH Grant EY023341

Edward Mallinckrodt Jr. Foundation

National Eye Institute Training Grant 5-T32-EY013360-13

Title: Diversity and light adaptation of parallel processing streams in the retina

Authors: *J. PEARSON, D. KERSCHENSTEINER;
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Abstract: The mammalian retina encodes different features of the visual world in the spike trains of approximately 20 retinal ganglion cell (RGC) types. These spike trains are the sole source of visual information to the brain. In spite of their fundamental importance to vision, the diversity of parallel processing streams from the retina is incompletely understood. A number of adaptive mechanisms in photoreceptors as well as downstream circuits enable to retina to support vision over a wide range of light levels. How light adaptation maps onto the diversity of parallel processing streams has not been explored in detail. Here, we perform large scale multielectrode

array recordings from mouse retinas and characterize and classify RGC responses to a broad set of stimuli. This allows us to reliable identify distinct functional RGC types. We then probe how visual encoding of RGC types changes across different light levels. In so doing, we find a surprising degree of diversity among light adaptation of different RGC types including changes in linearity/nonlinearity, spatial integration, temporal tuning and persistence or switch of trigger features.

Disclosures: **J. Pearson:** None. **D. Kerschensteiner:** None.

Poster

059. Retina: Circuits and Coding

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 59.02/Y29

Topic: D.04. Vision

Support: Wellcome Trust studentship 096975/Z/11/Z

EU FP7 project OptoNeuro (249867)

Title: Blockade of pathological ganglion cell hyperactivity improves optogenetically evoked responses in a mouse model of retinitis pigmentosa

Authors: ***J. M. BARRETT**[1], L. BARCA[1], P. DEGENAAR[2], E. SERNAGOR[1];
[1]Inst. of Neurosci., [2]Sch. of Electrical & Electronic Engin., Newcastle Univ., Newcastle Upon Tyne, United Kingdom

Abstract: Retinitis pigmentosa (RP) is a hereditary progressive retinal dystrophy that causes visual impairment and eventually blindness. Retinal prosthesis is the primary methodology providing hope for vision restoration. Presently, electrical prostheses restore only crude vision, but newer approaches based on optogenetics - genetic expression of light-sensitive proteins in neurons - may potentially offer higher-quality vision. However, alongside photoreceptor loss, retinal degeneration also involves significant inner retinal remodelling that leads to spontaneous, rhythmic bursting activity in retinal ganglion cells (RGCs). This source of biological noise is often overlooked in retinal prosthetic research. Recently, Toychiev et al (2013) were able to improve the signal-to-noise ratio of photoreceptor-mediated full-field stimulation and electrically-evoked responses by dampening this activity. We sought to extend these results to optogenetic stimulation and more sophisticated stimuli than full-field illumination. We crossed a

transgenic mouse line expressing the light-sensitive cation channel channelrhodopsin2 (ChR2) in RGCs with the rd1 model of retinal degeneration, creating a strain of mice with retinal degeneration and ChR2-expressing RGCs (ChR2rd1 mice). We recorded RGC activity from ex-vivo ChR2rd1 retinas using 60-channel ITO multielectrode arrays, while stimulating ChR2 using a 256-pixel Gallium Nitride microLED (μLED) array. The stimuli used included full-field flashes, bars moving in eight directions, and spots and annuli of different radii and thicknesses. We blocked spontaneous oscillations and bursting using either the gap junction blocker meclofenamic acid (MFA) or the Kv7 potassium channel opener flupirtine. Before application of the drugs, most recorded RGCs showed very strong bursting that made it difficult to discern responses to external stimuli. Both MFA (80uM) and flupirtine (100uM) greatly decreased the spontaneous firing rate, thus increasing the signal to noise ratio of responses to optogenetic stimulation and increasing the mutual information between the stimulus and the μLED-evoked responses. Our results show that spontaneous hyperactivity in the rd1 retina decreases the signal-to-noise ratio and information content of prosthetically-evoked responses and that these can be improved by reducing this activity. This may partially explain the poor quality of vision returned by current prosthetics and suggests that future retinal prosthetic research should explore methods of reducing or compensating for this pathological hyperactivity.

Disclosures: **J.M. Barrett:** None. **L. Barca:** None. **P. Degenaar:** Other; Spin-out company, OptoNeuro, with interest in retinal prosthesis. **E. Sernagor:** None.

Poster

059. Retina: Circuits and Coding

Location: Halls A-C

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Program#/Poster#: 59.03/Y30

Topic: D.04. Vision

Support: NIH Grant R21 EY022146

Title: Correlations between interneuron and output neuron populations of the retina

Authors: ***M. D. MENZ**, S. BACCUS;
Neurobio., Stanford Univ., STANFORD, CA

Abstract: Understanding the function of diverse neurons within a neural circuit has benefited from methods that record simultaneously from a neural population. In the retina, amacrine cells are inhibitory interneurons that shape the output of the retina as conveyed by retinal ganglion

cells. Both populations are diverse, but many of their specific functions are unknown. We recorded the visual responses from a population of amacrine cells optically using second harmonic generation (SHG) imaging, while simultaneously recording electrically from a population of retinal ganglion cells with a multielectrode array (MEA). From those recordings we analyzed correlations to infer the effect interneurons have on output cells, the location of noise sources, and connectivity. In the isolated salamander retina, we recorded amacrine and ganglion cell responses using a custom two-photon microscope that imaged the retina through a transparent MEA. We recorded the SHG signal generated by FM 4-64 applied to the bath from somas in the amacrine cell layer proximal to the inner plexiform layer. Ganglion cell spiking responses were recorded using an MEA. To measure correlations between the two populations, the visual stimulus was a moving dark bar against a light background. Population results were recorded from 122 ganglion cells, 365 amacrine cells, and nearly 5000 cell pairs were pooled across four retinas. By correlating many cell pairs for specific amacrine and ganglion cell types, we computed the average spatiotemporal pattern of activity of an amacrine cell population relative to the spike of a ganglion cell. These space-time plots show a wave of amacrine activity relative to ganglion cells as the bar moves across the retina, with different patterns for different amacrine cell types. Results indicated that in response to a moving edge, a significant fraction of the amacrine population was activated in advance of the ganglion cell, likely delivering retrograde inhibition. We further analyzed the noise correlations between amacrine and ganglion cell pairs by analyzing responses recorded on the same trial and on different trials. By averaging many cell pairs, we saw a strong average effect that nearby cell pairs with positive stimulus-driven correlations had negative noise correlations. This result was inconsistent with the dominant source of noise being in photoreceptors as has been previously suggested, and instead implies that for these cell pairs and stimulus, the dominant source of noise is in the inner retina. These results will be useful in understanding the contribution of specific amacrine populations, and in constraining models of how interneurons shape retinal output.

Disclosures: **M.D. Menz:** None. **S. Baccus:** None.

Poster

059. Retina: Circuits and Coding

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 59.04/Y31

Topic: D.04. Vision

Support: NSF GRFP

Title: A successful model for visual responses of alpha retinal ganglion cells to natural stimuli

Authors: *B. KRIEGER[1,2], M. QIAO[2], X. DUAN[2], J. R. SANES[2], M. MEISTER[1];
[1]Biol., Caltech, Pasadena, CA; [2]Harvard Univ., Cambridge, MA

Abstract: The retina sends many parallel channels of visual information to the brain through the axons of >20 retinal ganglion cell (RGC) populations. The purpose of these distinct circuits for vision remains an open question. Recent results suggest that each cell type responds selectively to a specific feature of the visual scene. These conclusions are derived primarily from experiments with artificial visual stimuli. It is unknown whether the insights gathered under such conditions extend to the natural environment in which the retina evolved. One can address this question by building a mathematical model of RGC responses to artificial stimuli and then testing how well that same model performs with natural visual input. For several RGC types this exercise has failed dramatically, indicating an imperfect understanding of their neural code. Here we focus on alpha RGCs, which possess large cell bodies, stout axons, and wide receptive fields. Three subtypes have been defined based on their responses to light steps: On, Off-sustained, and Off-transient. In the mouse, these alpha cells provide most of the input to layer 4 of the visual cortex and thus constitute an important pathway for cortical processing. The Off-transient type has been proposed as a looming detector involved in defense from predators. We targeted these RGCs for recording using a transgenic mouse line in which GFP is expressed in all three alpha subtypes. Using both artificial stimuli and natural movies, we measured the visual responses of the mouse alpha cells. We then constructed a simple cascade-style model to link the stimulus to the firing rate. The model includes an antagonistic center and surround, each with partial rectification. The center is further divided into nonlinear subunits. This model accounted very well for the visual responses of all three alpha RGC subtypes, correctly predicting about 70% of the variance in firing. The same model worked for both artificial stimuli (e.g. random flicker) and natural stimuli (mouse-cam and simulated-mouse movies). Across conditions there were only minor adjustments to parameters, as expected from adaptation processes. Under natural movie stimulation, the antagonistic surround of the receptive field played a relatively small role whereas the spatial subunit processing within the receptive field center made a larger contribution. This successful account of alpha cell function will be valuable as a retina model for understanding cortical vision in the behaving mouse.

Disclosures: **B. Krieger:** None. **M. Qiao:** None. **X. Duan:** None. **J.R. Sanes:** None. **M. Meister:** None.

Poster

059. Retina: Circuits and Coding

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Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 59.05/Y32

Topic: D.04. Vision

Support: NEI Grant EY05725

Title: Exclusive cholinergic excitation of on-off ganglion cells in rat retina

Authors: S. SETHURAMANUJAM[1], *M. M. SLAUGHTER[2];
[1]Neurosci., [2]SUNY Buffalo Sch. Med., BUFFALO, NY

Abstract: The objective was to ascertain the role of cholinergic synaptic input to retinal ganglion cells (RGCs).. Acetylcholine is released only by starburst amacrine cells, which are stratify in two narrow bands in the inner plexiform layer. Most RGCs express functional nicotinic acetylcholine receptors (nAChRs), but their role in light-evoked spike activity is unclear. Experiments were performed in the whole-mount mature rat retina. RGCs were classified based on responses to a spot of light (500nm, 200µm diameter). We recorded from ON sustained (ONs), ON-OFF transient and OFF sustained (OFFs) cells. 100µM hexamethonium chloride, a nAChR antagonist, did not significantly affect the light-evoked spike activity of ONs and OFFs cells. However, in a sub-population of ON-OFF cells, hexamethonium completely blocked both the ON and OFF light-evoked spike activity. Similar effects were seen using 100µM D-tubocurarine. Whole cell recordings revealed that hexamethonium drastically reduced the voltage response and excitatory synaptic currents in both the ON and OFF responses. These results indicate that in a sub-population of ON-OFF cells are driven exclusively or predominantly by acetylcholine excitation and lack direct bipolar cell input under these stimulus conditions. The lack of glutamate excitation was unexpected. We tested if this was due to pre-synaptic inhibition. Picrotoxin, TPMPA, and strychnine were used to block GABAA, GABAC and glycine receptors in the retina. With inhibition blocked, glutamate synaptic currents dramatically increased, indicating that pre-synaptic inhibition can suppress bipolar cell input to these cells, regulating the acetylcholine/glutamate excitation ratio. It has been shown that mGluR2 receptors are co-expressed with starburst amacrine cells. DCG-IV (10µM, a mGluR2 agonist) increased the light evoked spike activity of hexamethonium sensitive RGCs. Interestingly, light evoked spike activity in DCG-IV was not completely blocked by hexamethonium indicating that DCG-IV increased the direct, bipolar cell glutamate input to these RGCs. In summary, we find that the glutamate excitation to some RGCs can be superseded by acetylcholine and can be modulated by pre-synaptic inhibition.

Disclosures: **S. Sethuramanujam:** None. **M.M. Slaughter:** None.

Poster

059. Retina: Circuits and Coding

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Program#/Poster#: 59.06/Z1

Topic: D.04. Vision

Support: FP7-ICT-2011-9, RENVISION, Grant 600847

Title: Large-Scale recording of light-evoked responses in the retinal ganglion cell layer of the explanted retina: A new HD experimental platform

Authors: *S. DI MARCO[1], A. MACCIONE[1], G. HILGEN[2], S. PIRMORADIAN[3], T. NIEUS[1], M. HENNIG[3], E. SERNAGOR[2], L. BERDONDINI[1];
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Abstract: Aims: We introduce an innovative setup to simultaneously evoke and record *in-vitro* extracellular visual responses from Retinal Ganglion Cells (RGCs) with micrometer/cellular resolution from a complete whole-mount mouse retina. We show the capabilities of this setup by investigating the effects of the extra-classical receptive field of RGCs. The applicability of this setup ranges from the study of a complete biological system, to optogenetic experiments that may benefit from the precise control of a light stimulus (structured or not) and from the ability to simultaneously record spikes from thousands of single cells. **Methods:** Two main parts constitute the setup: the optical stimulator and the recording system. The recording system is a customized version of the commercially available Biocam (www.3brain.com) adapted to fit the optical stimulator geometrical constraints. Briefly, the system is composed by a CMOS 4096-electrode array chip (electrode size 21 μm x 21 μm, inter-electrode separation 21 μm), mounted on hardware for real-time filtering and simultaneous recording of all the electrodes at a sampling rate of about 7KHz/channel. With an active area of 7.12 mm^2 the system allows pan retinal recording from mouse explanted retina. Since the chip is not transparent, visual stimuli are delivered from the top of the chip by means of a DLP-projector, with 3-axis of free-movement, aligned with a single-convex lens that focuses the image on the biological sample. A beam-splitter, positioned in the optic pathway, direct the reflected light to a camera allowing to precisely align the stimulus with the recording area. To precisely control and synchronize the timing of the photo-stimulation with the data acquisition, custom hardware and software tools

were developed. These also allow generation of complex photo-stimulation protocols, and to record the timestamp of the photo-stimulations with millisecond precision. **Results:** Here we show that a visual stimulus confined to a small region of the retina does affect the spontaneous activity in parts of the retina not exposed to the stimulus. In particular, spontaneous activity is reduced in the area not exposed to the stimulus for during stimulus presentation, and increases sharply and transiently at stimulus offset. **Conclusions:** Our integrated platform enables successful recording of a large number of RGCs in the mouse retina during photo stimulation. Our results show that such dense and large-scale recordings can reveal spatial processing mechanisms in the retina that are difficult to characterize using conventional technology.

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Poster

059. Retina: Circuits and Coding

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 59.07/Z2

Topic: D.04. Vision

Support: RENVISION Grant FP7

Title: Characterising retinal population response with high density multielectrode arrays

Authors: *S. PIRMORADIAN[1], G. HILGEN[2], O. MUTHMANN[1,3], A. MACCIONE[4], U. BHALLA[3], L. BERDONDINI[4], E. SERNAGOR[2], M. HENNIG[1];
[1]Inst. for Adaptive and Neural Computation, Univ. of Edinburgh, Edinburgh, United Kingdom; [2]Inst. of Neuroscience, Med. Sci., Univ. of Newcastle upon Tyne, Newcastle upon Tyne, United Kingdom; [3]Natl. Ctr. for Biol. Sci., Tata Inst. of Fundamental Res., Bangalore, India; [4]Neurosci. and Brain Technologies, Italian Inst. of Technol., Genova, Italy

Abstract: Retinal ganglion cells are categorized according to a range of physiological criteria. So far, however, this classification largely relied on pooling data from multiple preparations due to limitations in the number of neurons that could be simultaneously recorded. Here, we present a new, efficient method for localizing ganglion cells recorded in the adult mouse retina during light stimulation with high density multielectrode arrays of 4096 channels. Recording channels were arranged on a 64x64 lattice and separated by 42 μm, enabling simultaneous sampling of activity at near cellular resolution of >800 units on a large patch of the retina. The method

exploited the fact that signals from the same cell were detectable on multiple neighboring channels. By averaging the signals of neighboring channels, we improved spike detection, which yielded more reliable and robust detection than is possible in single channels. The relative contributions of the different channels to each spike are then used to localize the signal source. Finally, spikes are iteratively clustered together by spatial proximity, thus identifying the activity of single units. A comparison with standard spike sorting techniques showed this method could substantially improve spike detection and correctly cluster spikes that would otherwise be assigned to different sources. We characterized the population response of single units, identified by the method, in a full field experiment (a sequence of dark/light stimuli) for comparison with previous work (Carcieri et al., J Neurophysiol, 2003). We observed that response latencies were broadly distributed from 50-950 ms, with faster responses in OFF cells. Distribution of the ratio of ON versus OFF responses were broad, with higher abundance of ON cells compared to OFF cells. The spatial distribution of ON and OFF cells in the dorsal retina was random. In summary, our results are a first step towards a comprehensive and statistically sound characterization of the activity of a large population of neurons without variability caused by combining data from multiple preparations.

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Poster

059. Retina: Circuits and Coding

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Program#/Poster#: 59.08/Z3

Topic: D.04. Vision

Support: MEXT-Supported Program for the Strategic Research Foundation at Private Universities (2011–2015 S1101013)

KAKENHI (22730586)

Title: Expansion-selective ganglion cells in frog retina

Authors: *H. ISHIKANE[1,2,3], M. MATSUZAKI[1];
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Abstract: Mice engage in protective behaviors in response to a looming stimulus. It has been reported that a subtype of ganglion cells (PV-5 cells) in the mouse retina exhibits approach sensitivity and that the involvement of PV-5 cells may be necessary for triggering these protective behaviors. Similarly, frogs exhibit escape behavior, a form of protective behavior, in response to a looming stimulus. We have already demonstrated that synchronized oscillatory spikes among class-4 neurons (dimming detectors) encode visual information that is necessary to induce escape behavior in frogs. However, it is still unclear what kinds of retinal outputs are sufficient for eliciting escape behavior. To elucidate this issue, we recorded spikes that occurred in frog retinal ganglion cells in response to a looming stimulus (a dark expanding disc). The frog's behaviors in response to this visual stimulus were also recorded using a video camera under infrared illumination. When a dark expanding disc was presented to the retina, all subtypes of ganglion cells generated spikes. Synchronous oscillatory spikes were observed among class-4 neurons. Then, we presented a dark ring that gradually filled in toward the center to become a solid disk. When this stimulus was presented, synchronized oscillatory spikes were observed among class-4 neurons. However, frogs exhibited no escape behavior in response to this stimulus. This result suggested that encoding of expansion is essential for eliciting escape behaviors. Thus, expansion selectivity of all subtypes of retinal ganglion cells was investigated. When both a dark expanding square and a dark square that was gradually filled in toward the center were presented to frog retinas, only class-3 neurons showed expansion selectivity. These results suggest that expansion-selective class-3 neurons might encode information that is necessary for eliciting escape behaviors in frogs.

Disclosures: **H. Ishikane:** None. **M. Matsuzaki:** None.

Poster

059. Retina: Circuits and Coding

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Topic: D.04. Vision

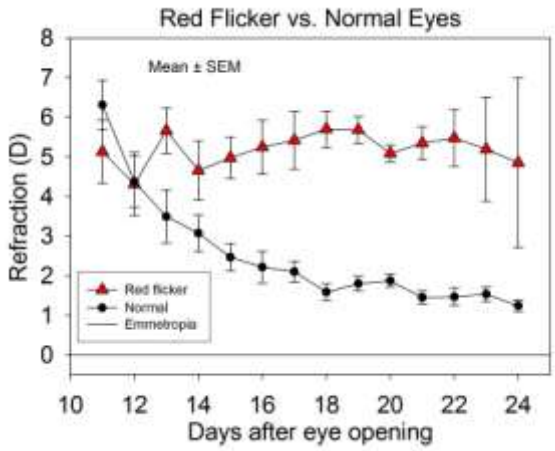
Support: NIH Grant EY005922

NIH Grant EY003039 (CORE)

Title: Temporally modulated long-wavelength light radically slows eye growth in young tree shrews

Authors: *T. J. GAWNE, J. T. SIEGWART, A. H. WARD, T. T. NORTON;
Dept Vision Sci., Univ. Alabama Birmingham, Birmingham, AL

Abstract: A post-natal eye will normally adjust its growth to achieve sharp focus. The retina can detect the sign of the defocus (hyperopia-eye too short, myopia-eye too long). It could use longitudinal chromatic aberration and compare the relative sharpness of the long and short wavelength images as a guide for whether it should increase or decrease its elongation rate to achieve well-focused retinal images. However, the sparseness of short-wavelength photoreceptors would seem to make it impossible to tell if the short-wavelength image is in focus. We hypothesize that the retina uses the temporal abruptness of changes in light at different wavelengths as a proxy for image focus: when an image is in sharp focus a photoreceptor will experience rapid changes in illuminance as the animal moves around the world and produce sharp transients in bipolar and amacrine cell membrane potentials. An out-of-focus image at a specific wavelength would reduce the illuminance transients and neural responses. Abrupt temporal illuminance transients at long wavelengths should cause the retina to slow the rate axial elongation. We tested this hypothesis on developing tree shrews (dichromatic mammals closely related to primates) at an age when the eyes are elongating rapidly and refractions are decreasing from hyperopia toward emmetropia. From day 11 after eye opening to day 24, tree shrews (n=3) were housed on a 14 h ON/10 h OFF light cycle in long-wavelength light (620 nm peak, 10 nm width at half height, mean illuminance at cage floor 325 lux) that was pseudo-randomly modulated with rapid, sharp increases and decreases of intensity. This illuminance regime radically slowed the refractive decrease and axial elongation compared to animals in standard fluorescent colony lighting (see figure - however, one animal showed signs that this effect was ebbing near the end of the period, suggesting some form of habituation). We speculate that the retina uses abrupt long-wavelength transients in the absence of abrupt short-wavelength transients as a signal to a postnatal eye that it should stop elongating.



Disclosures: **T.J. Gawne:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); IP rights are being

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Poster

059. Retina: Circuits and Coding

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Title: Signal recombination in the inner retina

Authors: *N.-W. TIEN[1], J. DEMAS[2], D. KERSCHENSTEINER[1];
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Abstract: In the mammalian retina, bipolar cells (BCs) relay signals from photoreceptors to ganglion cells (GCs), the output neurons of the eye. In cone-mediated vision, signals first diverge as each photoreceptor provides input to 10 types of BCs, and then are recombined in the inner retina to give rise to distinct spike trains of approximately 20 types of GCs. While it is known that BC types differ in their responses to photoreceptor input, how the output of individual BC types shapes spikes trains across GC types remains incompletely understood. This is in part, because in addition to providing direct excitation to GCs, BCs also drive specific sets of interneurons (amacrine, ACs) which in turn have varied influences on BCs, other ACs and GCs. To probe the logic and mechanisms of signal recombination in the inner retina, we generated mice in which a single BC type is removed. We then combine multielectrode array and patch clamp recordings to determine the influence of this processing stream from the outer retina on the spike trains of different GC types, and characterize the underlying mechanisms (direct excitation, indirect inhibition and indirect excitation). In addition to providing insights into visual processing in the retina, our findings may be relevant to other circuits in the nervous system that share architectural and computational features.

Disclosures: **N. Tien:** None. **D. Kerschensteiner:** None. **J. Demas:** None.

Poster

059. Retina: Circuits and Coding

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 59.11/Z6

Topic: D.04. Vision

Support: NIH Grant P1316189

Title: Roles of retinal circuits in innate visual behaviors of mice

Authors: *M. YILMAZ[1,2], X. DUAN[2], J. SANES[2], M. MEISTER[1];
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Abstract: The mouse retina has >20 different types of retinal ganglion cell (RGC), each of which reports a specific feature of the visual scene. Despite anatomical and physiological characterization of many of these neural circuits, their behavioral roles are mostly unknown. A few RGC types are dedicated to specific visual behaviors, for example pupil constriction and light entrainment of circadian rhythms [1]. Here we explore whether distinct RGC types can also be linked to more elaborate visual behaviors, such as predator defense, cliff avoidance, and the optokinetic response. All these behaviors rely on the interpretation of image motion. We ablated two different retinal cell types, alpha ganglion cells and starburst amacrine cells, using genetically targeted expression of the diphtheria toxin receptor followed by intraocular injection of diphtheria toxin. Toxin was injected in adulthood to avoid developmental effects. Alpha cells constitute ~6% of mouse retinal ganglion cells, encompassing 3 recognized cell types. They have the largest cell bodies and receptive fields in the retina, respond sensitively to fast motion, but are not selective for motion direction. Starburst amacrine cells provide asymmetric inhibition to 7 recognized types of direction-selective (DS) cells in the retina, which altogether make up ~20% of mouse RGCs. Ablation of the starburst amacrine cells has been shown to abolish the direction-selectivity of DS cells as well as the optokinetic nystagmus [2]. We show that ablation of alpha RGCs selectively impairs the mouse's defensive responses to looming objects (freezing and fleeing), whereas it leaves the optokinetic reflex and pupil construction largely intact. On the other hand, ablation of starburst amacrine cells completely abolishes the optokinetic reflex, while leaving looming avoidance and pupil constriction intact. This double dissociation suggests that the targeted retinal circuits indeed contribute to distinct visual behaviors, supporting the neuroethological hypothesis that each circuit in the retina evolved in response to a specific behavioral need. 1.Chen S.K., Badea T.C., and Hattar S. (2011). Photoentrainment and pupillary

light reflex are mediated by distinct populations of ipRGCs. Nature. 476, 92-95. 2.Yoshida K., Watanabe D., Ishikane H., Tachibana M., Pastan I., and Nakanishi S. (2001). A key role of starburst amacrine cells in originating retinal directional selectivity and optokinetic eye movement. Neuron. 30, 771-780.

Disclosures: **M. Yilmaz:** None. **M. Meister:** None. **X. Duan:** None. **J. Sanes:** None.

Poster

059. Retina: Circuits and Coding

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 59.12/Z7

Topic: D.04. Vision

Support: Foundation Fighting Blindness

French State program "Investissements d'Avenir"

Title: Targeting channelrhodopsin-2 to ON-bipolar cells with vitreally administered AAV restores ON and OFF visual responses in blind mice

Authors: ***E. MACÉ**[1], R. CAPLETTE[1], O. MARRE[1], A. SENGUPTA[1], A. CHAFFIOL[1], P. BARBE[1], M. DESROSIERS[1], E. BAMBERG[2], B. ROSKA[3], J.-A. SAHEL[1], S. PICAUD[1], J. DUEBEL[1], D. DALKARA[1];
[1]Vision Inst., Paris, France; [2]Max Planck Inst. of Biophysics, Frankfurt am Main, Germany; [3]Friedrich Miescher Inst. for Biomed. Res., Basel, Switzerland

Abstract: Inherited retinal degenerative diseases are a clinically promising focus of adeno-associated virus (AAV)-mediated gene therapy. These diseases arise from degeneration of photoreceptors eventually leading to blindness. Optogenetic reactivation of surviving retinal neurons is a promising strategy to restore vision. However, targeting ganglion cells neglects the visual processing done by the inner retina. Targeting upstream neurons is more suited for preserving retinal processing but up to now could only be addressed by a subretinal injection, which may damage the fragile degenerated retina of patients. Here we used an engineered AAV variant that can target the bipolar cells of the retina after safe administration into the eye's easily accessible vitreous. This capsid variant called AAV2-7m8, in combination with a cell-type specific promoter allowed strong and specific expression of channelrhodopsin (ChR2/H134R) in ON bipolar cells. Our results show that, after viral delivery in blind mice (rd1), different types of

ON-bipolar cells express efficiently and specifically the opsin. Functional efficacy of our transfection was controlled with multielectrode array (MEA) recordings. On the MEA, ON responses were recorded when stimulating treated retinas with blue light and the responses withstand pharmacological block at the cone to ON-bipolar pathway. OFF responses were also observed and were suppressed by blocking inhibitory responses of amacrine cells. To assess whether the new retinal photo-responses are transmitted to higher brain centers, we performed extracellular recordings in the visual cortex. Both ON and OFF responses were restored in treated animals as seen on VEPs and, for the first time, on multiunit spiking activity. The shorter latency of ON responses was consistent with the bypass of photoreceptors. Finally, treated mice also retrieved light induced locomotory behaviour. Our results support the clinical relevance of a minimally invasive AAV-mediated optogenetic therapy for visual restoration. By targeting inner neurons, both ON and OFF pathways are reactivated; which is a promising avenue for restoring a vision as close to natural vision as possible.

Disclosures: **E. Macé:** None. **R. Caplette:** None. **O. Marre:** None. **A. Sengupta:** None. **A. Chaffiol:** None. **P. Barbe:** None. **M. Desrosiers:** None. **E. Bamberg:** None. **B. Roska:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); GenSights Biologics. **J. Sahel:** F. Consulting Fees (e.g., advisory boards); GenSight Biologics, Pixium Vision, Sanofi Fovea. **S. Picaud:** F. Consulting Fees (e.g., advisory boards); GenSight Biologics, Pixium Vision. **J. Duebel:** None. **D. Dalkara:** F. Consulting Fees (e.g., advisory boards); GenSight Biologics.

Poster

060. Striate Cortex: Neural Coding

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 60.01/Z8

Topic: D.04. Vision

Support: Paul G. Allen

Title: Electrophysiological characterization of cortical neuron subtypes in mouse visual cortex

Authors: *J. BERG, T. JARSKY, S. SORENSEN, C. ANASTASSIOU, A. OLDRE, A. BERNARD, C. KOCH, H. ZENG;
Allen Inst. For Brain Sci., Seattle, WA

Abstract: The Allen Institute has initiated a 10-year program to take a systematic and comprehensive set of experimental and computational approaches to understand the circuitry involved in the mouse visual system. A foundational aspect of this project is to create a comprehensive classification scheme for neuronal classes within the mouse visual cortex and lateral geniculate nucleus, dorsal part (LGd). Part of this classification scheme is to understand how diverse neurons integrate synaptic input to generate output signals, a function of their intrinsic electrical properties. To characterize these cell classes electrophysiologically we will utilize the Institute's recently established *in vitro* slice electrophysiology platform to systematically record the intrinsic electrophysiological properties from individual neurons. For our initial data set, we will target layer-specific projection neuron Cre mouse lines as well as interneurons that span lamina. We will use a robust 'core' set of electrophysiology protocols designed to efficiently characterize the most salient electrophysiology properties of each neuron. This stimulus set focuses on: 1) subthreshold properties, including the linear range of a neuron's voltage response to current injection, 2) action potential threshold and kinetics, given both square and ramp pulse current injections, 3) spiking due to suprathreshold stimuli and the frequency to injected current relationship, and 4) subthreshold and suprathreshold response to derivative rich noisy stimuli. We will use custom software to extract relevant electrophysiological features and use principal component analysis to identify different classes of neurons. Together with complementary projects within the Allen Institute, these data will help us to understand the diversity of electrophysiological cell types within the cortex.

Disclosures: J. Berg: None. **T. Jarsky:** None. **S. Sorensen:** None. **C. Anastassiou:** None. **A. Oldre:** None. **A. Bernard:** None. **C. Koch:** None. **H. Zeng:** None.

Poster

060. Striate Cortex: Neural Coding

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 60.02/Z9

Topic: D.04. Vision

Support: Allen Institute for Brain Science

Title: Dynamics of neural activity in mouse cortex during visual behavior

Authors: *D. R. OLLERENSHAW, P. A. GROBLEWSKI, M. E. GARRETT, J. ZHUANG, J. WATERS, S. R. OLSEN;
Allen Inst. For Brain Sci., Seattle, WA

Abstract: Behavior and cognition are the result of dynamic neural activity in the neocortex and interconnected subcortical structures. We are using the mouse visual system as a model to understand the mechanistic basis of these processes. We train mice to perform visual behavioral tasks using the linear foraging paradigm, and monitor/manipulate neural activity in the visual cortex during the task. In the foraging task, a head-fixed mouse runs on a circular treadmill, the motion of which is coupled to the movement of stimuli across the visual display (Olsen & Scanziani, 2013). The mouse is trained to approach and stop at target stimuli, whereupon it receives a water reward. The foraging task has features that simulate environmental interactions including optic flow, object approach behavior, and decisions to stay or go. Mice are capable of completing hundreds of trials per day, allowing rapid estimation of psychophysical thresholds. Using this task we have characterized the psychophysics of visual stimulus detection for a variety of stimulus parameters including grating contrast, spatial frequency, size, and location in the visual field. In addition, we have assessed the impact of distractor stimuli on target detection thresholds. In another task, we characterized the mouse's ability to perform orientation discrimination at multiple spatial locations in the visual field. In order to characterize the neural activity that underlies these behaviors, we monitor the activity of neuronal populations in the primary visual cortex and higher visual areas using wide-field fluorescence imaging and 2-photon imaging of genetically-encoded calcium sensors including GCaMP6. Finally, we use optogenetics to perturb activity in the visual cortex and find that visual behavior is impaired by this manipulation. Together, these techniques provide a powerful experimental system in which behavior can be mechanistically linked with the underlying neural code. Reference: Olsen & Scanziani, 2013, Society for Neuroscience

Disclosures: **D.R. Ollerenshaw:** None. **P.A. Groblewski:** None. **M.E. Garrett:** None. **J. Zhuang:** None. **J. Waters:** None. **S.R. Olsen:** None.

Poster

060. Striate Cortex: Neural Coding

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 60.03/Z10

Topic: D.04. Vision

Support: NINDS R01 NS074015

HFSP

Title: On spike detection and the development of quantitative measures for spike clustering using "ground truth" data: A computational and slice electrophysiology study

Authors: C. MITELUT[1], S. L. GRATIY[2], S. DURAND[2], K. MIZUSEKI[2], K. GODFREY[2], C. LEE[2], T. BLANCHE[2], N. SWINDALE[1], C. REID[2], M. HAWRYLYCZ[2], C. KOCH[2], *C. ANASTASSIOU[2];
[1]Univ. of British Columbia, Vancouver, BC, Canada; [2]Allen Inst. for Brain Sci., Seattle, WA

Abstract: The quantity and quality of neural recordings is the cornerstone for many experimental investigations of the brain. Specifically, extracellular spiking in the living brain is typically measured using metal electrodes either in the form of single or groups of wires (tetrodes, etc.) or, alternatively, through metal contacts on silicon probes. The basic algorithmic steps after data collection are spike detection, extraction of distinctive features from the extracellular spike waveform, and clustering of the spikes by these features. With regards to this process, two important questions arise: (1) what is the optimal electrode size, configuration and geometry to allow for maximal detection of proximal spiking given a particular cell type of specific morphological and electrical features and (2) based on the detected signals, what are the quantitative measures to compare the "goodness" of spike sorting across different methods [Hill et al, JNeurosci, 2011]? To address both questions we use morphologically and functionally detailed compartmental models of cortical and thalamic neurons to simulate extracellular spike waveforms [Gold et al, J Comput Neurosci, 2006]. We systematically characterize the impact of cell type, extracellular medium as well as electrode size and configuration to the yield and quality of extracellular measurements. In a subsequent step, we test several clustering algorithms including: Klustakwik (EM), K-means, SIGAC, template matching pursuit, Superparamagnetic and bagged clustering, using in silico benchmarks to generate quantitative measures of goodness among such algorithms. Finally, we perform a blind analysis of several spike sorting methods using a unique *in vitro* data set where intracellular and extracellular spikes were simultaneously recorded from rodent cortical slice in a cell type-specific manner [Anastassiou et al, SfN, 2013].

Disclosures: **C. Mitelut:** None. **S.L. Gratiy:** None. **S. Durand:** None. **K. Mizuseki:** None. **K. Godfrey:** None. **C. Lee:** None. **T. Blanche:** None. **N. Swindale:** None. **C. Reid:** None. **M. Hawrylycz:** None. **C. Koch:** None. **C. Anastassiou:** None.

Poster

060. Striate Cortex: Neural Coding

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 60.04/Z11

Topic: D.04. Vision

Title: Building a biophysically detailed computational model of the layer 4 of mouse primary visual cortex

Authors: *A. ARKHIPOV[1], J. BERG[1], N. MAÇARICO DA COSTA[1], S. DURAND[1], D. FENG[1], T. P. FLISS[1], K. B. GODFREY[1], M. L. HINES[2], L. LI[1], A. OLDRE[1], S. R. OLSEN[1], S. A. SORENSEN[1], Z. ZHOU[1], C. A. ANASTASSIOU[1], A. S. SHAI[1], A. BERNARD[1], C. DANG[1], L. NG[1], H. PENG[1], J. W. PHILLIPS[1], R. C. REID[1], H. ZENG[1], S. MIHALAS[1], M. J. HAWRYLYCZ[1], C. KOCH[1];
[1]Allen Inst. For Brain Sci., Seattle, WA; [2]Yale Univ., New Haven, CT

Abstract: The Mindscope project at the Allen Institute for Brain Science aims to elucidate mechanisms underlying cortical function in the mouse, with the primary focus on the visual system. This involves concerted efforts of a large team of scientists employing experimental, computational, and theoretical techniques to characterize cell types, connectivity, and neuronal activity in behaving animals. An integral part of these efforts is the construction of a detailed biophysical model of the cortical tissue. Here we present preliminary progress in building such a model, with current emphasis on the first step in transformation of sensory information by the cortex: namely, on the transformation of visual signals by layer 4 of primary visual cortex (area V1). The present biophysical model accounts for a significant portion of layer 4 in V1 and includes ~10,000 neurons (80% excitatory and 20% inhibitory), each consisting of hundreds of compartments representing morphologically realistic dendritic arbors. The current generation of the model relies on preliminary, but broad experimental characterization of layer 4, including both *in vitro* and *in vivo* data. Using this model, we have performed simulations of spiking behavior of layer 4 neurons in response to putative visual stimuli. Simulation results are compared to experimental data from *in vivo* patch-clamp intracellular recordings as well as extracellular microelectrode recordings. For example, the magnitude of the excitatory postsynaptic currents in layer 4 neurons and levels of cortical amplification are studied. Potential effects of the various connectivity modes, including preferential connectivity between co-tuned cells, on the neuronal dynamics are investigated. We also extensively characterize effects of optogenetic manipulations of neuronal populations within layer 4 and investigate the efficiency of such manipulations for a number of scenarios.

Disclosures: **A. Arkhipov:** None. **J. Berg:** None. **N. Maçarico da Costa:** None. **S. Durand:** None. **D. Feng:** None. **T.P. Fliss:** None. **K.B. Godfrey:** None. **M.L. Hines:** None. **L. Li:** None. **A. Oldre:** None. **S.R. Olsen:** None. **S.A. Sorensen:** None. **Z. Zhou:** None. **C.A. Anastassiou:** None. **A.S. Shai:** None. **A. Bernard:** None. **C. Dang:** None. **L. Ng:** None. **H. Peng:** None. **J.W. Phillips:** None. **R.C. Reid:** None. **H. Zeng:** None. **S. Mihalas:** None. **M.J. Hawrylycz:** None. **C. Koch:** None.

Poster

060. Striate Cortex: Neural Coding

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 60.05/Z12

Topic: D.04. Vision

Support: NINDS Grant NS078067

Allen Institute for Brain Science

Title: Cholinergic neurons modulate performance of a visual discrimination task in mice

Authors: *B. DANSKIN, P. A. GROBLEWSKI, D. R. OLLERENSHAW, S. R. OLSEN, J. WATERS;
Allen Inst. For Brain Sci., Seattle, WA

Abstract: Sensory processing depends not only on sensory inputs, but also on the internal brain state of the animal. We aim to better understand the changes in cortical function with arousal and attention in awake, behaving mice. The neurotransmitter acetylcholine (ACh) is widely thought to be involved in arousal and attention and is released into neocortex primarily by cholinergic neurons from the basal forebrain. We used optogenetic techniques to manipulate the activity of cholinergic neurons in a mouse performing a visual discrimination task. We crossed the Chat-IRES-Cre mouse line (B6;129S6-Chattm2(cre)Lowl/J) to the floxed reporter line Ai35D to produce mice which express archaerhodopsin (Arch) in cholinergic neurons. We implanted a head restraint bar and guide cannula in mice at P40-90. The tip of the guide cannula was above posterior basal forebrain, 2 mm lateral, 0.5 mm posterior and 4.3 mm ventral to bregma. The mice were allowed a week of recovery prior to 1 week of handling and habituation to the training environment, followed by daily training (a single session of up to 1 hour per day) for 3-5 weeks. During behavioral sessions the mouse was head-restrained and ran on a disk while visual objects were presented to the right eye on a monitor centered 15 cm from the eye. The mouse was trained to discriminate between two 20-degree objects: a vertically-oriented stationary-grating target and a horizontally-oriented distractor, with only the vertically-oriented target being rewarded. Movement of the objects was yoked to running speed via the rate of rotation of the running disk, and the mouse collected a reward by slowing its running to select the target, while maintaining running speed to reject a distractor. The reward for successful target selection was 5-10 µL of water, with mice detecting 150-250 targets in a single session. To maintain motivation, mice were restricted to 1-1.5 mL of water per day. To test the role of cholinergic neurons, we used a 300 µm diameter optical fiber, inserted through the guide cannula to illuminate nucleus

basalis with 30 mW of 640 nm light. Illumination occurred on a randomized subset of 50 % of trials within a behavioral session, beginning immediately before the target appeared on screen and ending after the target left the screen. Consistent with a previous study (Pinto et al., Nature Neuroscience 16, 2013), optogenetic suppression of cholinergic activity decreased performance by impairing discrimination between the target and the distractor.

Disclosures: **B. Danskin:** None. **P.A. Groblewski:** None. **D.R. Ollerenshaw:** None. **S.R. Olsen:** None. **J. Waters:** None.

Poster

060. Striate Cortex: Neural Coding

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 60.06/Z13

Topic: D.04. Vision

Title: *In vitro* single cell morphology in mouse V1 and LGN

Authors: *S. A. SORENSEN, H. PENG, J. BERG, S. SUNKIN, A. OLDRE, N. DEE, S. CALDEJON, Z. ZHOU, C. ANASTASSIOU, M. FISHER, K. JOINES, D. SANDMAN, A. M. HENRY, T. DESTA, W. WAKEMAN, C. KOCH, C. DANG, A. BERNARD, J. HOHMANN, J. W. PHILLIPS, H. ZENG;
Neurosci., Allen Inst., SEATTLE, WA

Abstract: The Allen Institute for Brain Science has initiated a large-scale effort to map and understand the mouse visual system. To begin addressing questions about the cell types present in the visual system, an *in vitro* single cell characterization platform has been established to systematically describe the intrinsic physiological and morphological properties of individual neurons in the primary visual cortex (V1) and lateral geniculate nucleus of the thalamus (LGN). Using layer-specific and interneuron-specific Cre driver mice crossed to tdTomato (tdT) reporter mice, we will march through the cortical layers recording from tdT+ and tdT- neurons in acute slices to survey the full complement of neurons in V1. When possible, a similar approach will be used in LGN. As part of each slice physiology experiment, neurons are filled with biocytin through the patch pipette. After staining, their detailed morphology is analyzed and used to study the presence of morphologically-defined cell types, and their relationship to physiologically- and genetically defined cell types. To capture morphological information, multi-tile, multi-plane digital images are acquired using a brightfield microscope. Images undergo customized enhancement, and an automated reconstruction is generated using the NeuronTracer tool in

Vaa3D (www.vaa3d.org). The reconstruction is then manually corrected to provide an accurate representation of the soma, dendritic and axonal tree. A spectrum of metrics can be extracted from these reconstructions, which can then be used to cluster neurons into putative classes or types. The functional significance of these findings can then be evaluated with respect to data from other modalities. This multi-modal data generation and classification approach will be used to create a taxonomy of cell types in mouse V1 and LGN.

Disclosures: **S.A. Sorensen:** None. **H. Peng:** None. **J. Berg:** None. **S. Sunkin:** None. **A. Oldre:** None. **N. Dee:** None. **S. Caldejon:** None. **Z. Zhou:** None. **C. Anastassiou:** None. **M. Fisher:** None. **K. Joines:** None. **D. Sandman:** None. **A.M. Henry:** None. **T. Desta:** None. **W. Wakeman:** None. **C. Koch:** None. **C. Dang:** None. **A. Bernard:** None. **J. Hohmann:** None. **J.W. Phillips:** None. **H. Zeng:** None.

Poster

060. Striate Cortex: Neural Coding

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Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 60.07/Z14

Topic: D.04. Vision

Support: NINDS R01 NS074015

Title: Simulating *in vivo* LFPs and intracellular [Ca2+] imaging data in mouse V1 using a detailed biophysical model of the cortical column

Authors: ***S. L. GRATIY**[1], A. GARNER[1], A. D. CHENG[1], S. DURAND[1], K. MIZUSEKI[1], J. BERG[1], S. SORENSEN[1], A. ARKHIPOV[1], M. L. HINES[2], A. SHAI[1,3], S. SUNKIN[1], J. W. PHILLIPS[1], H. ZENG[1], R. C. REID[1], M. HAWRYLYCZ[1], C. KOCH[1], C. A. ANASTASSIOU[1]; [1]Allen Inst. for Brain Sci., Seattle, WA; [2]Yale Univ., New Haven, CT; [3]Caltech, Pasadena, CA

Abstract: Despite recent progress in characterizing the properties of individual cells and their connections, the question of how these give rise to circuit processing and, eventually, computation remains largely unanswered [Koch, 1999; Oberlaender et al, *Cerebral Cortex*, 2012]. To understand how single-neuron activity and electrogenesis underlie brain function, one approach is to combine the available anatomical and physiological information within a biophysically realistic neuronal model and assess the extent to which it can replicate a host of experimental observations [Linden et al, *Neuron*, 2011; Anastassiou, Reimann et al, *Neuron*, 2013]. Eventually, a faithful and useful model must both emulate experimental observations and

make testable predictions regarding the role of cells and cell assemblies in circuit processing in a multitude of relevant spatiotemporal scales. We developed a large-scale, biophysically realistic model of a mouse V1 cortical column consisting of more than 10,000 neurons, utilizing reconstructed single cell morphologies, cell type-specific connectivity and active membrane conductances. The model is capable of simulating electrophysiological recordings such as unit activity and local field potentials [Buzsaki et al, *Nat Rev Neurosci*, 2012] and optical imaging of intracellular concentration of calcium ions $[Ca^{2+}]_i$ (e.g., via GCaMP6 monitoring [Chen et al, *Nature*, 2013]) as routinely recorded *in vivo*. Using this model we aim to (a) reproduce *in silico* stereotyped V1 activity as recorded electro-physiologically and optically *in vivo*, (b) deconstruct experimental recordings into their main constituents [Anastassiou, Reimann et al, *Neuron*, 2013], and (c) make experimentally testable predictions of whole-column cortical activity during visual processing. Which features of experimental recordings are captured by such a large-scale model? Which features remain unaccounted for and for what reason? Toward this end, we will provide quantitative comparisons between computational results and a host of experimental data.

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Poster

060. Striate Cortex: Neural Coding

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 60.08/Z15

Topic: D.04. Vision

Support: Paul Allen

Title: Characterization of cell-type specific role in circuit structure and function of mouse visual cortex *in vivo*

Authors: *L. LI, R. IYER, C. TEETER, S. DE VRIES, B. LONG, J. BERG, S. MIHALAS, L. MADISEN, H. PENG, C. KOCH, C. REID, H. ZENG;
Allen Inst. For Brain Sci., Seattle, WA

Abstract: Cortical neurons display various morphological, physiological and genetic features and can be categorized into different ‘types,’ but how these types of neurons contribute to

cortical (micro)circuit construction and neural information processing remains largely unknown. The Allen Institute has recently begun its MindScope initiative: a multi-year project to understand how the mouse visual system functions. With the great support of transgenic mouse lines developed and acquired by the Allen Institute, we have set up a Mouse Cell Types program aimed at understanding morphological, physiological, genetic and connective properties of genetically defined cell types in the mouse visual system. In order to understand how different types of neurons are interconnected and assembled into microcircuits for neural information processing, we employ two-photon targeted whole-cell recording *in vivo* in transgenic mice in which individual types of neurons are fluorescently labeled. We characterize intrinsic membrane properties, spontaneous and visually evoked activities of neuronal types, and integrate these *in vivo* physiological data with gene expression patterns and morphology data. Since the Allen Institute also houses an initiative focusing on measuring intrinsic electrophysiological properties of neuronal types in brain slices, we can correlate our *in vivo* data with data generated from the *in vitro* pipeline, in order to obtain in-depth understanding of the construction and function of mouse visual circuits and how this leads to higher cognitive functions.

Disclosures: **L. Li:** None. **R. Iyer:** None. **C. Teeter:** None. **S. De Vries:** None. **B. Long:** None. **J. Berg:** None. **S. Mihalas:** None. **L. Madisen:** None. **H. Peng:** None. **C. Koch:** None. **C. Reid:** None. **H. Zeng:** None.

Poster

060. Striate Cortex: Neural Coding

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 60.09/Z16

Topic: D.04. Vision

Support: NSF GRFP

Title: The influence of long-range inputs on single-cell dendritic signaling

Authors: *A. SHAI[1,2], C. A. ANASSTASIOU[2], M. LARKUM[3], C. KOCH[2];
[1]Bioengineering, Caltech, Pasadena, CA; [2]Allen Inst. for Brain Sci., Seattle, WA; [3]Neurocure Cluster of Excellence, Berlin, Germany

Abstract: Here we explore the role of dendritic signaling in mouse primary visual cortex (V1) layer 5 (L5) pyramidal neurons during the integration of network inputs from the lateromedial (LM) higher order visual area. L5 pyramidal neuron dendrites extend along all six cortical layers

and receive inputs from both local and long-range axons and serve as a main output of columnar computation, making them one of the main integrators of cortical computation. We wish to understand how the anatomy of long-range connections relates to single neuron morphology and biophysics by focusing on axons that extend from LM to V1 L5 pyramidal neurons. First, we evaluate connectivity from LM to V1 using the method of subcellular channelrhodopsin assisted mapping (sCRACM) (Petreanu, Mao et al. 2009). We find that projections from LM synapse directly onto the tuft dendrites of V1 L5 pyramids, though these inputs seem to be substantially weaker than inputs onto their basal dendrites (Yang, Carrasquillo et al. 2013). However, due to limitations of the sCRACM method where tuft inputs might be underestimated and dendritic nonlinearities are left unaccounted for, we next tested the physiological influence these distal synapses might have. Continuing the optogenetic approach, we control the spatio-temporal pattern of inputs from LM onto L5 pyramidal neurons in V1 during somatic current injections using single photon laser stimulation in an *in vitro* prep. By monitoring the influence distal (in layers 1 and 2/3) LM inputs have on dendritic calcium-spiking (Larkum, Nevian et al. 2009), we show that these synapses do indeed play an important role in single-cell computation despite their substantial distance from the axon initial segment. In particular, we show that distal excitatory input from LM reduces the threshold for dendritic nonlinearities, ultimately allowing these long-range axons from LM to control burst-firing in L5 pyramidal neurons in V1. Larkum, M. E., T. Nevian, et al. (2009). "Synaptic integration in tuft dendrites of layer 5 pyramidal neurons: a new unifying principle." Science 325(5941): 756-760. Petreanu, L., T. Mao, et al. (2009). "The subcellular organization of neocortical excitatory connections." Nature 457(7233): 1142-1145. Yang, W., Y. Carrasquillo, et al. (2013)."Distinct balance of excitation and inhibition in an interareal feedforward and feedback circuit of mouse visual cortex." J Neurosci 33(44): 17373-17384.

Disclosures: **A. Shai:** None. **M. Larkum:** None. **C.A. Anasstasiou:** None. **C. Koch:** None.

Poster

060. Striate Cortex: Neural Coding

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

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Topic: D.04. Vision

Support: NIH Intramural Research Program

Whitehall Foundation

Alfred P. Sloan Foundation

Title: Spiking responses in V1 are coupled to the phase of infragranular alpha LFP

Authors: *K. DOUGHERTY[1], M. A. COX[1], D. A. LEOPOLD[2], A. MAIER[1];
[1]Dept. of Psychology, Vanderbilt Univ., Nashville, TN; [2]Lab. of Neuropsychology, Natl. Inst. of Mental Hlth., Bethesda, MD

Abstract: Visual sensitivity varies considerably within and across experimental trials. Some of these fluctuations in visual task performance have been shown to correlate with cortical slow-wave activity in the alpha range (7-14 Hz), which has led to the hypothesis that visual sensitivity covaries with gradual changes in the excitability of cortex. In line with this hypothesis, recordings in primary visual cortex (V1) of monkeys revealed that the magnitude of the alpha-band local field potentials (LFP) in deep cortical layers couple with gamma-range (>30 Hz) amplitude within the same column. This finding is consistent with optogenetic work in rodents that demonstrates that infragranular neurons can control neural excitability in other layers. However, the laminar pattern of coupling between alpha-band LFP and spiking responses in primate V1 is largely uncharacterized. Here, we used a linear multielectrode array to simultaneously record LFP and multiunit spiking activity (MUA) across all layers of V1 of two macaque monkeys. Animals fixated while static grating stimuli were presented inside the neurons' receptive field. Congruent with previous reports, alpha-band LFP fluctuations were more prominent in infragranular layers relative to other layers. These fluctuations in the alpha range were not accompanied by overt changes in gaze position or microsaccade frequency. We found that within each trial the visually evoked MUA exhibited an increase in magnitude around amplitude peaks of alpha-band LFP. Circular statistics revealed that MUA responses in all cortical layers covaried with the phase of the infragranular alpha rhythm in both animals ($p < 0.05$). This robust interlaminar coupling of alpha-band LFP and MUA persisted for several hundred milliseconds while the stimulus remained on the screen. The slow modulation of columnar responses reported here is consistent with the notion of dynamic changes in cortical excitability during visual processing.

Disclosures: **K. Dougherty:** None. **M.A. Cox:** None. **D.A. Leopold:** None. **A. Maier:** None.

Poster

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Robin and Richard Patton through the E. Bronson Ingram Chair in Neuroscience

Title: Microcircuitry of agranular frontal and granular occipital cortex: Testing the generality of the canonical cortical microcircuit with cross-frequency phase-amplitude coupling during resting-state

Authors: *T. NINOMIYA, K. DOUGHERTY, D. C. GODLOVE, J. D. SCHALL, A. MAIER; Dept Psychol, Ctr. Integr & Cog Neuro, Vanderbilt Vision Res. Ctr., Vanderbilt Univ., Nashville, TN

Abstract: Diverse lines of research have been guided by the hypothesis that the cerebral cortex is comprised of a canonical laminar microcircuit. Evidence for such a circuit is based principally on anatomical observations of primary sensory cortex, especially early visual cortex. However, confidence in the generality of this hypothesis is weakened with recognition of the notable cytoarchitectural difference between granular sensory cortex and agranular frontal cortex. To test whether evidence for such a circuit is uniform across cortex, we used interlaminar cross-frequency phase-amplitude coupling as an assay of cortical circuitry. Using a linear microelectrode array, spontaneous local field potentials (LFPs) were recorded from all laminae of granular V1 and agranular supplementary eye field (SEF) while monkeys rested in darkness. We found substantial differences in the relationship between the amplitude of the gamma-band (>30 Hz) and the phase of alpha-band LFP (7-14 Hz) between these areas. In V1, gamma amplitudes in L2/3 and L5 were coupled with the alpha-band LFP phase in L5, as previously described. In contrast, SEF phase-amplitude coupling was most prominent within L3. These results suggest that laminar interactions in agranular frontal cortex are unlike those in granular V1. Thus, the canonical cortical microcircuit does not generalize across cortical areas.

Disclosures: **T. Ninomiya:** None. **K. Dougherty:** None. **D.C. Godlove:** None. **J.D. Schall:** None. **A. Maier:** None.

Poster

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Support: Whitehall Foundation (RW)

NSF CRCNS 1308174 (RW)

Title: Dynamics of cortical correlation during vision

Authors: *N. WRIGHT, T. CROCKETT, J. POBST, R. WESSEL;
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Abstract: Cortical circuits deal with time-varying sensory inputs via specific recurrent connections, from which coordinated and stochastic cortical population activity emerges dynamically. The stimulus-modulated population activity modifies circuit properties via adaptation mechanisms, which, in turn, reorganize the distribution of cortical correlations. Pairwise correlation is a measure of neuronal association that dwells at the core of how groups of neurons act in concert and how population coding might be optimized. Thus, understanding how visual stimulation impacts the co-varying activity among populations of neurons is a fundamental issue that remains to be fully addressed. To investigate the dynamics of cortical correlations during visual processing, we projected visual stimuli to the retina, recorded the local field potential (LFP) from the primary visual cortex of turtle using the *in vitro* eye-attached whole-brain preparation, and, simultaneously, monitored the membrane potential (V) from up to two cortical neurons via whole-cell recordings. We calculated normalized cross-correlation functions, C(d), with up to d = 500 ms lag from the band-pass filtered recordings (0.1 to 100 Hz) after trial averages had been subtracted. Our analysis of pairs of V-LFP and V-V recordings revealed four significant features of cortical correlation. First, cross-correlation functions, C(d), differed from pair to pair. These differences were not adequately reflected in the zero-lag cross-correlation coefficient, C(d=0). Cross-correlation functions reached extrema values of up to +/- 0.6. Second, for each pair tested, the cross-correlation function during visual stimulation differed from the C(d) at resting state. Third, resting state activity of small LFP amplitude was punctuated by distinct bursts of synaptic inputs accompanied by large LFP oscillations of up to 5 s duration, indicating a network state of high correlation. For a given pair, the cross-correlation function at resting state differed from the C(d) during bursts of LFP oscillations. Fourth, differences in C(d) from pair to pair or from small to large LFP amplitude network states were similar in magnitude to changes in C(d) evoked by visual stimulation, when compared to resting state. Together, these observations reveal a broad distribution of pair-wise correlations, with correlations for an individual pair changing under varying conditions. However, with the present data set, no obvious changes were detected for the distribution of correlations as a whole.

Disclosures: **N. Wright:** None. **T. Crockett:** None. **J. Pobst:** None. **R. Wessel:** None.

Poster

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NSF CRCNS 1308174

Title: Cortical pyramidal neuron subtype classification and visual response diversity

Authors: ***T. CROCKETT**[1], N. WRIGHT[1], S. THORNQUIST[2], M. ARIEL[3], R. WESSEL[1]; [1]Washington Univ. In St Louis, Saint Louis, MO; [2]Harvard Univ., Cambridge, MA; [3]St. Louis Univ., Saint Louis, MO

Abstract: A detailed inventory of the cortex's constituent pieces is essential to effectively understand the principles underlying cortical signal processing and computation. The classification of pyramidal neurons into functional subtypes remains a crucial part of this survey since the subtype-specific division of labor by the cortex provides manifold combinatorial possibilities, creating a rich substrate for computation. However, the extreme degree of integration of individual neurons into the collective cortex suggests that cellular individuality may represent a smaller component of computational role in the context of the larger network. This possibility raises an important question: is the computational function of a neuron determined by its individual type or by its circuit connections and network role? We investigate the problem of cellular organization using the ancestral model of the turtle primary visual cortex. The turtle's pyramidal neurons are arranged in a single layer in a three-layer visual cortex and are known to contain layer 4/input and layer 5/output homologs of the mammalian six-layer cortex. Electrophysiological profiles were created from measuring passive neuronal properties and responses to current injection using whole-cell patch-clamp recordings. These parameters were derived from the passive membrane properties, the shape of the action potential, the shape of the hyperpolarization, the time course of spike-rate adaptation, and the grouping of spikes. A blind clustering algorithm exercised on the data revealed the presence of two principle classes of neurons, presumably coinciding with L4/I- and L5/O-type neurons. To address functional profiles, we recorded visual responses of identified neurons to diffuse light flashes and applied a

cluster analysis to this data set. Visual responses consist of a smooth early response and a more dominant and fluctuating late response. These response epochs are hypothesized to correspond to thalamic and intracortical inputs, respectively. Based on the existing data set, we found that a cluster analysis of the dominant late response does not support evidence for subtypes. Our study shows that the answer to the posed question, whether cell type identity translates into functional identity when integrated into a network, appears to be both yes and no. Visual responses to whole-field flashes show likely cell-type specific inputs from the lateral geniculate nucleus, but late responses mediated by intracortical inputs are similar for both types.

Disclosures: **T. Crockett:** None. **N. Wright:** None. **S. Thornquist:** None. **M. Ariel:** None. **R. Wessel:** None.

Poster

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Title: Characterization and proposed mechanisms of intermittent oscillations in cerebral cortex

Authors: ***M. HOSEINI**[1], J. POBST[1], W. CLAWSON[2], W. SHEW[2], R. WESSEL[1]; [1]Physics, Washington Univ. In St. Louis, Saint Louis, MO; [2]Physics, Univ. of Arkansas, Fayetteville, AR

Abstract: Rhythmic oscillations are ubiquitous in cerebral cortex and their potential functional roles continue to excite the imagination of neuroscientists. These oscillations are (i) intermittent, are (ii) of variable durations and frequencies, and (iii) typically are accompanied by sparse and irregular single neuron spiking. To our knowledge, no one spiking model has succeeded in capturing these three characteristics of cortical oscillations. What combination of neuronal and network properties mediates the characteristic features of observed cortical oscillations? To address this question, we recorded spontaneous and evoked neuronal oscillations in the visual cortex of turtle. This preparation was chosen because the local field potential (LFP) oscillations generated in this cortex are sufficiently large to allow single-trial analysis of characteristic

features without the need for averaging. We determined the frequency profiles for LFP oscillations, as well as the variability in the amplitude and the frequency of oscillations across trials, recording sites, and visual stimuli. Importantly, we designed a network of spiking model neurons with the objective to investigate model parameter values such that the network reproduces the observed features of cortical oscillations. The primary results of this study are that (a) visually-evoked activity often exhibits very large power increases with peaks in multiple narrow frequency bands, (b) from trial to trial, these peaks in relative power occur among different sets of frequencies within the 0.7 - 100 Hz range, and (c) for individual trials, spectral peaks are often shared among groups of electrodes across the electrode array, but these electrode groups may vary across trials and by frequency. The intermittent oscillations of variable duration and frequencies, accompanied by sparse and irregular spiking were reproduced with a model network consisting of excitatory neurons, and fast and slow inhibitory neurons. Model neurons with spike-rate adaptation were connected randomly to form a sparse network. Our model results indicate that fast interneurons help to keep the balance between excitation and inhibition, while slow interneurons play a critical role in turning off oscillations and causing intermittency. Adding dendritic non-linearity to the model allows for intermittency over a broader range of network parameters and makes the system more robust to noise. Our investigation demonstrates the possibility of generating intermittent and variable gamma-band oscillations in a network with realistic parameters and irregular and sparse single-neuron spiking.

Disclosures: **M. Hoseini:** None. **J. Pobst:** None. **W. Clawson:** None. **W. Shew:** None. **R. Wessel:** None.

Poster

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Title: Scale-free cortical resting state activity *in vivo* at single-cell resolution

Authors: *Y. KARIMIPANAH[1], A. C. KWAN[2], Y. DAN[3], R. WESSEL[1];
[1]Washington Univ. In St.Louis, University City, MO; [2]Yale Sch. of Med., New Haven, CT;
[3]Univ. of California, Berkeley, Berkeley, CA

Abstract: Mounting evidence from fMRI, EEG, and LFP recordings of resting state activity *in vivo* reveals a high level of coordination among the neuronal populations at the recording sites and specifically indicates a lack of a characteristic scale in the spatiotemporal patterns of activities. This scale-free nature of cortical activity suggests the attractive hypothesis that the cortex operates near a critical state between order (large-scale activity) and disorder (small-scale activity), which, on theoretical grounds, has long been suggested to be optimized for computation. The coarse spatial resolution (>100 μm) of the fMRI, EEG, and LFP recording methods, raises the question whether the scale-free nature of cortical activity extends to a small cortical volume consisting of some 40 neurons. To address this question, we labeled layer 2/3 cells in the primary visual cortex of urethane-anaesthetized adult mouse by bolus injection of the calcium indicator dye Oregon Green 488 BAPTA-1 AM, used two-photon calcium imaging to monitor ensemble activity, and inferred spikes as described previously (Kwan, Dan 2012). We thus obtained the inferred spike trains of several minutes duration from up to 40 simultaneously recorded neurons in primary visual cortex from 42 mice. Recordings of ongoing cortical L2/3 activity at single-cell resolution revealed pronounced coordinated activity among the population of some 40 closely-spaced neurons. First, temporal correlations for both single neuron and network activity were exposed using the Detrended Fluctuation Analysis, which showed a linear trend for a long range of time windows, indicating the existence of long-term memory. Second, the cross-correlation coefficients among the spike trains of pairs of neurons were generally small with a skewed non-Gaussian distribution dominated by a long tail. Third, correlations in time and among neurons were further revealed using the neuronal avalanche concept. The avalanche size and duration distributions were best fit by a power law function (both truncated and with exponential cutoff), compared to other commonly tested functions (e.g., exponential, lognormal, etc). Fourth, consistent with the properties of a dynamical critical state, the avalanche sizes scaled with avalanche duration. Fifth, a critical model network with synaptic depression qualitatively reproduced the four observed hall marks of coordinated activity. Taken together, the data and model investigations support the hypothesis that the mouse primary visual cortex operates near a critical state including at the cortical microcircuit level.

Disclosures: **Y. Karimipanah:** None. **A.C. Kwan:** None. **Y. Dan:** None. **R. Wessel:** None.

Poster

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Title: Cell-type specific differences in high and low frequency synchronization during behavior in the awake mouse

Authors: *A. E. CASALE, B. J. HANSEN, J. F. MITCHELL, J. H. REYNOLDS, E. M. CALLAWAY;
Salk Inst., La Jolla, CA

Abstract: Changes in behavioral or cognitive state have been shown to dynamically modulate the responsiveness of cortical neurons to sensory stimuli *in vivo*. In the mouse primary visual cortex (V1), simple motor behaviors such as locomotion are correlated with increases in visually-evoked firing rates. Recordings of cortical local field potentials (LFP), a measure of population activity, also show alterations in power at certain frequency bands during different states. Under periods of quiet rest or sleep delta oscillations (1 - 4 Hz) are prominent, whereas periods of attentive locomotion are associated with increases in gamma-band (30 - 80 Hz) power. These observations suggest changes in the way information is processed across different states. To investigate the mechanisms underlying these changes we compared the activity patterns of mouse V1 pyramidal cells and parvalbumin-positive (PV+) interneurons, cells characterized by differences in their membrane physiology, during rest and locomotion. Using extracellular laminar recordings and optogenetic tagging we identified PV+ cells and pyramids throughout all layers of V1. Spiking activity and LFPs were simultaneously recorded during periods of quiet rest or locomotion in the absence of visual stimuli. Spectral analysis of the LFPs showed an increase in gamma and decrease in delta power during periods of locomotion across lamina. During locomotion we found a significant increase in firing rate for both cell types across all layers, which was especially pronounced for PV+ neurons. Using pairwise phase consistency we quantified the phase locking of action potentials from each cell type. During locomotion, PV+ cells showed decreased phase consistency at low frequencies and increased phase consistency in the gamma-band. Pyramidal cells showed a weak trend to reduce phase consistency at low frequencies during locomotion, but no changes were significant at any frequency. PV+ cells had significantly more phase consistency in the gamma-band than pyramidal cells during both locomotion and rest. These findings suggest that the phase locking of PV+ neurons may play a

critical role in mediating changes in visual cortical state during locomotion. We are currently addressing the impact of visual stimulation on phase locking behavior.

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Poster

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The Gatsby Charitable Foundation Research Grant

Title: Locomotion-induced changes in noise correlations in mouse primary visual cortex

Authors: ***B. J. HANSEN**, A. E. CASALE, J. F. MITCHELL, J. H. REYNOLDS, E. M. CALLAWAY;
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Abstract: A fundamental question is how changes in behavioral state alter sensory processing in populations of neurons and what individual circuit elements mediate such changes in processing. Work in primates have employed spatial cueing paradigms to show improved signal-to-noise ratios when attention is directed to a stimulus, much of which is due to reductions in noise correlation, reflected in reductions of low frequency power in the local field potential (LFP). In addition, high frequency synchronization has been observed to increase, potentially leading to increases in the gain for downstream neurons. Despite their importance, the mechanisms that generate these changes remain unknown and the tools presently available for use in primates do not provide a means of modulating the activity of neuronal types thought to be involved. In contrast, the set of molecular and genetic tools available in the mouse are revolutionizing the study of neural circuits. Published studies show that locomotion in mice modulates visual processing: when mice are running, there is an increase in gain and changes in the LFP. These changes in processing may reflect improved sensory processing under active conditions, and be similar to the kinds of changes mediated by spatial attention in primates. But it is unknown whether locomotion also affects noise correlations. We tested the functional significance of

locomotion-induced changes in brain state in the mouse primary visual cortex (V1). We investigated whether and how locomotion-induced changes in brain state influence the accuracy of the population code via changes in the structure of correlations across networks in mouse V1. Head fixed mice ran freely on a wheel, while multi-contact laminar probes recorded extracellular activity of visually responsive neurons and LFPs. Cortical layers were identified by measuring the evoked-response potential and computing the current-source density. Visual stimuli consisted of drifting sine-wave gratings for 1.5 s. We examined the state-dependent changes in correlation structure by calculating spike count correlations (rsc) between pairs of neurons during trials when mice were running or stationary. In stationary mice noise correlations were high (rsc= 0.24 ± 0.03; mean ± SEM), similar to values in primates that are not attending a visual stimulus. During running, noise correlations decreased significantly (rsc= 0.07 ± 0.12) similar to the reduction induced by attention in primates. These results, confirmed across our population of 21 neuron pairs, show a remarkable property of decorrelation amongst neurons during a particular brain state, which is likely to strongly impact information coding.

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Poster

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Topic: D.04. Vision

Support: NSC 102-2410-H-002 -050 -

Title: Intermodulation between broad- and narrow-band visual stimuli in visually evoked potential

Authors: *C.-C. CHEN;
Psychology, Natl. Taiwan Univ., Taipei, Taiwan

Abstract: The visibility of a periodic pattern (target) deteriorates when the pattern is embedded in white noise (mask). The conventional interpretation of such noise masking effect is that the external noise perturbs the internal response and thus makes it more difficult for the visual system to separate the target from the noise. Recently, it is also suggested that the noise mask is to suppress the internal response to the target. We investigated the mechanisms underlying noise

masking by observing event related potential evoked by various combination of periodic targets and noise masks. The target was a vertical Gabor patch (1.3cyc/d). The mask consisted of random dots (4'x4' pixel size) whose luminance drew from a uniform distribution. The contrast of the target or the mask was either 0, 6%, 12%, 25% or 50%. There were, thus, 36 target-mask combinations. Each combination was repeated 40 or 80 times for each participant. In each trial, the target and the mask appeared simultaneously for 300ms, followed by a blank period of variable length (mean=2s, sd=.25s). The participants performed an irrelevant attention task by indicating the change of color of the fixation dot (0.1 chance at stimulus onset, 300ms duration). The event-related potential (ERP) was acquired with an EGI 256-channel recorder at 500Hz sampling rate. The waveform was sorted and time locked at the stimulus onset and was band-pass filtered between 0.1 and 50Hz. Our data analysis focused on the P1 (85-115ms after stimulus onset) and P2 (180-220ms) components of the waveforms from the occipital electrodes. A standard equal-variance test failed to find a significant difference between the variances of the amplitude from different conditions. Hence, the hypothetic response perturbation by external noise was not manifested in ERP. The ERP amplitude increased with both target and mask contrasts. However, the target contrast effect decreased with mask contrast, suggesting a suppression of the response to the target by the mask. In P2, the mask contrast effect increased with target contrast, suggesting a lack of suppression on the response to the mask by the narrow-band target. In sum, our result is consistent with the notion that noise masking is due a suppression of the target response mechanism by the noise mask through a broadband contrast normalization process.

Disclosures: C. Chen: None.

Poster

060. Striate Cortex: Neural Coding

Location: Halls A-C

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Topic: D.04. Vision

Title: The scaling of contrast discrimination under temporal constraints

Authors: *J. R. FLYNN[1], H. SHOUVAL[2];
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Abstract: Weber's law is a well-established phenomenon in the field of psychophysics. It states that the smallest detectable change in the intensity of a stimulus is proportional to the original intensity of the stimulus. This level of change is called the Just-Noticeable-Difference, or JND, and the ratio of the JND to the intensity of the stimuli is called the Weber Fraction. What is striking about Weber's law is that it holds for many, if not most, sensory modalities. This near universal feature across the senses suggests that there may be an unknown physiological basis for Weber's law. One model for this physiological mechanism is that innate noise in the firing rates of sensory system neurons may give rise to this linear scaling in perceptual errors. This model can also be adapted to non-linear scaling in pertinent systems. A useful aspect of this model is that, using experimentally determined spike count statistics of the relevant cortical neurons, it makes a very specific prediction on how the Weber Fraction will increase when the presentation time of the stimuli is reduced. It is this aspect of the model that we are testing here. We have chosen to perform the experiment in the contrast domain using sinusoidal gratings, because cortical neurons respond strongly to such stimuli. To determine the contrast JND for a subject, a reference contrast grating is shown, followed by test grating that has a slightly different contrast level. The subject is forced to decide if the test grating has a higher or lower contrast level. This is repeated multiple times, and a Bayesian based adaptive algorithm is used to adjust the difference between the test and reference grating in order to quickly find the JND. Black and white checkerboard masks are placed between the gratings in order to mask after images. Presentation times of the gratings are varied - the stimuli is left on the screen for 50, 75, 100, 125, 150, or 300 ms. We find that, for most subjects, a presentation time of at least 75 ms is necessary for performing above chance. As the presentation time increases further, the Weber fraction decreases until around 200 ms. Presentation times longer than this do not show any further decreases in subjects' Weber Fraction. While this result qualitatively corresponds to the theory being tested, the quantitative relationship between the Weber Fraction and the presentation time was not in line with our theoretical predictions. We suggest several alternative models that are in better agreement with the data.

Disclosures: **J.R. Flynn:** None. **H. Shouval:** None.

Poster

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DFG Research Fellowship

Title: Population encoding and decoding of visual motion in primary visual cortex from naturalistic visual scenes

Authors: *G. B. STANLEY[1], S. T. KELLY[1], J. KREMKOW[2], J. JIN[2], Y. WANG[2], S. J. KOMBAN[2], J.-M. ALONSO[2];
[1]Coulter Dept. of Biomed. Engin., Georgia Inst. Technol. & Emory Univ., Atlanta, GA; [2]Col. of Optometry, State Univ. of New York, New York, NY

Abstract: Although the large majority of what we currently know about the functional properties of neurons in the early visual pathway has been learned through the use of highly simplified, artificial visual stimuli, there is a growing body of complementary work involving natural visual scenes. However, the relationship between the classical tuning properties and natural scene responses is far from clear. Specifically, although artificial stimuli such as sinusoidal gratings are a fast and accurate way to capture the response of such neurons to particular single and highly controlled features such as orientation or spatial frequency, even in the simplest of real-world conditions these features strongly co-vary. Ultimately it is unclear how natural scenes, and more importantly the motion within them, are encoded by neuronal populations and how this encoding is transmitted between different brain regions. Using modern computer animation tools, we have created a novel stimulus that mixes natural scene properties (perspective and motion boundaries) with efficient artificial properties (sinusoidal gratings) to capture some of the complexities of the natural visual environment, while still maintaining some simplicity of the classical stimuli. These stimuli were presented to the visual pathway of the paralyzed, anesthetized cat, while recording single- and multi-unit activity in V1 using a multi-electrode array. Through a controlled sequence of navigation of the camera within the synthesized visual environment, we directly assess to what extent neuronal responses are predicted from classical tuning properties in this rich environment. Preliminary results indicate that while some V1 neuron responses are well predicted from classical tuning properties, a large portion are not. The data suggest that one key element is the proximity of the neuron's receptive field to motion boundaries, where there are potentially conflicting inputs to the neuron. The responses of these populations of neurons are, however, predictive of the motion in the scene, when utilized in a population decoding framework. Taken together, the results here suggest that V1 neurons do collectively capture the gross motion in the visual scene, but even simple extensions of traditional stimuli challenge notions of encoding.

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Poster

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Program#/Poster#: 60.21/Z28

Topic: D.04. Vision

Title: Exploring 200-1000 Hz field potentials with microelectrodes, ecog and meg

Authors: *B. F. HANDEL[1], C. A. BOSMAN[2], T. WOMELSDORF[3], P. FRIES[1];
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Abstract: Magnetoencephalography (MEG) has primarily been used to record event related fields or ongoing brain rhythms, both reflecting neuronal population activity, specifically, synchronized post-synaptic potentials (PSPs). This PSP synchrony generates signal power primarily in the classical EEG frequency bands below 200 Hz. By contrast, the individual post-synaptic potential contains power in a broad frequency band, reaching substantially beyond 200 Hz. Interestingly, microelectrode recordings and human electrocorticography (ECoG) recordings show activation-dependent increases in broadband high-frequency (BHF, 200-1000 Hz) power, reminiscent of the PSP spectrum. We explored whether we could trace such BHF power increases from microelectrode and ECoG recordings in monkeys to human MEG recordings. Both monkey and human subjects fixated and monitored a moving grating stimulus while recordings were obtained from visual cortex. Microelectrode recordings in V1 showed stimulus-driven sustained increases in firing rates, narrow gamma-band power and BHF power. The peak BHF power showed trial-by-trial correlations to peak firing rate increases (R=0.48, P=0.1^-10). When signals were first averaged in the time domain and then spectrally analyzed, the BHF component persisted and showed a post-stimulus peak around 100 ms with a similar correlation to firing rates. ECoG recordings showed stimulus-driven sustained increases in narrow gamma-band power and BHF power as well. The signal showed BHF power peaking at a similar latency as the microelectrode data. The ECoG grid covered large parts of the left hemisphere. The topography of BHF power increases was specific to the early visual system. MEG recordings showed stimulus-driven increases in narrow gamma-band power, but only very weak increases in

BHF power. However, when signals were first averaged in the time domain and then spectrally analyzed, there was a BHF power increase with a similar latency as in the microelectrode data and a topography suggesting a focus in the visual system. The BHF component was lateralized showing a higher power contralateral to the visually stimulated hemifield. The fact that the BHF response was visible in the MEG power only after time-domain averaging suggests limitations in MEG signal resolution. Yet, the comparison across the three approaches strongly suggests that MEG reflects in principle neuronal signals up to over 1000 Hz. This BHF signal strongly correlated with spike count and might prove useful in interpreting non-invasive electrophysiological data serving as proxy for spiking activity in the MEG.

Disclosures: **B.F. Handel:** None. **P. Fries:** None. **T. Womelsdorf:** None. **C.A. Bosman:** None.

Poster

060. Striate Cortex: Neural Coding

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 60.22/Z29

Topic: D.04. Vision

Support: NEI

Office of the NIH Director

Title: Visual stimulation triggers temporally-structured cortical cell assemblies in awake mice

Authors: ***L. CARRILLO**, J.-E. KANG MILLER, J. JACKSON, R. YUSTE;
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Abstract: Although primary visual cortex has been intensely studied in the context of development and experience dependent plasticity, little is known about multineuronal sequential activity patterns during spontaneous network activity that could encode natural visual responses. We used *in vivo* two-photon calcium imaging to record simultaneous activity of hundreds of neurons with single cell resolution in mouse visual cortex. Sequential presentation of natural stimuli defined different network states depicting closed cycles of activity. Network dynamics reliably described the neural correlate of visual stimuli. Without sensory stimulation we observed endogenous generated network dynamics, displaying temporally structured sequential activity patterns showing cell assembly signatures. Cell assemblies have recurrent activity over long

timescales and persist after network perturbations indicating robust neural circuits as the substrate of visual responses. Our results point out that sequential representation of visual percepts use spatiotemporal attributes of imprinted neural circuits opening the possibility to understand the emergent encoding of visual information in a multidimensional cortical space. The connections, modulation and pathological states of these emergent patterns deserve further study since they may reveal general properties of neural networks.

Disclosures: **L. Carrillo:** None. **J. Kang Miller:** None. **J. Jackson:** None. **R. Yuste:** None.

Poster

060. Striate Cortex: Neural Coding

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Topic: D.04. Vision

Support: NIH EUREKA PROGRAM

Title: Improved perceptual performance and coding accuracy following optical stimulation of V1 populations

Authors: *A. R. ANDREI, S. POJOGA, R. JANZ, V. DRAGOI;
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Abstract: The detection of subtle changes in the environment can challenge the limits of the organism's sensory systems. Studying neural activity and behavior at this sensory limit allows us to titrate out the contributions of unique cellular subpopulations to the formation of sensory percepts. In this study, we used optogenetic methods to investigate how the population activity of glutamatergic neurons in primary visual cortex (V1) impacts an animal's ability to detect near-threshold visual stimuli. We delivered the ChR2 gene to multiple sites in V1 using a lentivirus vector with a CaMKII promoter in two monkeys (macaca mulatta). Starting 4 weeks after the injection, single and multi-unit activity were recorded using laminar electrodes and a custom built laser positioned 0.5mm from the nearest recording site. Monkeys performed a visual detection task - while maintaining fixation, oriented gratings with differing contrast levels were presented parafoveally over the receptive fields of the neurons of interest for 1300 ms. The monkey signaled the presence or absence of a stimulus by releasing or holding a response bar. Half of the trials were paired with simultaneous optical stimulation (20-50Hz, for ~300ms). We recorded a total of 36 sessions, and a total of 473 light-responsive single and multi-units. 22/36

sessions activated neuronal populations closely tuned to the stimulus orientation, while 14/36 sessions activated distally-tuned populations. We found that optical stimulation of populations of excitatory neurons tuned to the visual stimulus resulted in an 8.0% ± 2.2 SEM improvement in behavioral detection of near-threshold stimuli (P=0.0022, Wilcoxon signed rank test). In contrast, optical stimulation of neurons unresponsive to the visual stimuli resulted in no change in task performance. At the neuronal level, while both session types showed robust firing rate augmentation and increases in signal to noise ratio, pairs of neurons from stimulus tuned sessions also showed a significant decrease in noise correlations (P<0.0001, Wilcoxon signed rank test, n=2436 pairs) and increase in coding accuracy following optical stimulation. These effects are unlikely to be caused by phosphenes elicited by optical stimulation as we did not observe changes in the false alarm rates or the percentage of aborted trials due to broken fixations. Our results suggest that the distinction between relevant and irrelevant information used in behavioral decisions is made at an early stage of visual processing and is reflected in differences in stimulus coding at the local network level.

Disclosures: **A.R. Andrei:** None. **S. Pojoga:** None. **R. Janz:** None. **V. Dragoi:** None.

Poster

060. Striate Cortex: Neural Coding

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Topic: D.04. Vision

Support: Max Planck Society

Human Frontier Science Program (JF)

Title: Hidden spatial selectivity of receptive fields in turtle visual cortex

Authors: *J. FOURNIER, C. M. MUELLER, G. LAURENT;
Max Planck Inst. For Brain Res., Frankfurt-am-Main, Germany

Abstract: As the primary cortical recipient of geniculate afferents, the three-layered dorsal cortex (DC) of turtles can be considered analogous to the primary visual cortex of mammals (V1). At a functional level however, this primary sensory area seems to process visual information in a very different way compared to mammalian V1. When stimulated with single flashes of light, DC neurons show wide receptive fields (RFs), generally covering most of the

contralateral visual field with no clear spatial preference. Moreover, thalamic axons projecting to dorsal cortex seem to lack the localized retinotopic arrangement of mammalian thalamo-cortical projections (Mulligan & Ulinski, J.Comp.Neurol. 296: 531, 1990), arguing for a distributed representation of the visual space across the entire dorsal cortex. Here we re-investigated the issue of spatial selectivity of DC neuron receptive fields using white noise analysis. Single units were recorded extracellularly in the dorsal cortex of anesthetized (0.5-1% isoflurane) and paralyzed turtles (Trachemys scripta) and their RFs were mapped with a two-dimensional white noise covering most of the contralateral visual field. Receptive fields were decomposed into 3 different components: a spatially non-selective component, contributing to the evoked response irrespective of the spatial pattern of the stimulus; a linear component, selective to the position and the polarity of contrast changes; and a nonlinear component, also spatially selective but independent of the polarity of the stimulus. Although the spatially non-selective component often dominates over the selective ones, we found localized and spatially structured linear and nonlinear RF components in a substantial fraction of neurons. Some of those RF components revealed alternating ON and OFF sub-regions, reminiscent of the spatial organization of V1 simple RFs, though considerably larger. These spatially selective components of the RF generally extend over large parts of the visual field and their locations can greatly differ between neurons recorded at the same cortical location. These results show that, despite the apparent absence of spatially tuned responses when mapped with single flashes of light, the RFs of turtle visual cortex neurons reveal spatial selectivity once the spatially non-selective component of their response is subtracted. This indicates that spatial information is indeed encoded across the DC neuron population although it is not mapped across dorsal cortex, consistent with the lack of hard-wired retinotopy of the thalamo-cortical afferent projections.

Disclosures: **J. Fournier:** None. **C.M. Mueller:** None. **G. Laurent:** None.

Poster

060. Striate Cortex: Neural Coding

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NARSAD Young Investigator award to UK

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NARSAD Young Investigator to JC

Title: Locomotion and arousal regulate activity patterns and visual encoding in V1

Authors: *M. VINCK, U. KNOBLICH, R. BATISTA-BRITO, J. CARDIN;
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Abstract: Neural function depends critically on behavioral state, such as sleep, wakefulness, arousal and focused attention. Previous work in rodents has found that the gain of cortical, but not thalamic, visual responses is strongly modulated by locomotion state (sitting vs. running), and that locomotion enhances area V1 gamma-band synchronization while reducing low-frequency synchronization. It remains unclear whether these changes in cortical state are driven entirely by locomotion or if concurrent changes in arousal contribute to state-dependent shifts in V1 activity. To investigate this, we recorded isolated single units and local field potentials (LFPs) from multiple sites throughout layers 2-6 of V1 in awake mice. Mice were head-fixed and mounted on a wheel apparatus. Visual stimuli of varying contrast were presented, interspersed with baseline periods without visual stimulation. Simultaneous measurement of pupil diameter allowed for a continuous assessment of global arousal levels. Fast-spiking (FS) and regular-spiking (RS) cells were identified based on extracellular waveforms and firing characteristics. Using the statistical method of change-point analysis, we identified transitions between quiet and active behavioral states with high temporal resolution. We found increases in firing rates of both FS and RS cells during locomotion, both in the absence and presence of visual stimulation. Locomotion especially increased the firing rate of cells that were also significantly activated by visual stimulation. Visual stimulation and locomotion caused enhancements of gamma-band synchronization in the same frequency range (~50 Hz). To assay the contribution of arousal to these changes in cortical activity, we studied the temporal trajectories of firing rate statistics and oscillatory dynamics around behavioral state transitions. Overall, we found a tight relationship between locomotion and pupil diameter. However, we also identified an additional behavioral state characterized by high levels of arousal in the absence of locomotion. Arousal-only periods were accompanied by spectral signatures similar to those observed during the locomotion state. We find that arousal explains a substantial amount of variation in firing rate statistics and oscillatory dynamics beyond locomotion state (quiet vs. active), while only a small fraction of the variance in firing rates is explained by locomotion parameters such as speed and acceleration. Our findings suggest that the changes in visual cortical activity associated with locomotion may reflect the combined influence of signals related to arousal and movement.

Disclosures: **M. Vinck:** None. **U. Knoblich:** None. **R. Batista-Brito:** None. **J. Cardin:** None.

Poster

060. Striate Cortex: Neural Coding

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Topic: D.04. Vision

Support: Wellcome Trust 095667

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Title: Effects of locomotion on somatostatin- expressing interneurons in mouse visual cortex

Authors: *M. DIPOPPA, A. RANSON, M. CARANDINI, K. D. HARRIS;
Univ. Col. London, London, United Kingdom

Abstract: An outstanding question in neuroscience concerns how processing in sensory cortex is modulated by multisensory and nonsensory information. In mouse primary visual cortex (V1) locomotion increases visual responses (Niell and Stryker, 2011), and decreases the suppression that cells receive from regions surrounding their receptive fields (Ayaz et al., 2013). Surround suppression has been ascribed to interneurons expressing Somatostatin (SOM, Adesnik et al., 2012), but it is unclear whether locomotion causes their activity to increase (Polack et al., 2013) or decrease (Fu et al., 2014). We asked how the responses of SOM neurons to stimuli of different size are modulated by locomotion. We used 2-photon imaging to record from superficial-layer neurons in V1 of head-fixed mice. Mice were free to run on a spherical treadmill in front of a screen showing drifting gratings within disks of different diameters. Viral expression of GCaMP6 revealed the calcium activity of all labelled neurons in the injection area. To identify SOM interneurons among these, we injected a flex-tdTomato virus into SOM-Cre mice. Cells not expressing tdTomato were unclassified, presumably consisting mostly of pyramidal neurons. Consistent with previous reports (Adesnik et al. 2012), SOM cells integrated over a larger region of space than unclassified cells (preferred size of 30 ± 20 s.d. vs. 22 ± 16 s.d. deg, , N = 24 and 87). Locomotion increased the stimulus-driven activity of all cells (both SOM and unclassified) in response to visual stimuli of optimal size (SOM: +46%, uncl.: +32%). It increased responses of unclassified cells and SOM cells by an approximately equal factor, which depended on stimulus size. Finally, consistent with previous results (Ayaz et al., 2013), locomotion decreased surround suppression in both SOM and unclassified cells. These results indicate that locomotion

enhances the activity of SOM cells. Because SOM cells provide both inhibition and disinhibition to pyramidal cells (Pfeffer et al., 2013), the ultimate effect of this enhancement is yet to be determined. Finally, these results suggest an intriguing hypothesis, that while active behavior affects the firing of different neuronal classes in sensory cortex, the ratio between their activity levels still remains constant.

Disclosures: **M. Dipoppa:** None. **M. Carandini:** None. **K.D. Harris:** None. **A. Ranson:** None.

Poster

060. Striate Cortex: Neural Coding

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Program#/Poster#: 60.27/Z34

Topic: D.04. Vision

Support: Howard Hughes Medical Institute

Title: Stimulus feature selectivity for ultraviolet light in mouse primary visual cortex

Authors: ***Z. TAN**, W. SUN, T.-W. CHEN, D. KIM, N. JI;
Janelia Farm Res. Campus, Ashburn, VA

Abstract: The mouse has become an important model for understanding the neural basis of visual perception. Although it has long been known that mouse retina cones express two types of opsins, with ultraviolet (UV) and visible peak sensitivity, the vast majority of work on mouse visual processing uses visible light stimuli. Consequently, little is known about how UV visual information is processed in mouse brain. Using a custom UV stimulation system and *in vivo* calcium imaging, we characterized the feature selectivity of layer 2/3 neurons in mouse primary visual cortex. In young mice (immediately post-eye-opening), a minority of layer 2/3 neurons respond to UV stimuli, compared to visible stimuli. In adult mice, a comparable percentage of the neuronal population responds to UV and visible stimuli, with similar pattern selectivity and receptive field properties, indicating that UV sensitivity provides an important pathway for mouse vision.

Disclosures: **Z. Tan:** A. Employment/Salary (full or part-time):; Janelia Farm Research Campus. **W. Sun:** None. **T. Chen:** None. **D. Kim:** None. **N. Ji:** None.

Poster

060. Striate Cortex: Neural Coding

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DFG KR 1844/2-1

DFG WE-5469/2-1

Title: Mapping RFs from chronically recorded low-SNR signals in monkey visual cortex

Authors: E. DREBITZ[1], B. SCHLEDDE[1], D. WEGENER[1], *A. KREITER[2];
[1]Brain Res. Inst., Univ. of Bremen, Bremen, Germany; [2]Univ. Bremen, FB2, Bremen, Germany

Abstract: Chronic recordings with multiple electrodes implanted over extended periods of time are an increasingly important prerequisite for understanding the dynamic mechanisms of distributed parallel processing in multiple brain structures. The same requirement arises for high performance brain computer interfaces resting on detailed information from local sets of neurons with defined response properties. A common problem for such chronic recordings of neuronal responses in higher mammals is the relatively weak signal-to-noise ratio of spiking activity in comparison to conventional recordings with electrodes inserted in each recording session anew. Often, considerable numbers of electrodes show no discernable, or much smaller, spikes than the typical well isolated single unit action potential acquired from an acutely and individually positioned electrode. This raises the question whether other signals than spike trains isolated by thresholding or spike-sorting approaches can be derived from such chronically implanted electrodes and how they compare to characteristic properties of well isolated single or multi-unit spike trains. Here we investigate a signal taking the entire high frequency activity into account [Gail et al. 2000, Cerebral Cortex 10:840-850] instead of individually selected action potentials. We recorded neuronal signals with chronically implanted lacquer-insulated tungsten microelectrodes, utilizing our recently developed miniature microdrives that allow for day-by-day depth adjustment. V1 recordings were carried out in two rhesus monkeys performing a fixation task, using automated mapping procedures to characterize receptive filed (RF) size, orientation selectivity, and direction selectivity. RF properties were investigated for thresholded single- and multi-unit spiking activity, the gamma-band LFP, and the rectified and low-pass

filtered high frequency activity between 350 Hz and 12.5 kHz (RLPF). We found that based on the RLPF-signal, receptive fields could be estimated at many recording sites for which this was not possible based on spiking activity. Day to day comparisons revealed a high reliability and good reproducibility of this signal. Orientation and direction selectivity estimates were in good agreement with the spike signals. In addition, we observed similar results for the RLPF-signal for recordings in areas V4 and MT with acutely inserted electrodes. These findings indicates that the RLPF-signal, which reflects the spiking activity of the population of neurons close to the electrode tip, could be a particularly useful signal in chronic multi-electrode recordings.

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Poster

061. Visual Processing: Contrast, Form, and Color

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 61.01/Z36

Topic: D.04. Vision

Title: Shift and gain of color-tuning in V4 neurons is modulated by hue distribution in natural scenes

Authors: ***P. RAMKUMAR**[1], H. L. FERNANDES[2], M. A. SMITH[3], K. P. KORDING[2]; [1]Physical Med. & Rehabil. and Neurobio., Northwestern Univ., Chicago, IL; [2]Rehabil. Inst. of Chicago and Northwestern Univ., Chicago, IL; [3]Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Neurons in macaque area V4 are known to respond selectively to color, but their tuning properties have only been characterized using artificial stimuli. Here, we present a method to characterize their hue-tuning properties from neural activity during the free viewing of natural scenes. Within independently characterized spatial receptive fields (RF) we assume cosine-tuning to hue and orientation, allowing us to fit a generalized linear model (GLM) to spike trains as a function of hue, saturation, luminance (HSL color space representation), and orientation. For each feature, we compare our full model that allows for temporal modulation by that feature as well as a generic response to saccade onset, against a simpler model that only captures the generic saccade response. This approach allows us to identify neurons tuned to each feature. For hue-tuned neurons, we found that both preferred hue and gain were modulated by the local hue distribution (within-RF variance of hue). These results suggest that the tuning curve is not a stationary attribute of a hue-tuned neuron, but is in fact modulated by the statistics of natural scenes during active vision. Our methods allow the characterization of neural tuning properties

under a wide range of conditions, paving the way for a richer understanding of how colors are perceived in natural scenes.

Disclosures: **P. Ramkumar:** None. **H.L. Fernandes:** None. **M.A. Smith:** None. **K.P. Kording:** None.

Poster

061. Visual Processing: Contrast, Form, and Color

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Topic: D.04. Vision

Support: MIUR Italy

Title: Electrophysiological modulation in an effort to complete illusory figures

Authors: ***M. GIRELLI**[1], T. POSCOLIERO[2];
[2]Neurolog. and Vision Sci., [1]Univ. Verona Dept. Neurolog. and Vision Sci., Verona, Italy

Abstract: Visual completion is a mandatory effortful cognitive operation when we attempt to discriminate illusory figures that miss some substantial parts of their contours. In this cognitive operation a crucial role is played by the Support Ratio (SR): The proportion of the real contour with respect to the total length of the contour of the figure; thus low SRs mean large gaps between successive real parts of the contour. ERPs in humans have revealed that the early negative component N1 (140-190 ms post-stimulus) might represent the cognitive operation leading to the emerging illusory figure by visual completion of the illusory contours. The N1 component has a lateral-ventral-occipital localization in the human brain which reminds a well known area involved in general object recognition i.e. the lateral occipital complex (LOC) encompassing the lateral occipital gyrus and the posterior fusiform gyrus. In this study the SR was systematically varied, across trials, in a discrimination task between illusory figures and figureless percepts in two different experimental conditions. Physical stimulation was kept constant throughout the different SRs in order to elicit the same visual evoked potential in the different experimental conditions allowing to emphasize only the effect of the cognitive operation leading to visual completion of the illusory contours. Behavioral performance of human observers showed that in the low SR trials the reaction times were slow and very similar in the two experimental conditions as if it was very difficult to discriminate the illusory figure from the figureless percept. On the other hand, Medium and high SRs trials showed a significant

difference between the illusory figure and the figureless percept favoring the highest SR trials where the RTs were faster than in the medium and low SR trials. The ERP results were inversely correlated to behavioral results in that the N1 component showed the largest amplitude difference between the illusory figure and the figureless percept in the lowest SR trials with respect to medium and high SR trials, likely representing an effortful cognitive operation. Peak voltages in current source density as well as voltage maps onto the difference waveforms between the ERPs elicited by the illusory figure with respect to the figureless percept in the N1 latency (140-190 ms), were localized in the lateral-ventral-occipital regions of the brain which is perfectly compatible with the LOC localization. Visual completion of an illusory figure, therefore, requires an effortful cognitive operation taking place in the visual system as early as 140-190 ms post-stimulus and likely represented in the LOC of the human brain.

Disclosures: **M. Girelli:** None. **T. Poscoliero:** None.

Poster

061. Visual Processing: Contrast, Form, and Color

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Topic: D.04. Vision

Support: NIH Grant EY07977

Title: Dual representations of a visual perceptual space

Authors: *J. D. VICTOR, S. M. RIZVI, M. M. CONTE;
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Abstract: A perceptual space is a representation of a sensory domain that serves as a substrate for discrimination, classification, and working memory. Color is a classic visual perceptual space; other well-known examples include visual texture and faces. Representing a perceptual space within biological constraints is challenging, primarily because the number of dimensions needed to describe the space can be very large. Thus, a brute-force approach, in which each region of the perceptual space is independently represented at the neuronal level, leads to a dimensional explosion: the resources required to represent a space grow exponentially with the number of dimensions. Two broad classes of alternative strategies can surmount the dimensional explosion: representations via projections onto coordinates, and distributed representations via broadly-tuned neurons. Here, using the perceptual space of local image statistics as a model, we

present psychophysical studies that imply that both of these strategies are used in parallel. We worked within a perceptual space of black-and-white visual textures, parameterized by their local statistics. Each image statistic (each parameter of the space) corresponded to a type of correlation within 2x2 neighborhoods of pixels. The texture space had 10 such independent coordinates, large enough to make a brute-force representation implausible. Moreover, the space captures many of the informative statistics of natural scenes, as well as the complex interactions of contrast, edge, and corner. In one experiment, we measured the perceptual distances between well-separated points in the space via a boundary salience paradigm (4-alternative forced choice, 4 subjects). We found that along some axes, distant points that were on opposite sides of the origin were perceived as similar. This behavior cannot occur in a coordinate-based representation, but is readily explained by a distributed representation whose resources are concentrated near the origin of the space. In a second experiment, we measured perceptual distances between nearby points in the space via a segmentation paradigm (also 4-AFC, same subjects). We found that thresholds in the space's periphery and near the space's origin were similar. This is inconsistent with the above distributed representation, but is readily explained by a representation based on coordinate projections. In sum, similarity judgments in a visual perceptual space reveal the parallel operation of two representation strategies: a coordinate-based strategy that supports near-threshold judgments and a distributed one that supports suprathreshold comparisons.

Disclosures: **J.D. Victor:** None. **S.M. Rizvi:** None. **M.M. Conte:** None.

Poster

061. Visual Processing: Contrast, Form, and Color

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Program#/Poster#: 61.04/AA3

Topic: D.04. Vision

Title: Reference frame of the tilt aftereffect measured by Pavlovian differential conditioning

Authors: *Y. NAKASHIMA[1], T. IIJIMA[1], K. TOHYAMA[2], Y. SUGITA[1];
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Abstract: It has been reported that tilt aftereffect (TAE) occurs in spatiotopic frame of reference. However, it was claimed that TAE is not spatiotopic but retinotopic. We investigated the reference frame of TAE, employing differential eyelid conditioning paradigm. In conditioning

trials, a vertical or horizontal gabor patch was first presented for 3 s. 500 ms later, a patch tilted clockwise or counterclockwise was presented for 200 ms, one of which (CS+) was always followed by an airpuff, whereas the other (CS-) was not. In test trials, a patch tilted clockwise or counterclockwise was presented for 3 s as an adaptation stimulus. After 500 to 1000 ms inter-stimulus interval (ISI), a vertical test patch was presented for 200 ms. In a full adaptation condition where the adaptation and the test patches were presented in the same position, eye blink responses were observed for the test patch, when the adaptation patch was tilted in the opposite direction to the CS+. The differential responses were also observed in a retinotopic adaptation condition, where the adaptation and the test patches were presented in the same retinal but different screen position. In a spatiotopic condition where the adaptation and the test patches were presented in the same screen but different retinal position, the response was very weak and decayed as increasing ISI. These results indicate that TAE is not only retinotopic but spatiotopic, but the spatiotopic effect is very weak and decays rapidly.

Disclosures: **Y. Nakashima:** None. **T. Iijima:** None. **K. Tohyama:** None. **Y. Sugita:** None.

Poster

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Topic: D.04. Vision

Support: FQRNT

NSERC

Title: An anisotropic gain control model replicates the orientation anisotropy of overlay masking

Authors: *B. RICHARD[1], A. P. JOHNSON[1], B. HANSEN[2];
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Abstract: The suppressive effect of a broadband, in spatial frequency, mask in overlay masking is anisotropic (Kim, Haun, & Essock, 2010). Suppression is greatest at horizontal, while smallest at oblique orientations. The horizontal anisotropy when the input stimulus is broadband is typically attributed to a cortical anisotropic gain pool that stems from the overrepresentation of horizontally tuned neurons in the striate cortex (Hansen, Haun, & Essock, 2008). Under higher

levels of activity (e.g., broadband input), greater gain adjustments are applied to horizontally tuned neurons than to neurons tuned to other orientations, which results in a reduced sensitivity to horizontal content. A cortical anisotropic gain pool may also explain the horizontal anisotropy witnessed in overlay masking. We developed a network of integrate-and-fire conductance based neurons with properties similar to those of simple cells in the striate cortex, and added an anisotropic gain control mechanism to recreate the horizontal anisotropy found in overlay masking. The network contained a total of 1890 (20% inhibitory) neurons separated into three independent spatial frequency layers (tuned to the same frequency as the target stimulus, plus an octave above and an octave below). Each spatial frequency layer (630 neurons) contained 12 orientations columns (0 - 165°) with an anisotropic population density - with more neurons tuned to horizontal (0°), followed by vertical (90°), while oblique (45°) tuned neurons were least populous. Excitatory synapses between neurons were short-range (+/- 11.5°) while inhibitory synapses were long-range (+/- 45°). Gain adjustments to a high-contrast stimulus within our network involved a feedback inhibitory pool from nearby neurons (+/- 45°), modelled as a shunt conductance, in addition to background synaptic activity (noise). The combination of a shunt and noise has a divisive effect on the slope of firing rates at increasing stimulation intensities, a trademark of gain modulation. External input to the network was a sinusoidal grating, and overlay narrowband or broadband mask, oriented at either 0°, 45° or 90°. The ability of the model to detect a target stimulus was inferred from the firing rate of neurons for mask alone or target plus mask trials. Suppressive anisotropies occurred both under narrowband and broadband masks. Under narrowband masks, accuracy was highest at horizontal and lowest at oblique, the inverse occurred with broadband masks. We found that an anisotropic population density and concordant gain pool can generate suppressive anisotropies, which renders it a likely contributor to the horizontal anisotropy of overlay suppression.

Disclosures: **B. Richard:** None. **A.P. Johnson:** None. **B. Hansen:** None.

Poster

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Topic: D.04. Vision

Support: JST Presto

Title: A hierarchical sparse-coding model of natural images explains shape tuning properties in V2 and V4

Authors: *H. HOSOYA[1], A. HYVÄRINEN[2];
[1]ATR Inst., Kyoto, Japan; [2]Computer Sci., Univ. of Helsinki, Helsinki, Finland

Abstract: In light of the tremendously high dimensionality of sensory inputs, a sensible hypothesis is that the sensory cortex might employ a coding strategy that is optimized to the input statistics stemming from the natural environment. Sparse coding and independent component analysis (ICA) are well-known statistical learning models that have successfully explained various properties of V1. However, it is not clear whether this line of modeling can be extended to extrastriate areas and, in particular, direct quantitative comparisons to neurophysiological experiments are rare. Here, we extend existing learning models and investigate the connection to experimental findings on V2 and V4. We trained a four-layer model consisting of a stack of ICA modules, with image patches extracted from natural movies of various types including wild and urban life, sport, documentary, film, etc. After learning, the upper two layers in the model exhibited response properties qualitatively and quantitatively compatible with several major neurophysiological results: 1) Third layer represented subfield orientation integration with a distribution biased to smaller orientation differences consistent with a V2 study by Anzai et al. 2) Third layer further exhibited tuning to angles with response specificity to one componential orientation as in a V2 study by Ito and Komatsu. 3) Fourth layer exhibited tuning properties to position-specific curvatures with a distribution biased to acute convexities as in a V4 study by Pasupathy and Connor. However, inspection of the internal representations revealed that units of these two layers did not quite represent angles or curvatures per se. Rather, the prominent structures of most units were fairly regular, combining local orientations in co-linear or parallel ways, and thus elicited strong responses to rather straight contours or artificial textures. Many units, however, also had quite complicated structure in the detail, combining slightly or largely different local orientations, which often contributed in explaining the angle or curvature selectivities. Taken together, our results offer alternative interpretations to the existing experimental data on shape tuning in the intermediate visual areas.

Disclosures: **H. Hosoya:** None. **A. Hyvärinen:** None.

Poster

061. Visual Processing: Contrast, Form, and Color

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 61.07/AA6

Topic: D.04. Vision

Support: BMBF Grant 01GQ1004A

Title: Lateral interactions in an anisotropic population code for color predict human color induction effects

Authors: *C. KELLNER[1,2], T. WACHTLER[1,2,3];
[1]Biol. II, [2]Grad. Sch. of Systemic Neurosciences, Ludwig-Maximilians-Universität München, Planegg-Martinsried, Germany; [3]Bernstein Ctr. for Computat. Neurosci., München, Germany

Abstract: The perceived color of an object depends not only on the spectral composition of the light reflected from its surface, but also on the visual context such as illumination and background color. Such contextual interactions are thought to underlie perceptual phenomena like color constancy. A possible neuronal basis may be lateral interactions which manifest in contextual influence on the tuning of color-selective neurons in the visual cortex (Wachtler et al., 2003). Here we present a model of cortical color processing that predicts color shifts induced by chromatic backgrounds as observed in psychophysical studies. The model assumes that stimulus hue is encoded by a population of neurons with Gaussian tuning curves and preferences distributed in color space, corresponding to the finding of distributed color preferences in primary visual cortex (Lennie et al 1990). Lateral inhibitory interactions between neurons sharing the same color preferences are modeled by a Difference-of-Gaussian interaction kernel. No interactions between color channels are assumed. Due to the contextual modulation the readout of the population response showed systematic shifts in the encoded stimulus hue when stimuli were presented on colored backgrounds. The induced shifts depended on the distance between stimulus and background hues in colorspace, but were always directed away from the hue of the background. The specific shapes of the resulting induction effects strongly depended on the configuration of the population code, like the density distribution and widths of the tuning curves. Specifically, anisotropic distribution of tuning curves resulted in dependencies of the induction strength and distribution on the location in color space, in line with effects observed in psychophysical studies. The results indicate that important computations in color vision that lead to perceptual hue shifts can be realized using simple neural mechanisms when color is represented by a distributed code. References: Wachtler, T., Sejnowski, T.J., and Albright, T.D. (2003). Representation of color stimuli in awake macaque primary visual cortex. Neuron, 37, 681-691. Lennie, P., Krauskopf, J., and Sclar, G. (1990). Chromatic mechanisms in striate cortex of macaque. Journal of Neuro- science, 10, 649-669.

Disclosures: **C. Kellner:** None. **T. Wachtler:** None.

Poster

061. Visual Processing: Contrast, Form, and Color

Location: Halls A-C

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Topic: D.04. Vision

Support: Wellcome Trust/DBT India Alliance (Intermediate Fellowship to SR).

Title: Comparison of spikes versus local field potential (LFP) and its implication on brain computer interfacing applications

Authors: *S. T. KANTH, S. RAY;
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Abstract: Neurons in the primary visual cortex respond preferentially to particular features of the visual stimulus, which allows us to infer the properties of the external world by observing the spiking activity. Because spiking activity is stochastic (often following Poisson statistics), the inference is incomplete when observing a single neuron, but can be improved by pooling the responses of a population of neurons. Previous studies have shown that like spiking activity, the local field potential (LFP) is also feature selective, and therefore the properties of the stimulus can be inferred using the LFP as well. However, single trial estimate of LFP power is extremely variable because of inherent limitations of spectral estimators, which limits its use for detection or discrimination of a stimulus on a particular trial. However, variability can be reduced by averaging the power over multiple frequencies. The overall performance is expected to depend on both the choice of spectral estimator and the frequency band to average. We recorded multiunit activity and LFP from microelectrode arrays implanted in the primary visual cortex of monkeys while presenting a stimulus of variable contrast, explored the choice of spectral estimator and frequency band that can maximize the detectability of the stimulus, and compared the performance of LFPs versus spiking activity. We found that when the stimulus contrast was high (above ~25%) and a salient gamma rhythm was generated, the performance was best when only the gamma range (30-60 Hz) was used for analysis, and this outperformed the spiking activity. At lower contrasts, gamma was weak or absent and did not reveal the presence of a stimulus; best performance was obtained by using the low frequency (<20 Hz) component of the LFP instead. Overall, our results suggest that LFP power in certain frequency bands could be a useful measure for inferring the properties of the external stimulus, which in some cases can outperform the discriminability of spiking activity. These make LFPs a suitable candidate for

brain machine interfacing applications, where changes in signal power due to changes in external stimuli or behavioural state can be used to control a device.

Disclosures: **S.T. Kanth:** None. **S. Ray:** None.

Poster

061. Visual Processing: Contrast, Form, and Color

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Title: Functional organization of colors, places and faces in alert macaque frontal cortex

Authors: ***M. C. ROMERO**, K. S. BOHON, R. LAFER-SOUSA, B. R. CONWAY; Neurosci., Wellesley Col., Wellesley, MA

Abstract: Both single-unit and fMRI evidence in macaque monkeys shows that frontal cortex contains domains that are biased for face processing. But it is not known whether the analysis of faces represents a special case, or whether frontal cortex is carved up with functional subdomains showing relative specialization for other kinds of visual computations. Such local specialization appears to be found within inferior temporal cortex (IT), a region of extrastriate cortex that plays an important role in high-level visual object perception. Anatomical evidence shows that frontal cortex receives input from diverse subcortical and cortical regions, including a strong projection from inferior temporal cortex. Cumulative evidence suggests that IT comprises a parallel multi-

staged processing network including regions that are functionally biased for faces, along with other regions biased for colors, and other regions biased for places. We sought to address the extent to which the architecture observed in IT extends to frontal cortex. We measured the fMRI response to color gratings, images of familiar scenes, and faces in four passively fixating rhesus macaque monkeys. Stimuli were presented in standard block design; animals were given an intravenous iron contrast agent to boost the fMRI signal. Three different color-biased regions were observed: an anterior patch located in area 12r (found in 7/8 hemispheres tested), a ventral region on the border of areas 12r and 47 (5/8 hemispheres) and a posterior patch in area 8Av in all four monkeys (8/8 hemispheres). These color-biased regions were adjacent and non-overlapping with the previously reported face-responsive frontal patches called PO, PA, and PL. In addition, we found a place-biased region dorsal to the posterior color patches, and not overlapping with the face patches (8/8 hemispheres). The most posterior color-biased, face-biased and place-biased regions were near and may partially overlap with the frontal eye fields. Preliminary results confirm the functional bias within each of the regions defined by an independent data set. These results suggest that the frontal cortex comprises a more precise functional organization than previously recognized.

Disclosures: **M.C. Romero:** None. **K.S. Bohon:** None. **R. Lafer-Sousa:** None. **B.R. Conway:** None.

Poster

061. Visual Processing: Contrast, Form, and Color

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 61.10/AA9

Topic: D.04. Vision

Support: EU SECO grant EU216593

ETH grant 2-73246-8

Title: The cat's curious computation of contrast adaptation and normalization

Authors: ***A. J. KELLER**[1], N. M. DA COSTA[2], K. A. C. MARTIN[1];
[1]Inst. of Neuroinformatics, Zurich, Switzerland; [2]Allen Inst. for Brain Sci., Seattle, WA

Abstract: Sensory neurons encode stimulus intensity in their spike rate and adjust their sensitivity by adaptation. In the visual cortex, adaptation is crucial because the dynamic range of

individual neurons is far narrower than the range of contrasts encountered in natural scenes. The dynamic range is shaped by normalization, which assumes that the output of a large number of excitatory neurons is pooled to provide drive for recurrent divisive inhibition. The normalization model, however, does not explain the slow adaptation, which shifts the contrast response function (CRF) over seconds according to average contrast in the scene. Here we use two-photon calcium imaging of a local network in cat primary visual cortex and found that the neurons exhibit a wide range of CRFs. This effectively extends their collective dynamic range without further adaptation. In addition, however, we discovered that the parvalbumin-expressing neurons and the neurons in the most superficial sites, about half of which were GABAergic, paradoxically increase their activity during contrast adaptation. These neurons may be critical components in the circuits that provide the slower adaptation.

Disclosures: **A.J. Keller:** None. **N.M. da Costa:** None. **K.A.C. Martin:** None.

Poster

061. Visual Processing: Contrast, Form, and Color

Location: Halls A-C

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Topic: D.04. Vision

Support: DGF: Program of German–Israeli Project cooperation (DIP Grant 185/1-1)

Israeli Center of Research Excellence (I-CORE) in Cognition (I-CORE Program 51/11)

Title: Unfilled hole in the V1 representation of a pure color surface

Authors: *S. ZWEIG[1], R. M. SHAPLEY[2], H. SLOVIN[1];
[1]Bar Ilan University, The Gonda Multidisciplinary Brain Res. Ctr., Ramat-Gan, Israel; [2]Ctr. for Neural Sci., New York Univ., New York, NY

Abstract: The perceived color and brightness of a uniform surface can be influenced by its surrounding background. This perceptual phenomenon suggests an interaction between the edges of the surface and its interior. We asked what are the interactions between edges and center responses of chromatic surfaces in the primary visual cortex (V1) and how does it compare with achromatic surfaces responses. To investigate edge vs. center responses, we presented two fixating monkeys with square patches of different sizes. The patches were either chromatic squares (CS) equal in luminance to the surrounding gray background or black and white

achromatic squares (AS) with equivalent contrast to the CS. Using voltage-sensitive dyes in V1, we imaged the evoked neuronal population response at high spatial and temporal resolution. Early responses (within 40-100 ms) evoked by CS and AS were similar and had a "rectangle" like spatial pattern in the imaged cortical area. The responses to the edges of the square were high while weaker responses were observed in its center. However, responses to CS and AS, at later times were different. The population response to the center of the AS showed a slow response increase. Its amplitude at late times (>150ms) was close to the edge response amplitude. Additional analysis suggested that the late AS-response increase progressed from edges to center as if there were a neural filling-in effect. The time required for the center to fill-in increased with the square size. The CS-response in the center, however, did not fill-in; it was edge-dominated at all times for all square sizes. In summary, during early times the spatial patterns of V1 population responses evoked by both CS and AS surfaces were edge dominated. However while the center of AS gradually filled-in later, the responses to CS were maximal at the edge-projections throughout. That is, we observed no color filling-in in V1. We conclude that while chromatic and achromatic surfaces processing in V1 may have different underlying mechanisms, both are sending to higher levels of the visual pathway figures that are edge-dominated.

Disclosures: **S. Zweig:** None. **R.M. Shapley:** None. **H. Slovin:** None.

Poster

061. Visual Processing: Contrast, Form, and Color

Location: Halls A-C

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Program#/Poster#: 61.12/AA11

Topic: D.04. Vision

Support: NIH Grant SC2GM099626

Title: Characterization of the effects of stimulus size and contrast on the initial afferent response in human primary visual cortex

Authors: *N. GEBODH, M. I. VANEGAS-ARROYAVE, A. BLANGERO, S. P. KELLY; Dept. of Biomed. Engin., The City Col. of New York, New York, NY

Abstract: The early visual system, particularly primary visual cortex (V1), is the most widely characterized part of the cerebral cortex owing to intracranial recordings in animals. In human visual evoked potentials (VEP), the initial “C1” (70-100 ms) component is thought to originate from V1. The latter is based on a line of studies starting with Jeffreys and Axford (1972),

characterizing the systematic shifts in topography with visual stimulus location, which together formed what came to be known as the Cruciform model. Despite this work, the C1 is famously elusive. Due to the anatomy of V1 and the individual differences in the folding of the calcarine sulcus, certain locations of the visual field might not elicit a detectable C1 at all for a given individual, because of the orientation of the portion of V1 that it activates. The location of stimuli in the visual field is therefore critical to be able to elicit and measure the C1. As a result of such signal reliability issues, the sensitivity of the initial afferent V1 response to elementary stimulus parameters such as contrast and size have not been characterized on a level anywhere near as fine as in animal intracranial work. Our aim was to achieve such a characterization by using individualized mapping for robust C1 measurements. We first identified the optimal individual location in the visual field that elicited the largest midline posterior C1 using multifocal, pattern-pulse mapping with m-sequence stimuli at 32 polar angles around the visual field. In a follow-up session, observers maintained fixation at the center of the screen while attending to Gabor stimuli that were presented at the optimal location derived from the multifocal mapping. The subjects' task was to keep track of the number of vertically or horizontally oriented stimuli and report which was the more numerous at the end of each trial. We tested 6 contrasts ranging logarithmically from 3.13% to 100%, and 4 sizes from 1 to 4 degrees of visual angle in diameter. Preliminary data show that monotonic scaling of both the amplitude and latency of the early C1 component as a function of both the contrast and size of Gabor stimuli can be observed robustly on the single-subject basis. Remarkably, for contrasts as low as 3.13% we are able to elicit a reliable C1 based on as few as 100 trials. Beyond the purposes of fundamental characterization, a practical implication of this is that once stimuli are placed so that they project to a cortical locus within the calcarine bank, very low contrasts close to perceptual thresholds can be used to elicit robust C1s just by increasing the size of the stimulus, opening new possibilities for principled studies of early visual perception and attention.

Disclosures: **N. Gebodh:** None. **M.I. Vanegas-Arroyave:** None. **A. Blangero:** None. **S.P. Kelly:** None.

Poster

061. Visual Processing: Contrast, Form, and Color

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Topic: D.04. Vision

Support: Brown University Center for Vision Research Postdoctoral Fellowship to O.R.

Title: Human contrast sensitivity in naturalistic conditions and the effects of saccadic eye movements

Authors: *O. RUIZ[1], T. R. LII[2], M. A. PARADISO[2];
[1]VCL, Salk Inst. For Biol. Studies, La Jolla, CA; [2]Dept. of Neurosci., Brown Univ., Providence, RI

Abstract: Visual contrast sensitivity is usually studied by flashing simple patterns on blank backgrounds or by having subjects examine charts displaying gratings or optotypes. The use of simplified stimuli without visual context and the possible neglect of normal saccadic eye movements make standard procedures different from natural vision. We investigated whether a more naturalistic test paradigm may yield measurements of contrast sensitivity more reflective of natural vision. This study was motivated by previous data from our laboratory, obtained in macaque primary visual cortex (V1), which showed that V1 responses are lower after saccades across complex scenes compared to responses elicited during steady fixation (vcl.salk.edu/~oruiz). Here, we tested the prediction that the complex scenes and eye movements of natural vision may reduce human contrast sensitivity. We found that human contrast sensitivity is indeed reduced after saccades on complex backgrounds. This post-saccadic suppression occurred in peripheral vision when tested with short-duration stimuli, and extended to foveal vision using exposure times typical of natural fixations. The decrease in contrast sensitivity depended on the visual scale of the test pattern: suppression was larger at low to mid spatial frequencies (1 to 2 cycles/deg) than at higher frequencies (4 to 8 cycles/deg). The post-saccadic reduction of contrast sensitivity was found for both short-duration stimuli and, surprisingly, stimuli presented for 300 ms (a typical fixation duration). Evidently the initial post-saccadic response is critical for the visual system and prolonged fixation cannot compensate for an early response altered by a saccade. We speculate that the initial visual response at the start of a fixation is critical for contrast sensitivity and possibly other aspects of visual perception. Our results suggest that, during natural saccadic exploration, a scene is perceptually high-pass filtered, presumably increasing the salience of foveated details. The basis of post-saccadic suppression appears to be a unique combination of adaptation and surround suppression that depends critically on natural visual input and timing. Our findings suggest a modified form of contrast sensitivity measurement that would be better at predicting real-world visual performance.

Disclosures: **O. Ruiz:** None. **T.R. Lii:** None. **M.A. Paradiso:** None.

Poster

061. Visual Processing: Contrast, Form, and Color

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

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Topic: D.04. Vision

Support: Well come Trust/DBT India Alliance (Intermediate Fellowship to SR).

Title: Effect of reference scheme on power and phase of the local field potential

Authors: *A. BORTHAKUR, V. SHIRHATTI, S. RAY;
Ctr. for Neurosci., Indian Inst. of Sci., Bangalore, India

Abstract: Spectral domain analyses are powerful tools to interpret brain signals and deduce information about the underlying neuronal networks. These analyses typically estimate the power and phase at different frequencies of the signal. The slope of the power spectral density (PSD) can indicate the noise and filtering properties of the network. Similarly, the phase alignment between two brain areas at particular frequencies has been implicated in neuronal communication. Electrophysiological signals are typically recorded with respect to a reference electrode, which is placed at a location away from the region of interest. However, if this reference wire picks up any activity, the signals recorded from other electrodes may get corrupted. Several different referencing schemes have been proposed to address this issue, such as average reference (where the reference signal is the average of all the electrodes), bipolar reference (the reference electrode is one of the neighboring electrode) and current source density (CSD; for a 2-D array the reference signal is the average of the four neighboring electrodes). For proper interpretation of the spectral measures such as PSD slopes and phase differences/consistency, it is essential to inspect their dependence on the reference scheme. We recorded Local Field Potential (LFP) signals from a 2-D array of microelectrodes chronically implanted in the primary visual cortex of monkeys. After referencing the signals using four reference schemes - single-wire, average, bipolar and CSD, we computed PSD slopes, coherence and phase difference between LFPs as a function of frequency and inter-electrode distances. We found that PSD varied with respect to reference scheme at low frequencies (<100 Hz), but remained unaffected by the scheme at high frequencies. Surprisingly, the mean phase difference between sites were found to be reference dependent and yielded contradictory results for the different schemes: 0º for single-wire, 180º for average reference, and 90º for bipolar and CSD. Analyses of the coherence profile across sites and properties of the spectral estimator explained these results. Our results show that PSD slopes and phase differences are sensitive to different reference schemes, necessitating careful interpretation of these spectral measures to gain insights into the network properties.

Disclosures: **A. Borthakur:** None. **V. Shirhatti:** None. **S. Ray:** None.

Poster

061. Visual Processing: Contrast, Form, and Color

Location: Halls A-C

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Program#/Poster#: 61.15/AA14

Topic: D.04. Vision

Support: Mind Science Foundation

Title: New insights from large-scale analysis of colored-sequence synesthesia

Authors: *D. M. EAGLEMAN;
Neurosci., Baylor Col. of Med., HOUSTON, TX

Abstract: Synesthesia is a perceptual condition in which stimulation of one modality triggers anomalous experiences in another modality. For example, synesthetes may experience colors in response to overlearned sequences such as letters, numbers, weekdays and months -- a form we term colored-sequence synesthesia. We here analyzed the data from 6,588 colored-sequence synesthetes rigorously verified using the Synesthesia Battery. We found that even while the colors in an individual's alphabet may be random, there are patterns in the relationship of the colors to one another within the alphabet. For example, while letters later in the alphabet are sometimes reported as having no color, early letters (e.g., A, B, and C) are almost always colored, with colors at the farthest points away from one another in color space. This suggests that synesthesia arises in part as a mnemonic device (perhaps unconscious) that is invested in the initial challenge of learning of sequences. Second, we found that several letter groupings tend to have the same colors (irrespective of what that color is) -- for example, B, P and 8; or E,F, and 3; or H, N, and Z; or L, 7 and T. These color similarities strongly suggest an influence of shape on the color of the letter, possibly belying a clustering of similar shapes on the cortical surface, and a corresponding connectivity of that region to a particular color. Finally, we verified a common observation that the letters I and O, as well as the numbers 1 and 0, tend to be uncolored or white. Is this because 0 and 1 form the beginning of a sequence, or instead because I, O, 1, 0 are natural shapes seen by children well before they learn the meaning of an alphabet? We found that the probability of a letter-color synesthete having uncolored I and O was the same whether or not s/he also had colored numbers -- thus supporting the natural shape hypothesis. We discuss implications for different theories of the neural basis of synesthesia, introducing a new anatomical hypothesis from our neuroimaging of areas involved in overlearned sequences, as well as new data about the heritability of synesthesia in twins. The capacity to perform large-scale analysis of the colors of verified synesthetes is made possible by our laboratory's advances in large-scale, online psychophysical testing.

Disclosures: D.M. Eagleman: None.

Poster

061. Visual Processing: Contrast, Form, and Color

Location: Halls A-C

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Topic: D.04. Vision

Support: NHMRC Grant APP1027258

ARC Future Fellowship FT110100150

ARC Centre of Excellence in Vision Science

Title: Orientation anisotropies in human early visual cortex depend on contrast

Authors: *R. T. MALONEY, C. W. G. CLIFFORD;
Sch. of Psychology, UNSW Australia, Sydney, Australia

Abstract: Mechanisms of orientation processing in mammalian visual cortex appear matched to the environment, such that larger populations of cells are tuned to the cardinal orientations (horizontal/vertical) than oblique orientations. Perceptually, this property appears to be manifested in poorer sensitivity to oblique compared to cardinal orientations in a variety of tasks: the so-called *oblique effect*. Some recent functional magnetic resonance imaging (fMRI) studies have however revealed an opposite pattern of anisotropy - namely, an increased response to the oblique orientations over the cardinals: the *inverse oblique* effect. This might reflect efficient coding strategies optimised to the particular diet of orientations encountered during natural viewing. Accordingly, it might be expected that the anisotropies would change as the quality/strength of the oriented stimulation changes. In two experiments, the fMRI blood oxygenation level dependent (BOLD) signal at 3T was measured in functionally-defined visual cortex (n=5 human subjects) as a function of the orientation of a sinusoidal grating, across different stimulus contrasts (10, 30 & 100% contrast in Experiment 1; 3 & 100% contrast in Experiment 2). The results revealed a shift from the previously observed inverse oblique effect at high contrast to an oblique effect at low contrast. In Experiment 1, a significant orientation by contrast interaction was evident only in primary visual cortex. There was a similar pattern in Experiment 2 that extended to subsequent visual areas. The qualitative change in the orientation

anisotropies as a function of contrast is consistent with the idea that early visual cortex adaptively changes its coding strategy as a function of stimulus signal-to-noise ratio.

Disclosures: **R.T. Maloney:** None. **C.W.G. Clifford:** None.

Poster

061. Visual Processing: Contrast, Form, and Color

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Topic: D.04. Vision

Support: Medical Research Council, UK, G0700976

Provincia di Trento, Italy, ATTEND grant

Title: Slow activity fluctuations alter the decoding strategies of an ideal observer

Authors: ***X. CHEN**[1], M. SANAYEI[1], D. CHICHARRO[2], S. PANZERI[2], A. THIELE[1]; [1]Newcastle Univ., Newcastle Upon Tyne, United Kingdom; [2]Ctr. for Neurosci. and Cognitive Systems, Italian Inst. of Technol., Rovereto, Italy

Abstract: The area under the receiver operating characteristic curve (AUROC) is a widely used measure of the discriminability of neuronal responses to a range of stimuli. It typically involves estimating distributions of activity levels by pooling across multiple trials for each stimulus, yielding an AUROC value which indicates the degree of overlap between these distributions of activity. However, for task paradigms in which the stimuli to be discriminated are presented within the same trial, this pooling process discards valuable trial-wise information which may be used in stimulus discrimination. In this report, we present a novel method of analysis, termed DICAF ('DIfferentiation of Correlated Activity Fluctuations'), which remains robust to inter-trial fluctuations in activity and offers superior discriminability capabilities over the AUROC technique. The DICAF approach is based on a comparison of within-trial activity and a simple calculation of the proportion of trials in which the response elicited by a particular stimulus was higher than that elicited by the other stimulus. We demonstrate that it can be used to extract stimulus-related information from spiking and multiunit data during a contrast discrimination task, and we show how it relates mathematically to the AUROC measure. Neuronal recordings were taken from chronically implanted multielectrode arrays in macaque V1 and V4 while subjects were presented with stimuli of various contrast levels, at peripheral and parafoveal

visual field locations. The DICAF method outperformed the AUROC technique at both the single channel and at the population level. Furthermore, the pooling of data across an ascending number of simultaneously recorded channels was accompanied by a corresponding improvement in signal discriminability.

Disclosures: **X. Chen:** None. **M. Sanayei:** None. **D. Chicharro:** None. **S. Panzeri:** None. **A. Thiele:** None.

Poster

062. Eye Movements: Cerebellum, Brainstem, and Muscles

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Topic: D.06. Eye Movements

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Title: Encoding of prediction error by complex spikes of the cerebellum

Authors: *D. J. HERZFELD[1], Y. KOJIMA[2,3], R. SOETEDJO[2,3], R. SHADMEHR[1]; [1]Dept. of Biomed. Engin., Johns Hopkins Univ., Baltimore, MD; [2]Physiol. and Biophysics, Univ. of Washington, Seattle, WA; [3]Washington Natl. Primate Ctr., Seattle, WA

Abstract: An important hypothesis is that learning in the cerebellum is driven by prediction errors that are communicated via climbing fibers to Purkinje (P) cells, resulting in complex spikes (CSs). However, numerous experiments have found that the probability of CSs does not change as would be predicted by this simple hypothesis. What is encoded by complex spikes? Monkeys were trained to produce saccades to targets in exchange for an applesauce reward. Stainless steel recording chambers placed over oculomotor vermis (OMV) were implanted and single-unit P-cell activity was recorded. In every trial, monkeys made saccades to a target, however in some trials, we shifted the target backwards, producing a prediction error (difference

between actual eye position at saccade end and current target position). Recent work has shown that P-cells in OMV are tuned for the error direction in foveal coordinates. We confirmed these results by observing the probability of CSs in the 200ms following the primary saccade across error direction. Indeed, we found that P-cells (n=20) were highly tuned for error direction, with a tuning width of 74.5+/-7.9° (half width at half maximum height, mean±SEM). Therefore, complex spikes strongly coded the direction of the vector representing prediction error. We tested whether the presence of a CS affected learning from error. In addition, we hypothesized that CSs might signal the appropriate direction of adaptation. To test this hypothesis, we divided our data into the two sets: pairs of trials in which two consecutive primary saccades were in the same direction, or in opposite directions. When two trials were in the same direction, the presence of a CS increased the magnitude of the learning compared to the condition in which a CS was not present (paired t-test, $p < 0.05$). That is, the monkey learned more from the error when CS was present. We noted the opposite trend when the primary saccades in two consecutive trials were in the opposing direction: presence of a CS decreased learning from error ($p < 0.05$). To confirm these results, we compared the probability of CSs across error directions for trials in which adaptation was larger or smaller than the mean adaptation. When adaptation in the P-cell's preferred error direction was larger, so was the probability of CSs. Therefore, at the level of a single P-cell, probability of CS encodes direction of the prediction error vector. When a CS occurs, it drives learning in the direction of the preferred error vector, regardless of the direction of saccade. Learning from error, measured as trial to trial change in behavior, can be predicted as the population coding of this error vector.

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Poster

062. Eye Movements: Cerebellum, Brainstem, and Muscles

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 62.02/AA18

Topic: D.06. Eye Movements

Title: Mechanistic underpinnings in the primate saccadic adaptation: A cerebellar standpoint

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Netherlands; [3]Hertie institute for clinical brain research, Tuebingen, Germany; [4]Computat. Neurosci., Okinawa institute for science and technology, Okinawa, Japan

Abstract: The cerebellum has crucial roles in adaptation and learning of motor function, and in addition to these roles, its efferents project massively to the spinal cord, basal ganglia, thalamus, midbrain and cortex. These outputs are relayed by Purkinje cells, whose variations in firing rate take the shape of either smooth modulations, or event-like perturbations to its efferent targets. Event like changes are due to pauses in purkinje cell activity, which originate from inhibitory interneurons, complex spike calcium transients or indirectly through glutamate spillover. In motor function, many of the correlates of online behavior have not yet been charted. In particular, a wide body of literature has observed that pauses, seen as reductions of firing rate in peristimulus time histograms will provoke coordinated disinhibition of selected cerebellar nuclear cells, and the contribution of such events are likely to be felt by the cerebellar multiple targets. Furthermore, reductions of firing, and pauses, have since long been observed as the concomitants of motor behavior. In addition, it has also been noted that the onset of pauses is closely linked with onset of behavior. However, the end of pauses, that is, resumption of inhibition of the cerebellar nucleus, has not been thus far linked to motor function, and thus the relevance of pauses to the system have not yet been fully elucidated. While most studies show pauses at the beginning or right before the onset of motion, we are not aware of any study that demonstrated a correlation between the duration of pauses and a specific motion. We have determined that, in primates, the end of pauses in purkinje neurons correlates with the end of the saccadic eye motion (the smooth period between the end of the saccade and the fixation, denominated glissade) which can last for hundreds of milliseconds. We have shown in Vermis lobule VIc and VII, that the end of glissades (when fixation resumes) are a tight correlate of pauses. During saccadic adaptation with an intra-saccadic step, we have verified that the otherwise highly stereotypical saccades are more variable, and that this variability mainly happens at the tail of the saccade. In addition to variations in peak velocity, we have observed substantial alterations of the end of the saccadic profile, the glissadic tail. We have thus set to test the hypothesis that pauses of firing underly motor adaptation in the cerebellum of the primate. Experiments were carried out to (1) verify whether the pause-glissade correlation exists during adaptation; and (2) measure the change of glissade parameters during adaptation.

Disclosures: **M. Negrello:** None. **G. Testa-Silva:** None. **M. Junker:** None. **A. Smilgin:** None. **S. Hong:** None. **E. Avila:** None. **P. Roelfsema:** None. **P. Thier:** None. **E. De Schutter:** None. **C. Dezeeuw:** None.

Poster

062. Eye Movements: Cerebellum, Brainstem, and Muscles

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 62.03/AA19

Topic: D.06. Eye Movements

Support: MRC UK(MR/G00458/1)

Title: Transcranial direct current stimulation of the dorsal cerebellum affects saccadic adaptation

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Abstract: Reactive saccades are fast and highly precise ballistic eye movements that allow us to rapidly redirect our line-of-sight. Sensory-motor adaptation is critical to maintain saccadic accuracy in a dynamic world. Depending of the direction of the error signal, saccadic adaptation can increase or decrease saccades amplitude. Several studies in human and non-human primate have demonstrated that these two processes may be underpinned by different mechanisms, probably relying on partially distinct neural substrates. One area of the brain known to be heavily involved in saccadic adaptation is the cerebellum. Interestingly, a recent patient study has proposed separate cerebellar substrates in the adaptive control of saccade amplitude increase and decrease. The aim of the present study was to assess the role of the oculomotor vermis in saccadic adaptation in healthy human by using transcranial direct current stimulation (TDCS). In different sessions, subjects' saccades were adaptively increase or decrease using the double-step target paradigm. This paradigm consists in jumping the saccadic target to a new location while the eyes are in flight. The consequence of this target step is that at the end of the saccade, there is a discrepancy between the eyes' position and the target position. By repeating this error for about hundreds of trials, the amplitude of the saccades will be progressively lengthened or shortened, depending of the target's jump direction, allowing the eyes to land closer to the new target location. Concurrently with the adaptation paradigm, anodal, cathodal or sham TDCS was applied to the scalp over the dorsal cerebellum. In control sessions, anodal or cathodal stimulation was applied in non-adaptation session, to assess whether TDCS over the dorsal cerebellum in the absence of adaptation could change saccades metrics. Preliminary results showed that anodal or cathodal TDCS over the dorsal cerebellum does not modify saccadic accuracy. However, anodal TDCS tended to slow down both adaptive processes, while cathodal TDCS seemed to boost mainly the adaptive process increasing saccade amplitude. These preliminary results suggest that the dorsal cerebellum could play a similar role in the adaptive lengthening and shortening of saccades.

Disclosures: **M. Panouilleres:** None. **C. Miall:** None. **N. Jenkinson:** None.

Poster

062. Eye Movements: Cerebellum, Brainstem, and Muscles

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 62.04/AA20

Topic: D.06. Eye Movements

Support: MEXT, Japan

Title: Contribution of the cerebellar dentate nucleus to the generation of anti-saccades

Authors: *J. KUNIMATSU, T. SUZUKI, M. TANAKA;
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Abstract: The anti-saccade task has been used to investigate the neural mechanisms of volitional oculomotor control. In this task, subjects suppress saccades to the sudden appearance of visual stimuli (pro-saccade) and make a saccade in the opposite direction. Previous studies have shown that many cortical and subcortical regions play roles in the generation of anti-saccades, including the frontoparietal cortices (Schlag-Rey et al., 1997; Gottlieb & Goldberg, 1999; Condy et al., 2007; Johnston et al., 2014), the basal ganglia (Yoshida & Tanaka, 2009; Watanabe & Munoz, 2009, Phillips & Everling, 2013) and the motor thalamus (Kunimatsu & Tanaka, 2010). The cerebellum is also interconnected with these regions, and in fact, recent imaging studies have revealed the enhanced activity in the cerebellum during anti-saccades (Tu et al., 2006; Jamadar et al., 2013). However, its role in anti-saccades remains unclear. Here, we recorded from single neurons in the cerebellar dentate nucleus in monkeys performing anti-saccades, and examined the effects of local inactivation. Among 28 task-related neurons, more than one third showed greater activity during anti-saccades than during pro-saccades. In the population as a whole, neuronal activity in the dentate nucleus was significantly greater during anti-saccades. In addition to the transient activity associated with saccades, more than half of dentate neurons also exhibited a gradual buildup of activity prior to the target onset. In the population, these preparatory activities were also greater during anti- than pro-saccades. Because the enhanced activity disappeared in erroneous anti-saccade trials, these signals might play a role in suppressing reflexive saccades to the visual stimulus. To examine the causal role, the recording sites were locally inactivated by injecting a small amount of muscimol (5μg in 1μl) in separate experiments. For all 3 sites tested so far, the number of erroneous pro-saccades increased significantly in the anti-saccade trials in either or both directions. Furthermore, the latency of saccades was also altered during inactivation; one site delayed, while the other two sites

shortened anti-saccade latency. Our results show that signals in the cerebellar dentate nucleus do play roles in the generation of anti-saccades. These signals may be sent to the prefrontal cortex or to the basal ganglia via the thalamus. The neuronal processes in the lateral cerebellum through the dentate nucleus to the frontal cortices might be essential for the proactive control of volitional eye movements.

Disclosures: **J. Kunimatsu:** None. **T. Suzuki:** None. **M. Tanaka:** None.

Poster

062. Eye Movements: Cerebellum, Brainstem, and Muscles

Location: Halls A-C

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Topic: D.06. Eye Movements

Support: DFG Research Training Group Grant 1091

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Title: GABAergic innervation of the monkey ciliary ganglion - an electron microscopy study

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Abstract: The ciliary ganglion (CG) of vertebrates contains postganglionic, cholinergic neurons that are known to be involved in pathways mediating pupillary constriction and lens accommodation by activation of the sphincter pupillae and ciliary muscle, respectively. CG neurons are controlled by cholinergic and peptidergic afferents that originate from preganglionic motoneurons of the Edinger-Westphal nucleus (EWpg). Recent immunocytochemical studies in avian, rat and monkey ganglia applying antibodies against the GABA synthetizing enzyme, glutamate decarboxylase (GAD) revealed a dense supply of GAD-positive nerve endings targeting a subpopulation of postganglionic neurons. From double-immunofluorescence staining for choline acetyltransferase (ChAT) and GAD there is evidence that these markers co-localize in many afferent terminals within the CG. To extend these findings, we examined the CG of 3 macaque monkeys by using electron microscopy techniques on paraformaldehyde/glutaraldehyde fixed tissue. After embedding in Durcupan, ultrathin sections were processed for GABA post-embedding using immunogold labelling with an antibody to glutaraldehyde fixed GABA.

Ultrastructural analysis revealed numerous GABA-positive terminals present in the CG. Most GABA-positive terminals were observed contacting dendrites in the perisomatic neuropil. A smaller number contacted dendrites in neuropil not associated with specific cells, and a few directly contacted somata. Most somata displayed nearly exclusively GABA-positive or GABA-negative contacts on their membranes or in the perisomatic neuropil. In fact, among these GABA recipient CG neurons, less than 1% of associated nerve endings did not show immunolabeling. Those with GABA-positive contacts represented approximately 25% of all CG cell bodies. Preganglionic terminals were generally large, were filled with clear, spherical vesicles, had occasional dense-cored vesicles, and displayed asymmetric synaptic terminals. The vesicles of GABA-positive terminals appeared somewhat more pleomorphic and the synaptic densities appeared less prominent. A quantitative analysis resulted in 5.1 terminals on average associated with one CG neuron in a given section. In conclusion, a direct synaptic input from GABAergic nerve endings to a subpopulation of postganglionic neurons in the CG is confirmed in primates. This points to the presence of heterogeneity within postganglionic cell population, as only a subpopulation would be modified by the GABA co-transmitter. The function of the GABAergic innervation of the CG remains to be clarified, and the presence and action of GABA-receptors needs to be verified.

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Poster

062. Eye Movements: Cerebellum, Brainstem, and Muscles

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Topic: D.06. Eye Movements

Support: R01 DC008585

Title: Corollary discharge of head motor commands mediates primate gaze control and eye-head coordination

Authors: ***W. ZHOU**[1,2], J. HUANG[1,4], Y. XU[1], I. SIMPSON[1], W. WEI[1], K. KOSEK[3], H. ZHU[1];
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Abstract: Corollary discharge (CD) of central commands is essential for internal models of motor control. However, neural mechanisms utilizing CD remain to be elucidated. Here we show evidence in primate vestibular-oculomotor system that CD of head motor commands supersede vestibular sensory signals for active gaze stabilization (AGS), which is superior to and independent of the vestibulo-ocular reflex (VOR). First, we showed that AGS generates compensatory eye movement that is temporally synchronized with active head rotations with virtually no latency. Since the VOR lags head rotation by ~7ms, AGS is not initiated by vestibular sensory signals, but by the corollary discharge of head motor commands. Second, motor learning experiments showed that the VOR can be independently modified without affecting AGS, indicating that AGS supersedes the VOR, rather than supplement it as presently believed. Third, extraocular motoneurons exhibit different discharge patterns during AGS and VOR, suggesting that motoneurons receive different premotor inputs in the two conditions. Furthermore, we identified a group of neurons near the nucleus prepositus hypoglossi (NPH) that encode active head rotation signals and meet the criteria of mediating AGS. Since all current gaze control models treat the VOR as the sole gaze stabilization mechanism that interacts with saccade system during combined eye-head gaze shifts, these behavioral and neuronal results call for constructing new gaze control models that take AGS into consideration in order to understand the neural mechanisms underlying eye-head coordination in natural conditions (e.g., head unrestrained).

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Poster

062. Eye Movements: Cerebellum, Brainstem, and Muscles

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Topic: D.06. Eye Movements

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ORIP ODO10425

Research to Prevent Blindness

Title: Calretinin-positive neurons within the macaque oculomotor C-group may represent cell bodies of palisade endings for medial and inferior rectus muscles

Authors: *K. LIENBACHER[1], M. MUSTARI[2], S. ONO[2], A. K. E. HORN[1];
[1]Inst. of Anat., Munich, Germany; [2]Dept. of Ophthalmology, Washington Natl. Primate Res. Center, Univ. of Washington, Seattle, WA

Abstract: Extraocular muscles (EOM) are composed of two major muscle fiber types based on their innervation pattern: singly-innervated twitch fibers (SIF) and multiply-innervated non-twitch fibers (MIF). SIFs are controlled by motoneurons within the motonuclei proper, whereas MIFs are supplied from neurons in the periphery of the motonuclei. This population may also house the cell bodies of palisade endings (PE). PEs are associated with MIFs at the EOM myotendinous junction and both are suggested to play an important role in gaze stabilization and eye alignment. Currently, it is unclear, whether the peripheral neurons represent a homogeneous group that give rise to PEs and the multiple endings or whether they represent two groups, MIF motoneurons and PE cell bodies. The C-group at the dorsomedial border of the oculomotor nucleus houses the MIF motoneurons of inferior rectus (IR) and medial rectus muscle (MR). Our discovery that in monkey PEs of the MR and IR express a selective immunoreactivity for the calcium-binding protein calretinin (CR) initiated a systematic study of the C-group for the identification of CR-positive putative PE cell bodies. Macaque monkeys received tracer injections (choleratoxin subunit B or wheatgerm agglutinin) either in the muscle belly or distal myotendinous junction of the MR or IR. Double-immunofluorescence methods were used to identify retrogradely labelled neurons in the C-group and CR expression. After a distal MR injection a considerable number of tracer-labelled CR-positive neurons (up to 45%) was found in the C-group, and only few after a distal IR injection. In general, much less tracer-labelled CR-positive neurons were found in the C-group after a belly injection of IR or MR. No topographical arrangement was found, instead CR-positive and CR-negative tracer-labelled neurons were intermingled with each other at all caudo-rostral planes. The absence of CR in SIF motoneurons goes along with the lack of CR in the central “en-plaque” endings in the SIFs. In conclusion, our study indicates that two different neuron populations are present within the C-group for IR and MR, respectively. CR- positive neurons may represent PE cell bodies and CR-negative MIF motoneurons, respectively. It cannot be ruled out that some CR-positive neurons give rise to PE and multiple endings. The confinement of the CR-expression to PEs of the MR and IR indicate the need for a specific calcium buffer system in these muscles that may contribute to vergence. Malfunction of this system may play a role in strabismus or nystagmus.

Disclosures: **K. Lienbacher:** None. **M. Mustari:** None. **A.K.E. Horn:** None. **S. Ono:** None.

Poster

062. Eye Movements: Cerebellum, Brainstem, and Muscles

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Title: Precise control of microsaccades by cerebellar purkinje cells

Authors: *M. A. JUNKER, D. ARNSTEIN, A. SMILGIN, P. W. DICKE, P. THIER;
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Abstract: Microsaccades, the small saccades made when we try to maintain fixed gaze, were once believed to be inconsequential for vision. However, recent studies suggest that the preferred fixation locus is an order of magnitude smaller than the fovea, and just as macrosaccades serve to bring objects of interest to the fovea, microsaccades bring task-relevant visual targets to the preferred subregion of the fovea (Putnam et al., 2005, J. Vis., Vol. 5; Ko et al., 2010, Nat. Neurosci., Vol. 13; Poletti et al., 2013, Curr. Bio., Vol. 23). It is known that the cerebellum is necessary for precise macrosaccades, so we investigated whether microsaccades may also exploit this neural machinery in order to precisely relocate gaze. We recorded the simple spike discharge of vermal Purkinke cells (PCs) while monkeys made macrosaccades (>1° saccades) and microsaccades (<1° saccades made during attempted fixation). The vast majority increased or decreased their spike rate during macrosaccades compared to the pre-saccade baseline (126/146, 86%). Surprisingly, almost as many PCs modulated their spike rate during microsaccades (119/146, 82%). The simple spike modulation during macrosaccades was systematically related to the modulation during microsaccades. Neurons with a burst (spike rate increase) during macrosaccades usually also responded with a burst for microsaccades, and neurons with a pause (spike rate decrease) during macrosaccades usually also responded with a pause for microsaccades. The timing and direction-selectivity of the discharge were also correlated

between macro- and microsaccades. Many PCs were selective for saccade amplitude, but the preferred amplitudes ranged from the smallest microsaccades to the largest macrosaccades, and the tuning curves indicated a continuous representation of amplitude. Finally, the population average spike rate correlated well with saccade duration across both the macro- and microsaccadic domains. In sum, the oculomotor vermis of the cerebellum is involved in the control of microsaccades in addition to macrosaccades. Recent behavioral studies have shown that microsaccades are not simple "busy work" but are rather directed purposefully in order to scrutinize the fine details of a visual scene. Our findings complement these behavioral studies by uncovering the neural basis of microsaccadic precision.

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Poster

062. Eye Movements: Cerebellum, Brainstem, and Muscles

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Research to Prevent Blindness

Title: Neurophysiological and modeling evidence that strabismus is associated with cross-axis oculomotor signals in brainstem

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Abstract: Pattern strabismus is characterized by horizontal and vertical eye misalignments that are correlated with eye position along the orthogonal axis. This is typically considered to be, and treated as, abnormal action of oblique muscles but we do not know whether or how this might be related to disordered brainstem oculomotor commands. A number of studies have reported that saccades are notably disconjugate in both humans and monkeys with strabismus. Postsaccadic

drifts have also been consistently reported. Interestingly, we have found that, for our strabismic monkeys, the horizontal and vertical disconjugacies were correlated with the amplitude of the orthogonal component, suggesting the possibility that this phenomenon might share a common mechanism with “A” and “V” patterns of static fixation. In an attempt to investigate this, we have recorded single units in PPRF (n=60) and nucleus prepositus hypoglossi (NPH, n=10) while strabismic monkeys (one esotrope and one exotrope) made saccades to targets presented on a tangent screen. For both animals, the PPRF recordings revealed an abnormally broad distribution of preferred directions on one side of the brain. An unexpectedly large percentage (20%) of units modulated their activity most strongly in association with the vertical component of the saccade. Preliminary data from NPH suggest that the integration of these disordered saccade velocity commands may cause horizontal eye position signals to become “contaminated” by vertical signals. For 6/10 neurons, the tonic firing rate was significantly correlated with both horizontal and vertical eye position. It has been suggested that the neural integrators in NPH and interstitial nucleus of Cajal (INC) are shared by various oculomotor systems. We suggest, therefore, that pattern strabismus may result from the integration of cross-axis eye movement commands in brainstem. As a predictive guide for future experiments, we constructed a Simulink model by making the following modifications of the Becker and Jurgens (1990) model: 1) the horizontal and vertical burst generator gains differ for the two eyes, 2) the population drive from PPRF and riMLF differ for the two eyes, carrying differently biased cross-axis signals and 3) the neural integrators are partially separate for the two eyes. The model replicated the known aspects of saccade disconjugacy in strabismus, including amplitude and directional disconjugacies (Walton et al., 2014), post-saccadic drifts and a pattern strabismus for static fixation. If this model is correct, future recordings from INC, NPH, and riMLF will reveal cross-axis eye position and velocity signals in strabismic monkeys.

Disclosures: **M.M. Walton:** None. **S. Ono:** None. **M. Mustari:** None.

Poster

062. Eye Movements: Cerebellum, Brainstem, and Muscles

Location: Halls A-C

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Topic: D.06. Eye Movements

Support: NIH Grant EY-13239

NIH T32 EY 7125-23

Title: Activity of neurons in brainstem nucleus reticularis gigantocellularis during head-unrestrained pursuit and gaze shifts

Authors: *A. PALLUS[1], M. M. G. WALTON[2], E. G. FREEDMAN[1];
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Abstract: The nucleus reticularis gigantocellularis (NRG) has been shown in cats to contain neurons with activity related to head movements made during gaze shifts (Isa and Naito 1995). Although recordings from individual neurons in primates have not been reported, microstimulation of the NRG in rhesus monkeys has been shown to produce a variety head movements (Cowie and Robinson 1994). The rostral portion of the NRG (rNRG), in particular, produces horizontal head rotation ipsilaterally when stimulated (Quessy and Freedman 2004), and likely participates in generating head movements during gaze shifts (Freedman and Quessy 2004). When the head is not restrained, monkeys make head movements that contribute to both saccadic gaze shifts and smooth pursuit, yet these behaviors are controlled by separate neural circuitry. It is unknown whether individual neurons in the NRG drive head movements associated with these very different eye movements. The purpose of this experiment, therefore, was to investigate the activity of single units in the rNRG of rhesus monkeys during horizontal head movements contributing to these different behaviors. We recorded from neurons in the rNRG in two rhesus monkeys while they performed both horizontal delayed gaze shift and head-unrestrained pursuit tasks during each recording session. Neurons with task-related activity were chosen for further analysis. Most of these were observed to have greater firing rates during movements in one horizontal direction (the preferred direction). By controlling the initial positions of the eyes in the orbits, we were able to observe a wide range of head movements associated with identical gaze movements, allowing us to dissociate gaze, eye and head kinematics. We determined that the observed task-related activity was best correlated with head velocity. The correlation between firing rate and head movement was observed during both behavioral tasks. Despite this, small but statistically significant differences were observed in this correlation between movement types. In addition to head velocity-related activity, some neurons also showed activity related to eye position, which was especially evident when gaze was stationary.

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Poster

062. Eye Movements: Cerebellum, Brainstem, and Muscles

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Topic: D.06. Eye Movements

Support: NIH EY021581

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Title: Structure/function of precerebellar eye-velocity sensitive neurons in the caudal hindbrain of larval zebrafish

Authors: *C. L. GROVE[1], M. LEE[2], R. BAKER[1], E. AKSAY[2];
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Abstract: Adaptations enabling precision in oculomotor behavior depend upon interactions between Purkinje cells and premotor neurons that provide mossy fiber inputs to the cerebellum. In *hoxb4a:yfp* zebrafish, a prominent mossy fiber projection arises from rhombomeres 7 and 8 in the caudal hindbrain. Here we examined the structure/function relationships in this brain region associated with a group of cells, termed eye-velocity sensitive neurons (VSNs), which are believed to be involved in generating (and perhaps storing) appropriate slow-phase eye-velocity command signals from visual and vestibular inputs (Beck et al., 2006). VSNs were identified using two-photon calcium imaging in 5-9 day post fertilization *vglut:dsRed* zebrafish. As previously observed for VSNs of adult goldfish, neuronal activity correlated with changes eye velocity, but showed little sensitivity to position, fast phase responses, and spontaneous saccades. The majority of VSNs were dsRed positive and mapped to the portion of the glutamatergic scaffold associated with *alx* (~33%) and *dbx1b*(~48%) transcription factors. Subsequent electroporation of VSNs revealed at least two distinct morphologies. Cells along the *alx* domain projected ipsilaterally, whereas those along the *dbx1b* domain projected across the midline. In both cases, cells projected to the cerebellum, consistent with an excitatory mossy fiber identity. These data help specify links between the molecular, genetic, structural, and functional properties of VSNs, a prominent precerebellar neuronal group that may play an important role in velocity storage and oculomotor plasticity.

Disclosures: **C.L. Grove:** None. **M. Lee:** None. **R. Baker:** None. **E. Aksay:** None.

Poster

062. Eye Movements: Cerebellum, Brainstem, and Muscles

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Topic: D.06. Eye Movements

Support: EY014263 (PJM&SW)

BX000278-01 (JFS)

AT2009-0039 (RB)

ES04/2010 (RB)

Title: The organization of extraocular muscle motoneuronal pools in the mouse

Authors: *M. O. BOHLEN[1], S. WARREN[2], R. BLUMER[3], J. F. STAHL[4], P. J. MAY[2]; [1]Psychiatry and Human Behavior, Program In Neurosci., Jackson, MS; [2]Dept. of Neurobio. & Anatom. Sci., Univ. of Mississippi Med. Ctr., Jackson, MS; [3]Ctr. for Anat. & Cell Biology, Integrative Morphology Group, Med. Univ. of Vienna, Vienna, Austria; [4]Neurol. Division, Cleveland Dept. of Veterans Affairs Med. Ctr. & Dept. of Neurol., Case Western Reserve Univ., Cleveland, OH

Abstract: While frontal-eyed animals, particularly monkeys, have been the model of choice for oculomotor research, recent advances in the use of genetic manipulations in the mouse has increased the importance of this rodent model for vision-related research. To undergird this approach, it is necessary to define the basic parameters of the mouse oculomotor system and plant. Towards this end, we have investigated the distribution of motoneurons supplying the extraocular muscles using two mouse strains: C57BL/6 (n=5) and 129S1/Svlm (n=7) by intramuscular injections of WGA-HRP. Histologically, the abducens nucleus (VI) is represented by a cluster of neurons ventromedial to the facial nerve, and the trochlear nucleus (IV) lies in a depression in the medial longitudinal fasciculus (MLF), immediately caudolateral to the oculomotor nucleus (III). While III maintains its relationship with the MLF, it sits as separate nuclei, with small non-motoneurons lying between the two sides. Injections of the lateral rectus muscle labeled cells within the confines of ipsilateral VI. Injections of the superior oblique muscle labeled cells in contralateral IV. Injections of the inferior oblique muscle labeled cells in ipsilateral III, located predominantly rostrally. The pool forms a comma shaped cap on III rostrally, but hooks medially as the column extends caudally, so that it occupies most of the medial edge of the nucleus. The labeled inferior rectus motoneurons were most common in the middle of the rostrocaudal extent of ipsilateral III. Labeled medial rectus motoneurons were found predominantly rostrally in ipsilateral III. They filled the rostral pole of the nucleus, and

extended ventrolaterally, adjacent to and sometimes within the MLF. Labeled superior rectus motoneurons were predominantly located caudally in contralateral III. They filled the mediolateral extent of III, and also extended into the MLF. A few labeled cells were found on the opposite side for many of the muscle injections. This general pattern of muscle-related motoneuronal pools is broadly similar to that observed in other mammals. The areas of the multipolar labeled motoneurons ranged in size from 75 to 490 square μm, with a mean of 243. Examination of the muscles themselves, using anti-neurofilament protein for nerve fibers and anti-synaptophysin for contacts, reveals that mice lack palisade endings at the muscle-tendon interface. However, they do display both multiply and singly innervating forms of motor axons. In light of the large range of cell sizes and the presence of two axon types, further investigation is needed to determine whether two morphologically distinct populations of motoneurons are present.

Disclosures: **M.O. Bohlen:** None. **S. Warren:** None. **R. Blumer:** None. **J.F. Stahl:** None. **P.J. May:** None.

Poster

062. Eye Movements: Cerebellum, Brainstem, and Muscles

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 62.13/BB5

Topic: D.06. Eye Movements

Support: EY08313

Research to Prevent Blindness

Title: Independent active contractile mechanical behavior of bovine extraocular muscle(EOM) compartments

Authors: *A. SHIN, L. YOO, J. DEMER*;
UCLA / JSEI, Los Angeles, CA

Abstract: Intramuscular innervation of horizontal rectus EOMs is segregated into superior & inferior (transverse), compartments while all EOMs are also divided into global (GL) & orbital (OL) layers with scleral & pulley insertions, respectively. Mechanical independence between both types of EOM compartments has been demonstrated during passive tensile loading (Shin et al., Invest. Ophthalmol. Vis. Sci. 53: 8414-23, 2012.) We examined coupling between EOM

compartments during active, *ex vivo* contraction. Specimens of the 6 anatomic EOMs were removed from fresh adult bovine orbits, and parts of the tendon end of each EOM were attached to two independent Grass strain gauges. EOMs were immersed in 50 mM CaCl2 labeled with sodium fluorescein dye and warmed to 37°C. One compartment was coated with hydrophobic petrolatum to avoid permeation, and contraction was induced by CaCl2 in the other compartment while tensions in both channels were monitored. Control experiments omitted petrolatum, so that the entire EOM contracted. After physiologic experiments, EOMs were sectioned transversely to demonstrate specificity of CaCl2 permeation by yellow dye fluorescence excited by blue light. In control experiments without petrolatum, both transverse and GL/OL compartments contracted similarly. Contraction forces by 300 s were typically 6 gm in both transverse compartments, and 2.5 gm in GL and 1.5 gm in OL (GL is larger than OL). Selective petrolatum coating caused markedly independent compartmental contraction whether measured at the OL vs. GL insertions, or for transverse compartments at the scleral insertion. In the first 100 s, force decreased in the petrolatum-coated compartment, while increasing in the other compartment. While some CaCl2 spread occurred after 100 s, force coupling for 5 specimens still averaged only 10.5±3.3% and 6.0±1.5% in GL/OL and transverse compartment experiments, respectively. Typical contraction forces were 250 & 30 mg in GL & OL compartments, and 320 & 18 mg in superior & inferior compartments with coated OL and inferior compartments. Fluorescein penetration confirmed selective CaCl2 permeation. This experiment confirms findings of mechanical independence of EOM compartments, and extends results to active contraction. EOMs behave actively as if composed of mechanically independent parallel fiber bundles that have different insertional targets, and so differing mechanical actions, endowing each EOM with potential for separately controllable actions as proposed in the active pulley & transverse compartmental hypotheses.

Disclosures: **A. Shin:** None. **L. Yoo:** None. **J. Demer*:** None.

Poster

062. Eye Movements: Cerebellum, Brainstem, and Muscles

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 62.14/BB6

Topic: D.06. Eye Movements

Title: Synchrony in the macaque brainstem: Oculomotor system

Authors: ***A. DALE**, K. E. CULLEN;
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Abstract: The brain employs a variety of strategies for encoding signals in an ensemble of neurons, and this can be observed by investigating the collective output of a population. For example, burst-tonic neurons in the nucleus prepositus integrate burst inputs encoding eye velocity to provide abducens motoneurons with an eye position signal. The generation of this signal occurs within the nucleus prepositus itself, and is likely accomplished through local feedback loops as suggested by single-unit characterizations (Delgado-Garcia et al. 1989) and labeling studies (Escudero et al. 1992). If this is the case, then the ensemble of burst-tonic neurons should exhibit correlated activity. To obtain direct electrophysiological evidence of local connections between neurons, we simultaneously recorded pairs (n=29) of burst-tonic neurons in the nucleus prepositus of rhesus macaque monkeys using 8-channel linear microelectrode arrays. We then computed cross correlations between their spike trains during fixations at different eye positions. We found that cross correlations between burst-tonic neurons corresponded to increased coincident firing during ipsilateral fixations, which reflects the eye position sensitivity of the neurons. By accounting for the expected chance level of correlations between the two neurons in a pair, however, we found that burst-tonic neurons also exhibited positive excess synchrony, and that this excess synchrony displayed a decreasing trend with increasing eye position. These results suggest that burst-tonic neurons receive common, synchronizing input and that there is a functional change in that input during the encoding of different eye positions. Interestingly, we lastly found that the magnitude of positive excess synchrony also decayed exponentially with increasing distance between neurons (inter-channel spacing), which provides insight into the anatomical range of projections within the nucleus prepositus. When we performed the same analyses on pairs of vestibular nuclei neurons or of their afferent inputs, we found no excess synchrony between any pairs either during spiking at their resting rates or during vestibular stimulation at various frequencies and with different behavioral goals. We interpret these findings to suggest that higher-order processing of self-motion signals is achieved by pooling the independent outputs of multiple neurons. Together, our results reveal contrasting strategies - synchrony in the oculomotor system and independence in the vestibular system - by which ensembles of neurons achieve accurate downstream function.

Disclosures: **A. Dale:** None. **K.E. Cullen:** None.

Poster

062. Eye Movements: Cerebellum, Brainstem, and Muscles

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Topic: D.06. Eye Movements

Support: USPHS EY 08313

Title: High poisson ratio of contracting human superior rectus muscle indicates reverse compressibility

Authors: *L. H. YOO, R. CLARK, A. SHIN, J. L. DEMER;
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Abstract: A fundamental parameter describing the mechanical behavior of any material is the PR, the ratio of transverse to axial strain, i.e., the ratio of change in cross sectional area to change length during uniaxial loading. Measurement of the PR requires accurate 3-D determination of specimen dimensions, typically by quantitative imaging during changes in mechanical loading. During passive, *ex vivo* tensile elongation, computed x-ray tomography showed the PR of bovine extraocular muscle to be ~0.45 (Kim et al., BioMed. Res. International, 2013), but optical coherence tomography demonstrated the PR of extraocular tendon to slightly exceed the ideal incompressible value of 0.5 (Shin et al, ARVO, 2013). A remarkably high PR of 2.88 has been reported for sheep flexor tendon (Lynch et al., J Biomech Eng, 2003). Since the PR of contracting extraocular muscle (EOM) is unknown, we used magnetic resonance imaging (MRI) to determine the PR of the living human SR whose axial dimension changes physiologically during vertical gaze change. Surface coil MRI of 8 orbits of 4 normal adults was performed in ~20 deg target-controlled up and down gaze positions to obtain sets of 20 contiguous images 2 mm thick in quasi-coronal planes perpendicular to the long orbital axis of each orbit at 312 micron resolution. SR length change from computed up to down gaze positions were obtained from reconstructed sagittal images. The SR was outlined in each coronal image plane to obtain the area centroid, and the cross sectional area, and areal strain by Green's theorem. To correct for path curvature, centroids were sequentially aligned to straighten the SR for analysis. EOMs were then discretized into elements 10-20 microns long. Changes in longitudinal thickness of each element were determined to calculate strain. Mean SR volume significantly increased by 24% from down to up gaze ($P<0.005$). The mean PR from discretized 3-D models for every microscopic element in the 8 SRs was 0.76±0.06 (SD), markedly exceeding the incompressibility criterion of 0.5. A material having a PR <0.5 is said to be compressible. However, the much higher PR value for the SR muscle implies that its total volume in the active contraction significantly exceeds that in relaxation, a behavior termed reverse compressibility. Heretofore demonstrated for tendons, reverse compressibility of EOMs would strongly impact accuracy of finite element analysis simulations of EOM cooperative biomechanics and also provides a strong rationale for use of EOM volume metrics as functional indices of contractility.

Disclosures: **L.H. Yoo:** None. **R. Clark:** None. **A. Shin:** None. **J.L. Demer:** None.

Poster

062. Eye Movements: Cerebellum, Brainstem, and Muscles

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Topic: D.06. Eye Movements

Support: NIH Grant EY08313

Title: Magnetic resonance imaging (MRI) demonstrates differential compartmental function in the superior oblique (SO) muscle during vertical fusional vergence (VFV)

Authors: *J. L. DEMER[1], R. A. CLARK[2];
[1]Jules Stein Eye Inst., Jules Stein Eye Inst., LOS ANGELES, CA; [2]Ophthalmoloyg, Univ. of California Los Angeles, Los Angeles, CA

Abstract: Slight vertical binocular misalignment is normally compensated by VFV. In light of recent findings suggesting potential for differential control of SO neuromuscular compartments preferential for infraduction vs. incycloduction based upon scleral insertion site, we employed MRI to clarify the SO contribution to VFV induced by a 1° base up prism before one eye of 6 normal young adult subjects binocularly fusing an accommodative target 20 cm distant. Viewing through such a prism requires 1° infraduction, without eye rotation by the aligned fellow. Scans were repeated without prism, and with prism shifted to the opposite eye. Contractility, indicated by change in extraocular muscle (EOM) posterior partial volume, was separately analyzed in the medial (equatorial insertion, torsion) and lateral (posterior insertion, vertical) SO halves, and the superior vs. inferior horizontal rectus EOM halves. Notwithstanding anatomical evidence against compartmentalized intramuscular innervation in vertical rectus EOMs, the medial and lateral halves were separately analyzed as a control. In the infraducting eye that viewed through prism, the medial (torsional) SO half significantly relaxed by 7.4±2.1% (SEM, $P<0.005$), while the lateral (vertical) half did not change. In the aligned eye, the lateral (vertical) SO half contracted significantly but paradoxically by 7.2±2.7% ($P<0.025$), while the medial (torsional) half did not change. The vertical component of VFV was implemented mainly by the inferior rectus (IR), whose medial and lateral halves contracted similarly ($P>0.3$) by 3.3±0.9% in the infraducting eye, but paradoxically relaxed by 2.1±1.0% in the aligned eye; the interocular difference was significant ($P<0.0001$). There was no significant contractility either the medial or lateral halves of the superior rectus in either the infraducting or aligned eye ($P>0.4$). There was also no significant contractility of the inferior or superior halves of the medial rectus in either the infraducting or aligned eye ($P>0.5$). However, there was similar ($P>0.1$) contraction in both the superior and inferior lateral rectus (LR) halves that was significantly greater in the infraducting

(4.9±0.2%) than aligned eyes (-0.9±2.0%, P<0.01). We conclude that VFV is associated with striking differential SO compartmental activity: relaxation limited to the medial SO of the infraducting eye would promote excycloduction, but contraction limited to the lateral SO of the aligned eye would promote infraduction without cycloduction appropriate to balance supraduction from IR relaxation. This pattern suggests that the SO overrides balanced binocular vertical vergence commands to the IR.

Disclosures: **J.L. Demer:** None. **R.A. Clark:** None.

Poster

062. Eye Movements: Cerebellum, Brainstem, and Muscles

Location: Halls A-C

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Title: Preferential expressions of specific nicotinic receptor subtypes are different in neurons exhibiting distinct neurotransmitter phenotypes in the medial vestibular and prepositus hypoglossi nuclei

Authors: ***Y. ZHANG**, Y. YANAGAWA, Y. SAITO;
Genet. and Behavioral Neurosci., Gunma Univ. Grad. Sch. of Med., Maebashi, Gunma, Japan

Abstract: Neurons in the medial vestibular nucleus (MVN) and prepositus hypoglossi nucleus (PHN) receive cholinergic inputs from the pontomesencephalic reticular formation. Such inputs are thought to play important roles in horizontal eye movements. In our previous studies, we found that cholinergic responses mediated via nicotinic ACh receptors (nAChRs) were larger than those mediated via muscarinic ACh receptors (mAChRs) in cholinergic neurons that projected to the cerebellum. In this study, we clarified whether the expression patterns of ACh receptors are different in neurons exhibiting distinct neurotransmitter phenotypes. Individual neuron types such as cholinergic, GABA/glycinergic, and putative glutamatergic neurons were identified using specific transgenic rats and cholinergic responses to the puff application of ACh in these three neuron types were investigated using whole-cell recordings in brainstem slices. We

found that the nAChR-mediated currents were also larger than mAChR-mediated currents in all these three types of neurons. To focus on the nAChR-mediated currents, we isolated the currents from mAChR-mediated currents by the application of atropine. We detected three types of nAChR-mediated current responses that exhibited the distinct activation and desensitization kinetics and therefore designated them as fast, slow, and fast & slow types. The fast type was found to be a preferred response in both MVN and PHN GABA/glycinergic neurons, while the slow type was a preferential response in both MVN and PHN glutamatergic neurons. The cholinergic neurons showed no preferred response type. Pharmacological analyses revealed that the fast, slow, and fast & slow types were mainly mediated by α7, non-α7, and both α7 and non-α7 nicotinic receptors, respectively. These findings suggest that MVN and PHN neurons express nAChRs predominantly and show three distinct current responses dependent on the expressions of α7 and/or non-α7 receptors. Furthermore, the preferential expression pattern of the nAChR subtypes may be different in the neuron types.

Disclosures: **Y. Zhang:** None. **Y. Yanagawa:** None. **Y. Saito:** None.

Poster

062. Eye Movements: Cerebellum, Brainstem, and Muscles

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Topic: D.06. Eye Movements

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FEDER

Title: Activities of premotor extraocular neurons during rapid eye movement (REM) sleep

Authors: *M. ESCUDERO, L. C. CERVANTES, A. SANCHEZ-LOPEZ;
Univ. of Seville, Seville, Spain

Abstract: REM sleep is mainly defined by rapid eye movements, high frequency and low amplitude EEG waves and a decrease or absence of muscular tonus interrupted by twitches. We recently showed that rapid eye movements mainly occur in the abducting direction of the horizontal plane and are generated by the activity of abducens motoneurons. Although it is now know that abducens motoneurons display a tonic inhibition interrupted by phasic activities timely

associated with ponto-geniculo-occipital (PGO) waves, the source of their premotor activities remains unknown. Now we characterized the activity of premotor extraocular neurons. Six cats were prepared for chronic recording of eye movements and unitary activities. Eye movements were recorded by the scleral search coil technique. Premotor neurons were identified by its localization with respect to the abducens nucleus, its activity during wakefulness and/or its antidromic activation from the abducens nucleus. We recorded burst neurons from the reticular formation related to the generation of saccadic eye velocity; and, from the medial vestibular and prepositus hypoglossi nuclei related to the generation of eye position signals. Burst neurons showed coherent activities with the phasic events during REM sleep. Vestibular neurons showed a decrease in their mean tonic discharge but remained active during REM sleep. Prepositus hypoglossi neurons displayed phasic and tonic discharge patterns during REM sleep similar to that showed during wakefulness. Almost all neurons showed phasic inhibitions or activations in association with PGO waves. As conclusion, the premotor extraocular system seems to be not inhibited during REM sleep but its activity is not conveyed to the eye due to the inhibition of the extraocular motoneurons. Although the inhibition of the motor output during REM sleep prevents the interpretation of the oculomotor activities, the study of premotor extraocular neurons could be used as a tool to analyze dreams imagery.

Disclosures: **M. Escudero:** None. **L.C. Cervantes:** None. **A. Sanchez-Lopez:** None.

Poster

062. Eye Movements: Cerebellum, Brainstem, and Muscles

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Topic: D.06. Eye Movements

Support: NIH grant K08DC011540

Title: Central neural processes in mouse vestibular system

Authors: ***N. SHIMIZU**[1], S. WOOD[2], A. PERACHIO[1], R. COOK[1], T. MAKISHIMA[1]; [1]Univ. of Texas Med. Br., Galveston, TX; [2]Azusa Pacific Univ., Azusa, CA

Abstract: Background The central vestibular system plays an important role in higher neural functions responsible for self-motion perception and spatial orientation. The velocity storage mechanism (VSM) is one such function used to store head angular velocity. For more than three decades, VSM has been thoroughly investigated across a wide range of species. Although

numerous studies on VSM has been done in other mammalian species, very little known is known regarding that of mice. One of the reasons why the mouse is understudied is because the mouse lacks optokinetic after-nystagmus or a dominant time constant of vestibulo-ocular reflex under traditional rotational paradigms, which are frequently used to evaluate VSM. Therefore, we sought to establish new stimulus paradigms to examine the eye movements related to VSM and to verify its characteristics in mice. Methods Wild type C57BL/6J mice were used. We used a novel stimulus paradigm, pseudo off-vertical axis rotation (pOVAR), which consists of dual yaw axis rotations to generate a similar rotating gravity vector as a traditional off-vertical axis rotation (OVAR) but with a larger resultant gravito-inertial force (1 g). In pOVAR, the semicircular canals do not respond to the ongoing constant rotation, while the otolith organs are activated by the rotating vector around the subject. To study the relationship between the induced eye movements during pOVAR and the central neural processes, we further investigated the effect of baclofen, a GABA B receptor agonist, which is known to reduce the central activities of the oculomotor and the vestibular systems. Results Similar to previously reported OVAR experiments, there were two components in the steady state eye movement during pOVAR: 1) a bias component, and 2) a modulation component. During OVAR, each response is considered to be generated by two different mechanisms; for the bias component, the VSM is responsible, whereas modulation components arise predominantly through linear VOR orientation mechanisms. Baclofen significantly reduced the bias component indicating that the drug affected the central neural processes. Conclusions We revealed the dynamic characteristics of mouse eye movements during pOVAR. The findings suggest the mouse also has a process in the central vestibular system working as the primitive VSM, similar to that of other mammalian species.

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Poster

062. Eye Movements: Cerebellum, Brainstem, and Muscles

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Topic: D.06. Eye Movements

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NEI R01 EY021581

Title: Connectivity underlying persistent firing in zebrafish

Authors: *A. VISHWANATHAN[1], K. DAIE[2], A. RAMIREZ[2], A. SHOWLER[2], E. AKSAY[2], S. SEUNG[3];
[1]BCS, MIT, Cambridge, MA; [2]Dept. of Physiol. and Biophysics, Weill Cornell Med. Col., New York, NY; [3]Princeton Neurosci. Inst., Princeton, NJ

Abstract: The ability to hold one's eyes at a particular location requires a command signal to hold the eye at a desired position, the effort of will according to von Helmoltz. This signal is generated by the oculomotor neural integrator, which temporally integrates upstream eye-velocity signals to eye-position commands. Previous work has led to the hypothesis that recurrent interactions between integrator neurons are essential for generating persistent firing and that this persistent neural activity encodes the eye position. However, little is known about the precise patterns of connectivity mediating temporal integration in this system. To better understand the connectivity of this circuit, we performed two-photon calcium imaging of the larval zebrafish integrator during spontaneous eye movements followed by serial electron microscopy in the same animal. To date, we have imaged at a nominal resolution of 5 nm in x-y and 45 nm in z a volume of the caudal hindbrain that includes ~1/4 of the integrator population and a motorneuron subgroups to which they project. Preliminary reconstructions of functionally-identified integrator cells reveal two morphological classes, one cell type with an ipsilaterally-projecting axon and widely- ramifying dendritic arbor, and a second cell type with a contralaterally-projecting axon and relatively compact dendritic arbor. Additionally, we observe somatic gap junctions, numerous axo-dendritic synapses, and notable heterogeneity in the sizes of presynaptic vesicles. Detailed reconstruction and annotation of this data set promises insights into the microcircuitry underlying temporal integration.

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Poster

062. Eye Movements: Cerebellum, Brainstem, and Muscles

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Topic: D.06. Eye Movements

Support: NIDCD R01-DC2390

RFFI Grant 13-04-01736

Title: Modeling of network neuronal processing in the central vestibular nuclei

Authors: *D. OGORODNIKOV[1,2], C. C. D. SANTINA[3], J. N. ERON[4];
[1]Mount Sinai Sch. Med., NEW YORK, NY; [2]FNND LLC, Elmwood Park, NJ; [3]2Department Otolaryngology Head-Neck Surgery, Johns Hopkins Sch. of Med., Baltimore, MD; [4]3Inst. of Higher Nervous Activity and Neurophysiol. of RAS, Moscow, Russian Federation

Abstract: The neural network underlying the vestibulo-ocular reflex (VOR) is considered to include a *fast* component (mediated by a *direct pathway* including second order eye movement related neurons in the vestibular nuclei (VN)) and a *slow* component (mediated by an *indirect pathway* via vestibular-only (VO) neurons in VN). While the neural basis of fast/direct component VOR is well understood, neuronal mechanisms underlying the slow/indirect component, which involve neural circuits mediating the velocity storage mechanism, remain unclear. Recent evidence suggests that the efferent vestibular system may be involved in changing excitatory influences to central vestibular neurons that encode the slow component of VOR. In the present study, we analyzed the VOR slow component of horizontal eye movements tested in five monkeys (three *Macaca fascicularis* and two *Macaca mulatta*) and nine mice (six C57BL6 wild-type vs. three $\alpha 9^{-/-}$ mutant mice) to estimate network neuronal processing in the vestibular nuclei. Measured time constants of horizontal VOR responses and neuronal activities of central vestibular neurons during constant velocity step rotations (30-250°/s) about an Earth-vertical axis in the darkness were used for model predictions of mechanisms of neural circuits. Our model includes the following components: 1) the number of active vestibular cells in neuronal circuits; 2) unilateral and bilateral activation of neuronal circuits; 3) inhibitory inputs from vestibular-cerebellum; and 4) modulated interactions of efferent vestibular neurons to neurons in VN. Our model results suggest that (1) positive feedback is a major mechanism contributing to the "neural integrator" and (2) the value of the VOR time constant during constant velocity step rotations is predominantly determined by the weight of activated cells in both hemispheres. ***Supported by NIDCD R01-DC2390, RFFI Grant 13-04-01736***

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Poster

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Topic: D.06. Eye Movements

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Research to Prevent Blindness

Title: Compartmental innervation scheme for the mammalian superior oblique (SO) and inferior oblique (IO) muscles

Authors: *A. LE, J. L. DEMER, V. POUKENS;
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Abstract: Innervation of horizontal rectus muscles in human, monkey, and non-primate mammals divides into superior and inferior non-overlapping zones. We sought evidence for a similar innervation scheme in SO and IO muscles. Whole orbits were obtained from 4 humans: two adults, a 17 mos old toddler, and a 33 wk fetus; 2 rhesus monkeys; 1 rabbit, and 1 cow. Each orbit was formalin fixed, embedded in paraffin, coronally sectioned at 10 μm thickness, and stained with Masson trichrome. Muscle fibers and trochlear (CN4) & oculomotor nerve (CN3) branches were traced in digitally imaged serial sections using Adobe Photoshop and projected using ImageJ to reconstruct intramuscular innervation in 3-D. After en bloc excision from fresh bovine & human orbits, SO tendons removed in continuity with muscles were unrolled to trace fiber continuity topographically from innervation zone to scleral insertion. In all 4 species, CN4 bifurcates prior to entering the SO muscle. In postnatal humans, CN4 arborizes in non-overlapping superomedial & inferolateral muscle fiber zones. Fetal CN4 exhibited 4 major branches with some overlap. Monkey and bovine orbits display bifurcation of intramuscular CN4 with minimal overlap between resulting zones of innervated fibers. Gross dissection shows that the superior bovine SO compartment inserts posteriorly on the sclera for mainly vertical action, while the inferior portion inserts anteriorly for mainly torsional action. The lateral human SO belly is in continuity with the posterior scleral insertion for mainly vertical action, while the medial belly is in continuity with the anterior insertion for mainly torsional action. The rabbit SO muscle has two heads, each innervated by separate CN4 branches whose branches remain non-overlapping more anteriorly where the two heads have fused. The nerve to the monkey IO bifurcates external to the muscle innervating non-overlapping anterior and posterior zones. Compartmental innervation exists for the mammalian IO and SO. CN4 arborizes into superomedial & inferolateral branches apparently specialized for torsional vs. vertical actions, while the IO motor nerve arborizes into anterior & posterior branches whose potential specializations remain unknown. Since there is little mechanical sheer coupling between adjacent parallel muscle fibers, bifurcation of the motor nerves of the horizontal rectus and oblique muscles allows independent mechanical actions of each of their compartments.

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Poster

062. Eye Movements: Cerebellum, Brainstem, and Muscles

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Title: Decreased susceptibility to pilocarpine in Rac-GAP α-chimaerin deficient mice

Authors: *A. KATOH[1], E. TAKEUCHI[1], T. HATANAKA[1], E. SASAGAWA[2], T. IWASATO[3], S. ITOHARA[4];
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Abstract: α-chimaerin (α-chimerin), a Rac-specific GTPase activating protein, plays a key role to wire neurons precisely in the brain. Indeed, $miffy$ mice, α-chimaerin spontaneous null mutant mice, have abnormal midline crossing of corticospinal tract axons and abnormal spinal central pattern generators, resulting in exhibiting a rabbit hopping gait (Iwasato, 2007). A more recent study reported that when α2-chimaerin, one of two major isoforms of α-chimaerin and known as a gene causing Duane Retraction Syndrome, was knocked-down in neurons in oculomotor nuclei, its axon guidance was impaired in zebrafish (Clark, 2013). Here we found that $miffy$ mice were significantly less sensitive to pilocarpine which induces myosis. Instillation of 1% pilocarpine hydrochloride into the eye shrank the pupil by 80% in wild-type mice, whereas $miffy$ mice exhibited less than 40% shrinking of the pupil. The pupillary response to light occurred in $miffy$ mice but the time course of the size change of the pupil was altered compared to wild-type mice. In addition, the effect of atropine to induce mydriasis seemed more profound in $miffy$ mice than in wild-type mice. Myosis is controlled by parasympathetic nerves innervating to sphincter muscles of the pupil, originated from oculomotor nuclei through ciliary ganglion, and mydriasis is controlled by sympathetic nerves. Our current results suggest that lacking of α-chimaerin altered mechanisms balanced by sympathetic and parasympathetic nerves to regulate the pupil size.

Disclosures: **A. Katoh:** None. **E. Takeuchi:** None. **T. Hatanaka:** None. **E. Sasagawa:** None. **T. Iwasato:** None. **S. Itohara:** None.

Poster

062. Eye Movements: Cerebellum, Brainstem, and Muscles

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 62.24/BB16

Topic: D.06. Eye Movements

Support: Development of BMI Technologies for Clinical Application and Construction of System for Spread of Primate Model Animals carried out under the Strategic Research Program for Brain Sciences Japan

PRESTO, JST, Japan

KAKENHI, Japan (26120715)

Title: Fixational-saccade related and periodic activity of the pedunculopontine tegmental nucleus neurons in behaving monkeys

Authors: *Y. KOBAYASHI[1,3,2], K.-I. OKADA[1];
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Abstract: The pedunculopontine tegmental nucleus (PPTN) is the major source of excitatory projections to the midbrain and thalamus. Classical literature emphasizes that the activity of PPTN neurons plays an important role for regulating cognitive states during behavior control. However, it remains unclear how do PPTN neurons fire phasically or tonically during various behaving conditions, including saccades and reward seeking. For the phasic response we analyzed saccade-related neuronal activity (including microsaccades) of the PPTN, and for the tonic response we analyzed dynamics of task-related tonic neuronal activity of PPTN. Fixational microsaccades are small, involuntary eye movements that occur during attempted visual fixation and are affected by several cognitive process. We recorded the activity of PPTN neurons in behaving monkeys during a reward-biased visually guided saccade task, and analyzed neuronal activity for small fixational saccades during visual fixation and compared it with the activity for large visually guided targeting saccades. A population of PPTN neurons exhibited a fixational saccade-related phasic increase or decrease in activity, and many of them also showed activity modulation with large targeting-saccades and showed tonic modulation with task execution and reward expectation. Thus, fixational microsaccade-related signals of PPTN neurons share information with large-targeting saccade signal and the tonic reward expectation signal, and

might contribute to the cognitive modulation of fixational saccades. The dynamics of the tonic response of PPTN neurons might be influenced by local property of the PPTN and reverberate large-scale circuit including the PPTN. To explore dynamical modulation of the tonic firing of PPTN, we analyzed their firing regularity and periodicity for each neuron. A population of PPTN neurons exhibited a tonic regular firing pattern in that the coefficient of variation of inter-spike intervals was lower than that of theoretical random and irregular spike trains. Furthermore, a group of PPTN neurons exhibited a clear periodicity of firing. Many of these neurons exhibited the tonic regular-periodic firing pattern during highly active states, either during the task execution or resting period. The periodic firing neurons exhibited longer action potential durations and were also mainly distributed in the caudal part of the PPTN, consistent with the reported feature of cholinergic neurons. These task context-related changes in tonic and periodic discharge of PPTN neurons might regulate the monkey's motivational and arousal level to perform the cognitive task.

Disclosures: **Y. Kobayashi:** A. Employment/Salary (full or part-time):; Osaka University. **K. Okada:** A. Employment/Salary (full or part-time):; Osaka University.

Poster

063. Somatosensory System

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 63.01/BB17

Topic: D.09. Tactile/Somatosensory

Support: YCG 183446

NMA 309010

Title: Lumbosacral ganglia in female rats

Authors: N. MIRTO-AGUILAR[1], *Y. CRUZ[2];
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Abstract: Afferent neurons are critical in supplying the central nervous system information about sensory events that occur in the periphery. Their cell body reside in the dorsal root ganglia (DRG), alongside the spinal cord. The aim of this study was to characterize the L3-S1 lumbosacral ganglia in female rats. Eight adult female Wistar rats were euthanized and the L3-S1

ganglia localized and measured. In 3 animals the ganglia were collected and fixed for histological study. The tissue was sectioned at 5 μm thickness and stained with Masson's trichrome stain. The number and distribution of neurons, as well as the area of neurons were determined. The results showed that L3-S1 DRGs differ in sizes, with L4 and L5 being longer ($p<0.01$), wider ($p<0.01$) and thicker ($p<0.05$) than L6 and S1. With exception of L6 (triangular shape), L3-S1 DRGs have an elliptical shape. The histology showed that the ventral root axons ran medial to the DRG neurons. Each ganglion had large (>30 μm in diameter) and small neurons (10 μm in diameter), most of the large neurons were located near the ventral root. The ganglion with more neurons was L5 (10678±839.8), and the ganglion with less neurons was S1 (3379±556.7). Around 60% of L3, L4, L5 neurons, and more than 80% of L6 and S1, are in a range of 101-500 μm^2. The described lumbosacral DRGs neurons are related to the innervation of reproductive and excretory organs (pelvic viscera and perigenital skin), as well as to the hind limbs. The detailed knowledge in DRG neuronal organization will allow to design experiments to determine neural circuits, effect of specific sensory neuronal damage and/or regeneration in future studies.

Disclosures: **N. Mirto-Aguilar:** None. **Y. Cruz:** None.

Poster

063. Somatosensory System

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Topic: D.09. Tactile/Somatosensory

Support: NIH Grant NS064046

James S. McDonnell Foundation

USC Provost Office

Title: Effects of vibratory feedback on efficient muscle use during a redundant, one-dimensional myocontrol task

Authors: *S. A. LIYANAGAMAGE[1], M. BERTUCCO[1], N. H. BHANPURI[1], T. D. SANGER[1,2,3];
[1]Biomed. Engin., [2]Neurology, Biokinesiology & Physical Therapy, USC, Los Angeles, CA; [3]Children's Hosp. of Los Angeles, Los Angeles, CA

Abstract: Application of vibratory feedback to a muscle, proportional to its level of activity, has been shown to significantly improve the motor function of children with cerebral palsy and upper extremity motor deficits, such as in dystonia. Understanding how vibration characteristics affect improvement is important for identifying possible mechanisms of action and optimizing feedback characteristics. To this end, we studied the effects of proportionally-scaled and constant modes of vibration on target muscle use. We conducted a redundant, one-dimensional myocontrol experiment on a group of children with dystonia and age-matched controls. Using Fitts' law, we designed a task combining target widths and movement amplitudes, which resulted in 5 different Indexes of Difficulty (IDs). Subjects were instructed to flex both their right and left biceps simultaneously to move a cursor on a computer screen, and reach each of the targets as quickly as possible. The experiment was designed in a manner that allowed for multiple solutions, of which, only a subset was energetically efficient. The vibration was delivered to the more dystonic arm in children with dystonia, and the non-dominant arm in controls. Vibration was provided at a frequency either scaled in proportion to measured EMG ("scaled") or fixed at a constant level of 50 Hz ("constant"). Results show that vibration leads to increased use of the vibrated arm across all groups. Scaled vibration helps subjects reach a more energetically favorable solution to the task, while constant vibration does not. These results were consistent across all IDs. Furthermore, Fitts' Law analysis showed no significant change in the index of performance (inverse of linear regression slope of movement time vs. ID) due to vibration. In other words, vibrational feedback can be employed to help people discover different, more efficient solutions without a reduction in performance. Thus, it is possible that scaled vibration provides an additional mode of salient sensory information, while constant vibration simply enhances attention to a specific muscle. These experiments are useful for understanding the underlying mechanisms of vibratory feedback as an adjunct therapy for childhood dystonia. We plan to conduct further experiments using other modes and frequencies of vibration in order to gain a better understanding of how vibration affects muscle use in children with dystonia.

Disclosures: **S.A. Liyanagamage:** None. **M. Bertucco:** None. **N.H. Bhanpuri:** None. **T.D. Sanger:** None.

Poster

063. Somatosensory System

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 63.03/BB19

Topic: D.09. Tactile/Somatosensory

Title: Selective optogenetic activation of ChAT-Cre/channelrhodopsin-expressing primary afferents

Authors: *I. A. SPEIGEL, S. HOCHMAN;
Emory Univ., Atlanta, GA

Abstract: Somatosensory inflow to the central nervous system is complexly regulated at the primary afferent synaptic relay to the spinal cord dorsal horn. Presynaptic nicotinic acetylcholine receptors (nAChRs) modulate synaptic transmission and are abundantly distributed on primary afferent terminals. Possible sources for cholinergic modulation of these nAChRs include dorsal horn islet-like interneurons, rostral ventrolateral medulla, and primary afferent fibers expressing a choline acetyltransferase (ChAT) splice-form specific to peripheral neurons (peripheral or pChAT). GM24-Gsat/ChAT-Cre mice (GENSAT) express Cre recombinase from the initiation site of the first coding exon shared by both ChAT/pChAT. We previously demonstrated reporter-based expression in known spinal cholinergic neurons as well as in a subpopulation of primary afferents that span the rostrocaudal neuraxis and includes cutaneous mechanoreceptors (Speigel et al. Society for Neuroscience 2013). Here, ChAT-Cre mice were crossed with the channel channelrhodopsin (ChR2) expressing Ai32D Cre-dependent line to examine the spinal actions of selectively recruited ChAT expressing primary afferents. We first show ChR2 trafficked to dorsal root ganglia (DRG), dorsal root axons, spinal projections and peripheral nerve fibers, enabling further studies on possible optical excitability. We then used the isolated hemicord to assess optical excitability in juvenile ChAT::ChR2 mice. In P7-10 preparations with attached hindlimbs, blue laser-diode light delivered through optical fibers positioned onto peripheral nerves evoked compound action potentials in recordings from sciatic nerve or lumbar dorsal roots. When recording from the thoracic or lumbar dorsal root entry zone of juvenile ChAT::ChR2 isolated hemicords, optical activation of afferents in DRG or dorsal roots elicited dorsal root potentials consistent with primary afferent depolarization, a form of presynaptic inhibition. We conclude that ChAT expressing primary afferents modulate spinal somatosensory transmission, and ongoing studies will determine whether evoked responses include activation of nAChRs. Support NS-065949 and NS086370-01A1

Disclosures: **I.A. Speigel:** None. **S. Hochman:** None.

Poster

063. Somatosensory System

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Program#/Poster#: 63.04/BB20

Topic: D.09. Tactile/Somatosensory

Title: Cutaneous silent period characteristics are dependent on the organization of upper limb muscles

Authors: ***N. R. ECKERT**[1], A. W. MEEK[2], K. SMITH[2], J. C. WILLIAMS[2], Z. A. RILEY[2]; [1]Sch. of Physical Educ. and Tourism, Conventions and Event Mgmt., Indiana Univ., Indianapolis, IN; [2]Indiana Univ. Purdue Univ. Indianapolis, Indianapolis, IN

Abstract: Cutaneous silent periods (CSPs) are inhibitory spinal reflexes mediated by small diameter A-δ fibers, serving to protect the body from harmful stimuli (Leis et al., 1992; Kofler, 2003). Previously, CSPs were believed to only inhibit the extensor muscles of the upper limb to impede motions such as reaching; while other spinal circuits simultaneously excite flexor muscles to withdraw the limb. The present study sought to determine if CSPs could be evoked in both extensor and flexor muscles throughout the upper limb, thereby providing further insight into the organization of the spinal circuitry associated with this reflex. 22 subjects performed isometric contractions with each of the following upper limb muscles: abductor pollicis brevis (APB), flexor carpi radialis (FCR), extensor carpi radialis (ECR), biceps brachii long head (BIC), triceps brachii lateral head (TRI), anterior deltoid (AD), and posterior deltoid (PD). Subjects were electrically stimulated (10x perceptual threshold) with 20 individual pulses delivered to digit II (radial nerve), digit V (ulnar nerve), and paired digit II+III (digit III at perceptual threshold 100ms preceding digit II) of the right hand during each contraction, while muscle activity was recorded with electromyography. Distal muscles presented with the earliest onset times for the CSP ($F[6,21] = 15.42$), longest duration of inhibition ($F[6,21] = 65.25$), least amount of inhibition ($F[6,21] = 91$), and greatest amount of post-inhibitory rebound ($F[6,21] = 14.8$), though there were no specific differences noted for flexor-extensor muscle pairs. Furthermore, there were no significant differences across the three stimulation conditions. Linear regressions depicted that the distance a muscle is from the spinal cord can serve as a significant predictor of CSP duration (digit II $r2 = 0.43$; digit V $r2 = 0.46$; digit II+III $r2 = 0.36$, all $p < 0.001$) and the amount of inhibition (digit II $r2 = -0.51$; digit V $r2 = -0.48$; digit II+III $r2 = -0.48$, all $p < 0.001$). These results demonstrate evidence of the CSP in both flexor and extensor muscles of the upper limb, with the greatest effect taking place within the distal muscles. We hypothesize that this distal_proximal organization of cutaneous inhibitory reflexes may be influenced by the number of direct cortico-motoneuronal connections within the corticospinal tract. Thus, the cutaneous feedback plays a larger role in modulating direct descending input in distal muscles involved in grasping and manipulation, versus proximal muscles coordinating reaching.

Disclosures: **N.R. Eckert:** None. **A.W. Meek:** None. **K. Smith:** None. **J.C. Williams:** None. **Z.A. Riley:** None.

Poster

063. Somatosensory System

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Topic: D.09. Tactile/Somatosensory

Support: NIH Grant NS16446

NIH Grant NS067017

Title: Congenital foot deformation altered the topographic organization in the primate somatosensory system

Authors: *C.-C. LIAO, H.-X. QI, J. L. REED, D. J. MILLER, J. H. KAAS;
Dept. of Psychology, Vanderbilt Univ., Nashville, TN

Abstract: For various reasons, part of a limb may fail to develop during early fetal stages. Here we describe the organization of the somatosensory system in a 6 years old monkey (Macaca radiata) that was born with a deformed left foot with toes 1, 3, and 5 missing, but with the most proximal parts of toes 2 and 4 present. We characterized the patterns of peripheral afferent inputs to the brainstem, thalamocortical connections, and somatotopic organizations in the primary somatosensory area 3b on both sides. Comparisons were made with results obtained from a normal monkey (Macaca fascicularis). Injections of cholera toxin subunit B (CTB) conjugated with horseradish peroxidase (B-HRP) into the matching locations of the two feet revealed the plantar representations in the lumbar spinal cord and gracile nucleus (GrN) of the brainstem. We mapped the area 3b of both hemispheres using multiunit recordings. Injections of wheat germ agglutinin horseradish peroxidase (WGA-HRP) and CTB made into the representations of toes and plantar foot of area 3b revealed the cortical projecting neurons in the ventroposterior lateral nucleus (VPL) of the thalamus. Contrary to the orderly-arranged foot representations throughout the lemniscal pathway in the normal monkey, the plantar representations of the deformed foot were significantly expanded and intruded into the expected representations of toes in the spinal cord, GrN, VPL, and area 3b. We also observed abnormal representations of toes and plantar pad in area 3b contralateral to the intact foot. Our results demonstrated that the congenital malformation greatly influences the development of topographic organization in the somatosensory system. Alteration not only involves the ipsilateral spinal cord and GrN, and the contralateral thalamus and cortex that represent the deformed foot, but also the cortex that represents the intact foot.

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Poster

063. Somatosensory System

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Support: Cerbomed GmbH

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Rutgers University Research Fund

Title: fMRI characterization of central vagal projection sites via external ear stimulation

Authors: *B. R. KOMISARUK[1], E. FRANGOS[1], N. WISE[1], W. BIRBANO[1], K. ALLEN[1], J. ELLRICH[2];
[1]Dept Psychology, Rutgers, The State Univ. of New Jersey, NEWARK, NJ; [2]Hlth. Sci. and Technol., Aalborg Univ., Aalborg, Denmark

Abstract: Neuroanatomical and functional studies provide different views of the organization of the nucleus of the solitary tract (NTS). Evidence of viscerotopic organization of the NTS is based on stimulation of different parts of the alimentary tract, with esophagus, stomach, and intestines activating NTS regions in sequence along the length of the NTS in rats (Altschuler et al, 1992). By contrast, HRP injected into the cymba conchae of the external ear, which receives afferent innervation via the auricular branch of the vagus nerve, resulted in widespread labeling of the subnuclei of the NTS in cats (Nomura & Mizuno, 1984). In the present study using fMRI, mild electrical stimulation of the cymba conchae activated the NTS along its entire length in humans rather than in a somato- or viscerotopically-related region. Furthermore, this stimulation activated the conventional projection sites of the NTS, as demonstrated by "effective connectivity" analysis. This analysis also demonstrated the persistence of activation of the vagus projection sites for 4+ min after cessation of the stimulation. The location of the NTS was estimated with reference to the brainstem atlas of Duvernoy (Naidich et al, 2009); functionally, the NTS was "triangulated" with reference to activation of its surrounding localizers, e.g., nucleus cuneatus (finger tap), and principal trigeminal nucleus (face tap), and conjunction

analysis using a gustatory stimulus (sweet, sour, bitter, salty mixture) as a localizer that showed congruence of activation of NTS by cymba conchae stimulation. It is noteworthy that marked activation of the paracentral lobule resulted from the cymba conchae stimulation. This is consistent with our previous findings (Komisaruk et al, 2011) that vaginal and cervical self-stimulation activated the same region of the paracentral lobule in able-bodied women, and that vaginal and cervical self-stimulation in women with complete spinal cord interruption at T10 and above activated NTS, indicating a genital sensory role for the vagus nerve (Komisaruk et al, 2004). The ability to access and modulate the activity of the projections of the vagus nerve non-invasively via stimulation of the external ear could have significant therapeutic potential.

Disclosures: **B.R. Komisaruk:** 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.; Cerbomed GmbH. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Cerbomed GmbH. F. Consulting Fees (e.g., advisory boards); Cerbomed GmbH. **E. Frangos:** F. Consulting Fees (e.g., advisory boards); Cerbomed GmbH. **N. Wise:** None. **W. Birbano:** None. **K. Allen:** None. **J. Ellrich:** A. Employment/Salary (full or part-time):; Cerbomed GmbH.

Poster

063. Somatosensory System

Location: Halls A-C

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Program#/Poster#: 63.07/BB23

Topic: D.09. Tactile/Somatosensory

Title: Effect of cutaneous silent period on cortical output in proximal-distal muscles in the upper limb

Authors: ***A. W. MEEK**[1], N. R. ECKERT[2], K. SMITH[1], J. C. WILLIAMS[1], Z. A. RILEY[1]; [1]PETM Dept., Indiana Univ. Purdue Univ. Indianapolis, Indianapolis, IN; [2]Indiana Univ., Bloomington, IN

Abstract: A single pulse of high intensity electrical current delivered to the digits of the hand produces a period of decreased electromyographic (EMG) activity, or a cutaneous silent period (CSP), in muscles of the upper limb (e.g. thenar, triceps brachii) during voluntary contractions (Caccia and Violini 1973; Uncini et al. 1991; Inghilleri et al. 1997). Pairing transcranial magnetic stimulation (TMS) with digit stimulation results in motor evoked potentials (MEPs)

with reduced amplitudes in a thenar muscle (Kofler, 2008), suggesting a direct inhibitory influence on the descending drive from supraspinal centers through noxious cutaneous stimulation. However, it is not known whether this occurs only in hand muscles where there are a greater number of direct cortico-motoneuronal connections, or whether similar behavior can be observed in more proximal upper limb muscles. The current study investigated the inhibitory influence of the CSP on the descending drive for several muscles throughout the upper limb. 14 subjects performed isometric contractions, while EMG was recorded, with the following muscles: abductor pollicis brevis (APB), flexor carpi radialis (FCR), extensor carpi radialis (ECR), biceps brachii (BIC), triceps brachii (TRI), anterior deltoid (AD), and posterior deltoid (PD). During the isometric contractions, subjects experienced three different stimulation conditions: electrical stimulation of digit II (10x perceptual threshold) only (CSP), transcranial magnetic stimulation (TMS), and a pairing of digit II stimulation and TMS (TMS+). 10 individual pulses were completed for each stimulation condition, in random order, resulting in 40 total stimulations for each of the seven muscles. Comparisons of the MEP size showed significant differences ($p< 0.05$) between the TMS and TMS+ conditions. Specifically, the TMS MEP was significantly greater than the TMS+ MEP for APB ($p <0.001$), FCR ($p =0.006$), and BIC ($p <0.049$) muscles, though the opposite relationship was seen within the PD ($p <0.047$) muscle. An ANOVA test of normalized MEP values (TMS+/TMS) compared across muscles showed significant differences in APB vs TRI ($p = 0.004$) and PD ($p = 0.003$), and in FCR vs TRI ($p = 0.046$) and PD ($p = 0.037$) muscles. The results suggest that the CSP plays a differential role in modulating descending drive resulting in suppressed MEP amplitudes within distal muscles while facilitating MEP amplitudes within more proximal muscles. We hypothesize that this differential response may be due to the number of direct cortico-motoneuronal connections to distal muscles versus proximal muscles and could help to coordinate grasp (distal) versus reach (proximal) motor control.

Disclosures: **A.W. Meek:** None. **Z.A. Riley:** None. **K. Smith:** None. **J.C. Williams:** None. **N.R. Eckert:** None.

Poster

063. Somatosensory System

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Program#/Poster#: 63.08/BB24

Topic: D.09. Tactile/Somatosensory

Title: Zona incerta regulates communication between superior colliculus and POm

Authors: *G. D. WATSON, J. B. SMITH, K. D. ALLOWAY;
Ctr. for Neural Engin., Penn State Univ., University Park, PA

Abstract: The zona incerta is known for receiving dense projections from the superior colliculus and for sending inhibitory projections to the medial posterior thalamic nucleus (POm). To delineate the anatomical and functional specificity of the tectoincertal projections, different anterograde tracers were separately injected into visual-sensitive and whisker-sensitive parts of the superior colliculus. We found that the superior colliculus projects to the zona incerta, but not to a neighboring structure, the subthalamic nucleus. A crossing pattern was also observed in which visual-sensitive neurons in the medial superior colliculus project to the lateral zona incerta, whereas projections from whisker-sensitive collicular neurons terminate in more medial sectors of the zona incerta. In separate tracing experiments, we found that whisker-sensitive regions in POm receive projections mainly from the lateral sector of the zona incerta. Collectively, these anatomical findings implicate the zona incerta with a role in mediating communication between the superior colliculus and POm. To address this hypothesis, we have begun a series of experiments in which the responses of neurons in superior colliculus, ZI, and POm are recorded during sensory stimulation. In our initial study, we have recorded whisker-sensitive neurons in superior colliculus and POm simultaneously. The results indicate that neurons in both superior colliculus and POm respond to low frequency whisker deflections. During continuous whisker stimulation at 8 Hz, however, neurons in superior colliculus show significant adaptation whereas neurons in POm become more responsive.

Disclosures: **G.D. Watson:** None. **J.B. Smith:** None. **K.D. Alloway:** None.

Poster

063. Somatosensory System

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Topic: D.09. Tactile/Somatosensory

Support: NIH Grant NSO72454

Title: Analysis of proprioceptive endings in the mouse soleus muscle using a transgenic mouse model

Authors: M. J. SONNER, *D. R. LADLE;
Neurosci, Cell Bio, Physiol, Wright State Univ., DAYTON, OH

Abstract: Muscle spindles and Golgi tendon organs (GTOs) are encapsulated, stretch-activated sensory receptors housed within skeletal muscles, yet their functional contributions to proprioceptive monitoring of limb movements are quite different. For example, muscle spindles are responsible for detecting stretch of muscle fibers, and GTOs provide feedback regarding muscle tension during contraction. Peripheral axons extending from proprioceptive sensory neurons in the dorsal root ganglia supply muscle spindles and GTOs. Muscle spindles are innervated by Group Ia and II sensory fiber endings, while GTOs are innervated by a single Group Ib sensory fiber. The developmental process guiding axons from these neurons to their targets and ultimate functional identities in skeletal muscle, however, remains to be elucidated. To study these proprioceptive sensory neurons and their peripheral endings more closely, we took advantage of existing transgenic mouse models using cre-lox recombination technology in which all proprioceptive afferents are labeled with red fluorescent protein (Parvalbumin-Cre/+; Rosa-CAG-LSL-tdTomato-WPRE). Here we present results of confocal microscopy analysis of proprioceptive endings from whole-mount neonatal mouse soleus muscle preparations. From our survey of the right soleus muscle of 19 mice at postnatal ages 3 to 7 days, we found the soleus muscle to contain 10.8 ± 0.3 (mean ± SEM) muscle spindles and 5.2 ± 0.2 GTOs. We then analyzed a subset of proprioceptive endings at higher magnification and observed that GTOs were consistently innervated by a single proprioceptive axon (n = 20 GTOs). Numbers of proprioceptive axons innervating muscle spindles were variable, ranging from 1 to 5 (2.3 ± 0.2, n = 39 spindles). A consistent characteristic of innervation of the soleus muscle is the presence of thin and thick branches of the common soleus nerve, which diverge prior to muscle entry. The thin branch only supplies sensory endings housed within the proximal compartment of the muscle, while the thick branch provides both sensory and motor innervation to the remainder of the muscle. Our analysis revealed 4 to 9 proprioceptive axons are found within the thin branch (6.0 ± 1.1, n = 4 muscles). Invariably, the thin branch contained axons terminating in GTOs. The number of thin branch spindle afferents was more variable, ranging from 0 to 7. Our preliminary analysis suggests the thin branch often contains a mix of Ia and/or II spindle afferents, but occasionally contains muscle spindle afferents of only one type. This model may provide a useful tool in analysis of muscle spindle and GTO maturation during embryonic and early postnatal development.

Disclosures: **M.J. Sonner:** None. **D.R. Ladle:** None.

Poster

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Topic: D.09. Tactile/Somatosensory

Support: NIH Grant NS16446

NIH Grant NS 08476

Title: Barreloid-like structures in the ventroposterior medial subnucleus of the somatosensory thalamus in prosimian galagos

Authors: *E. K. SAWYER[1], C.-C. LIAO[1], H. QI[1], P. BALARAM[1], D. MATROV[2], J. H. KAAS[1];
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Abstract: Galagos (Otolemur garnetti) are prosimian primates that appear to more closely resemble ancestral primates than any of the present-day anthropoids. As in many other mammals, facial whiskers of galagos are distributed across the upper and lower jaws and above the eye. In rats and mice, the mystacial macrovibrissae are famously represented throughout the ascending trigeminal lemniscal pathway as arrays of cytoarchitecturally distinct modules with each module having a one-to-one relation with a specific facial whisker. The macrovibrissa representations are termed barrelettes, barreloids, and barrels in the trigeminal somatosensory brainstem, the ventroposterior medial subnucleus of the thalamus, and primary somatosensory cortex, respectively. Despite the presence of facial whiskers in all non-human primates, similar barrel structures have not been reported in primates. Using cytochrome oxidase, Nissl stains and immunohistochemistry for vesicular glutamate transporters, we show a distinct array of barreloid-like modules in the ventroposterior nucleus of galagos. Labeled thalamocortical and corticothalamic connections demonstrate that barreloid-like modules are located in an area of the somatosensory thalamus that is topographically consistent with a role in facial touch. The presence of barreloid-like modules in galagos suggests the existence of broad similarities in the processing of sensory information in rodents and primates.

Disclosures: **E.K. Sawyer:** None. **C. Liao:** None. **H. Qi:** None. **P. Balaram:** None. **D. Matrov:** None. **J.H. Kaas:** None.

Poster

063. Somatosensory System

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 63.11/BB27

Topic: D.09. Tactile/Somatosensory

Support: NIH Grant EY02686

NIH Grant NS16446

Title: VGLUT1 and VGLUT2 identify driving (class 1) and modulatory (class 2) glutamatergic projections in the somatosensory system of prosimian, New World, and Old World primates

Authors: *P. BALARAM, E. K. SAWYER, J. H. KAAS;
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Abstract: Vesicular glutamate transporters (VGLUTs) regulate the uptake and release of glutamate from presynaptic vesicles of glutamatergic neurons in the central nervous system. Two VGLUT isoforms, VGLUT1 and VGLUT2, are differentially expressed in subsets of glutamatergic projections across the mammalian brain and appear to confer distinct properties of glutamate release to their host neurons. Previous studies of VGLUT1 and VGLUT2 expression in the primate visual system suggest that glutamatergic projections involving VGLUT2 correspond to Class 1 projections, which are capable of driving the activity of their post-synaptic targets. In contrast, glutamatergic projections involving VGLUT1 correspond to Class 2 projections, which primarily modulate the activity of postsynaptic cells. In order to determine whether VGLUT1 and VGLUT2 identify similar functional segregations of glutamatergic circuits in other primate sensory systems, we examined the distribution patterns of VGLUT1 and VGLUT2 across major subcortical nuclei and cortical areas of the dorsal column and trigeminal pathways in prosimian galagos, New World monkeys, and Old World monkeys. From the spinal cord to the cortex, these pathways contain a multitude of functionally distinct glutamatergic projections that have been shown to utilize VGLUT1, VGLUT2, or both VGLUT1 and VGLUT2 in nonprimate species. Thus, we examined the expression patterns of VGLUT1 and VGLUT2 mRNA and protein in the cuneate, gracile, and principal trigeminal nuclei of the brainstem, the ventroposterior nucleus of the thalamus, and areas 3b and 1 of somatosensory cortex. Similar to glutamatergic visual projections, driving somatosensory projections primarily utilize VGLUT2 while modulatory somatosensory projections primarily utilize VGLUT1. In addition, somatosensory nuclei of the thalamus appear to express both VGLUT1 and VGLUT2 in overlapping populations of glutamatergic projections, similar to thalamic visual projections in primates and thalamic somatosensory projections in rodents. Glutamatergic projections from somatosensory cortex predominantly utilize VGLUT1, but some subsets of cortical projections appear to utilize VGLUT2, similar to findings in the primate visual system and the rodent somatosensory system. These findings demonstrate that VGLUT1 and VGLUT2 differentiate between driving and modulatory glutamatergic projections across primate and nonprimate sensory systems.

Disclosures: **P. Balaram:** None. **E.K. Sawyer:** None. **J.H. Kaas:** None.

Poster

063. Somatosensory System

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Strategic Research Program for Brain Sciences from the MEXT of Japan

Title: The precuneus is involved in the detection of incongruency between tactile and visual texture information: A functional MRI study

Authors: *R. KITADA[1], A. T. SASAKI[2], Y. OKAMOTO[3], T. KOCHIYAMA[4], N. SADATO[1]; [1]Div. of Cerebral Integration, Natl. Inst. For Physiological Sci., Okazaki, Japan; [2]RIKEN Ctr. for Life Sci. Technologies, Kobe, Japan; [3]Fukui Univ., Fukui, Japan; [4]ATR Brain Activity Imaging Ctr., Seika-cho, Japan

Abstract: Visual information can lead to incorrect conclusions regarding the physical substance of manufactured objects. For example, a plastic ring can look like a real gold ring. However, we can avoid misidentifying an object's substance by comparing visual and tactile information. As compared to the spatial properties of an object (e.g., orientation), little information regarding physical object properties (material properties) is shared between vision and touch. How can such different kinds of information be compared in the brain? One possibility is that visuo-tactile comparison of material information is mediated by associative memories previously learned between the two modalities. Previous studies suggested that a cortical network involving the medial temporal lobe, lateral prefrontal cortex and precuneus plays a critical role in the retrieval of information in associative memory. Here, we conducted a functional MRI study to test if these brain regions are involved in the visuo-tactile comparison of material properties. Stimuli consisted of surfaces in which an oriented plastic bar was placed on a background texture. Twenty-two healthy subjects determined whether the orientations of visually- and tactually-

presented bar stimuli were congruent in the orientation conditions, and whether visually- and tactually-presented background textures were congruent in the texture conditions. The texture conditions revealed greater activation of the fusiform gyrus, medial temporal lobe and lateral prefrontal cortex compared with the orientation conditions. In the texture conditions, the precuneus showed greater response to incongruent stimuli than to congruent stimuli. This incongruency effect was greater for the texture conditions than for the orientation conditions. These results suggest that the precuneus detects incongruency between tactile and visual texture information via the medial temporal lobe and lateral prefrontal cortex. Our findings highlight the contribution of the cross-modal association to the visuo-tactile texture comparison.

Disclosures: **R. Kitada:** None. **A.T. Sasaki:** None. **Y. Okamoto:** None. **T. Kochiyama:** None. **N. Sadato:** None.

Poster

063. Somatosensory System

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Topic: D.09. Tactile/Somatosensory

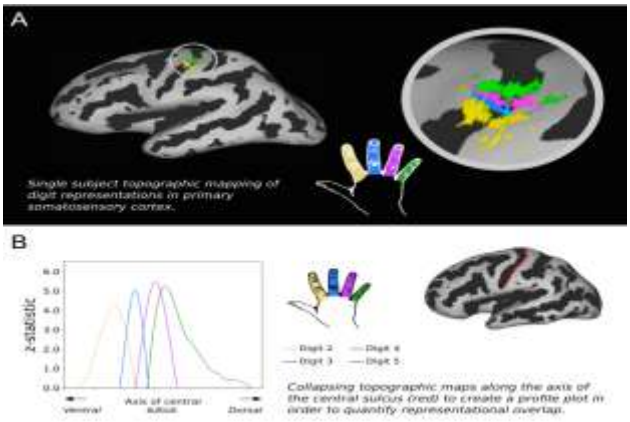
Support: Wellcome Trust Senior Research Fellowship to HJB

Title: Short-term use-dependent remapping of digit topography in human primary somatosensory cortex

Authors: ***J. KOLASINSKI**, T. MAKIN, S. JBABDI, C. STAGG, H. JOHANSEN-BERG; Univ. of Oxford, Oxford, United Kingdom

Abstract: The regions comprising primary somatosensory cortex (SI) are organized somatotopically, forming multiple sensory body map representations. The cortical representations of the digits are much exaggerated relative to their actual size, reflecting their dense sensory innervation and their potential role in the tactile acuity required for complex dexterous tasks. Electrophysiological studies in non-human primates have demonstrated the potential for use-dependent remapping of these representations in response to altered sensory inputs over the course of months. In this study we considered the potential for use-dependent plasticity in SI digit representations in human subjects, induced by a manipulation in which digit 2 (index) and digit 3 (middle) of the right hand are glued together. To map SI sensory representations of individual digits in human participants we applied BOLD fMRI at 7 tesla with

an isotropic resolution of 1.2mm using a self-administered phase-encoding paradigm. This approach produced robust and reproducible maps of SI digit topography (Fig. 1A) with high inter-session reliability. We further collapsed the digit maps along the axis of the central sulcus, producing a profile plot of the digit representations and their overlap for each session (Fig. 1B). Digit topography was mapped in nine participants before and after a 24-hour control period, and a 24-hour glued period using a repeated measures (RM) counterbalanced design. The gluing manipulation induced a reduction in the overlap of digits 3 (middle) and 4 (ring), and an increase in the overlap of digits 4 (ring) and 5 (little). These changes were not identified in an equivalent 24-hour control period (RM ANOVA: Significant interaction between condition and digit overlap, $F(4,32)=7.96$, $p<0.001$). This remapping provides a fundamental insight into the nature of plasticity in the human brain, demonstrating the potential for use-dependent reorganisation in digit representations not directly involved in the glued manipulation, and the propensity for such changes over a period as short as 24 hours.



Disclosures: **J. Kolasinski:** None. **T. Makin:** None. **S. Jbabdi:** None. **C. Stagg:** None. **H. Johansen-Berg:** None.

Poster

063. Somatosensory System

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Topic: D.09. Tactile/Somatosensory

Support: NIH Grant NIGMS5R25GM096161

Rutgers University Research Fund

Title: Transition to orgasm/ejaculation and to the refractory period in men: fMRI evidence

Authors: *K. ALLEN, N. WISE, E. FRANGOS, P. LAKSHMIN, W. BIRBANO, B. R. KOMISARUK;
Dept. of Psychology, Rutgers Univ., Newark, NJ

Abstract: After ejaculation, most men experience a "refractory period" during which they find it difficult or impossible to stimulate a subsequent orgasm. The neural processes underlying this period are still unknown. In the present study, we compared neural activity during orgasm to the activity during the post-orgasm refractory period. Functional Magnetic Resonance Imaging (fMRI) data were collected using a Siemens Trio 3T head-only scanner with each participant immobilized with a custom-fitted, thermoplastic whole-head stabilization mask. During genital self-stimulation to orgasm, subjects used a button-press to indicate the start of orgasm/ejaculation, the end of orgasm, and the end of any residual post-orgasmic feelings. The end of these residual feelings was the criterion we used to identify the onset of the refractory period. Neural responses to the first 6 seconds (3 TRs) of the refractory period were modeled and compared to the first 6 seconds of orgasm (preprocessing and analysis completed using AFNI). The brain regions found to have significantly different activations (cluster-corrected $p < 0.05$) during orgasm/ejaculation versus during the refractory period included the frontal cortex, temporal lobe, lentiform nucleus, hippocampus, septum, operculum (SII), anterior and posterior cingulate cortex, precuneus, pons, and cerebellum. Neural activity during the first 6 seconds of orgasm was also compared to the 6 seconds immediately preceding orgasm. A significant difference was observed in pons. The present findings provide evidence of distinctly different regional brain activity correlated with the physiological and subjective differences that distinguish between orgasm/ejaculation, the moments immediately prior to orgasm/ejaculation, and the refractory period following orgasm/ejaculation.

Disclosures: **K. Allen:** None. **N. Wise:** None. **E. Frangos:** None. **P. Lakshmin:** None. **W. Birbano:** None. **B.R. Komisaruk:** None.

Poster

063. Somatosensory System

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Topic: D.09. Tactile/Somatosensory

Support: Rutgers University RUBIC

NIH-NIGMS 5R25 GM 096161

Rutgers University Research Fund

Title: Brain regional activation upon transition to self- and partner-induced orgasm in women: An FMRI analysis

Authors: *N. J. WISE, E. FRANGOS, K. ALLEN, W. BIRBANO, P. LAKSHMIN, B. R. KOMISARUK;
Psychology, Rutgers Univ., Newark, NJ

Abstract: The present study analyzed differences in regional brain activity occurring during self-induced and partner-induced orgasm in women (Ns=5). Functional Magnetic Resonance Imaging (fMRI) data were collected using a Siemens Trio 3T head-only scanner with custom-fitted, thermoplastic whole-head stabilization masks. Preprocessing and analysis were performed using FSL. In order to sample equivalent time points across the participants' variable durations of stimulation and orgasm, the first 10sec of orgasm was compared to the 10sec of genital stimulation immediately preceding orgasm. No significant deactivations in frontal cortical or other brain regions were noted for either the self-induced or partner-induced orgasm group. When the two groups were combined to assess the common activity during the first 10sec of orgasm compared to the global baseline, widespread activations (cluster corrected, $p < 0.05$) were noted, including the paracentral lobule (genital sensory cortex), primary sensory cortex (face and viscera regions), secondary sensory cortex, precuneus, supplementary motor areas, primary motor cortex, frontal pole, anterior and posterior cingulate, insula, nucleus accumbens, hypothalamus, hippocampus, amygdala, ventral tegmentum, corpus callosum, cerebellum, and pons. Comparison of the first 10sec of orgasm with the 10sec immediately preceding orgasm for the combined group (N=10) indicated that the activity of many of these regions increased significantly upon the onset of orgasm. These findings provide evidence that the activity of multiple brain regions, including primary sensory, motor, sensory- integration, and reward regions reach peak activity at orgasm.

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Poster

064. Thalamocortical Mechanisms

Location: Halls A-C

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Program#/Poster#: 64.01/BB32

Topic: D.09. Tactile/Somatosensory

Support: BIOTACT EU FP7

Title: Changes in whisking behaviour during object exploration in cortically altered mice

Authors: *R. A. GRANT[1], N. H. GAMBLES[1], T. J. PRESCOTT[2];
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Abstract: Rodents rhythmically sweep their whiskers back and forwards in a behaviour termed 'whisking'. During surface exploration, rodents employ active control strategies, which increases the information acquired from an object. The emergence of these behaviours in neonates coincides with the development of upstream cortical regions; however, limited studies have specifically examined the role of the cortex in active vibrissal sensing. This study examines the effect of cortical alterations on inquisitive whisker behaviours in three different transgenic mice: Barreless, RIM-DKO-sert and a Robo3 Knockout. Vibrissae movements were tracked in video recordings during contact and non-contact episodes and whisker kinematics were measured including; frequency, amplitude, asymmetry, retraction, offset and spread. All strains of mice showed behaviours that differed from controls during object contact. In particular, the RIM-DKO-sert mice showed a reduction in exploratory behaviour during active touch exploration. These findings suggest that the cortical regions of the brain are involved in whisking behaviours during object exploration, and, in particular, the thalamocortical circuits effect inquisitive, exploratory whisker movements.

Disclosures: **R.A. Grant:** None. **N.H. Gambles:** None. **T.J. Prescott:** None.

Poster

064. Thalamocortical Mechanisms

Location: Halls A-C

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Topic: D.09. Tactile/Somatosensory

Support: MEXT 23115515, 23115721, 26430022,

Japan-US BRCP

NIH NS048884

Title: Topographical precision in the thalamocortical projection mediated by cannabinoid receptor in the developing barrel cortex

Authors: C. ITAMI[1,2,3], J.-Y. HUANG[2,3], H.-C. LU[2,3], *F. KIMURA[4];
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Abstract: Cannabinoids, the major psychoactive component of marijuana, have involved with human lives for more than thousands of years, but how they affect neuronal circuit formation is not well understood. Here, we provide evidence that cannabinoid plays a major role in the development of precise formation of thalamocortical projection. We previously reported that before P14, thalamocortical terminals to L2/3 cells express cannabinoid receptor (CB1R), and these synapses exhibit CB1R-mediated spike timing-dependent LTD (STDP-LTD, or tLTD). In addition, L4-L2/3 synapses, which are undergoing massive synaptogenesis at this age, exhibit spike timing-dependent LTP (tLTP) (Itami, 2012, J. Neurosci.), Thus, two STDPs with opposite directions converge onto L2/3 cells from L4 and thalamus, and we showed evidence that these STDPs interact each other, shaping the precise thalamocortical projection. To explore whether CB1R-tLTD is the underlying cellular mechanism, pharmacological studies were conducted. Consistent with our hypothesis, neonatal treatment with WIN (CB1R agonist) strongly suppressed the thalamocortical projection. In contrast, blocking CB1R signaling with AM281 (CB1R antagonist) reversed the suppression of thalamocortical projection. To obtain further evidence and quantification, we examined the morphology of individual thalamocortical axons of CB1R-KOs at P12 using DiI-labeling technique. In wild type littermate controls, each thalamocortical axons were well-confined within L4 barrels, as consistent with previous studies. In CB1R heterozygotes and KO mice, however, significant increase in the number of thalamocortical axons were found in L2/3. Moreover, those axons were often beyond the barrel boundary, too. Taken together, these results provide evidence that cannabinoid signaling plays an important role in wiring neural circuits in the primary somatosensory cortex during early postnatal development.

Disclosures: **C. Itami:** None. **H. Lu:** None. **F. Kimura:** None. **J. Huang:** None.

Poster

064. Thalamocortical Mechanisms

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Topic: D.09. Tactile/Somatosensory

Support: Bogazici Univ. BAP 6747S

Bogazici Univ. BAP 13XP8

Title: Multivariate tactile processing in children and adolescents with obsessive-compulsive disorder

Authors: ***B. GUCLU**[1], C. TANIDIR[2], E. CANAYAZ[1], B. GUNER[1], H. IPEK TOZ[2], O. UNERI[2], M. TOMMERDAHL[3];
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Abstract: Obsessive-compulsive disorder (OCD) is considered to be associated with basal ganglia dysfunction, and was previously shown to reduce sensory gating in cortical areas. We tested whether OCD may alter tactile processing in the somatosensory cortex by using a battery of psychophysical experiments which applied mechanical vibrations on the skin. 32 patients with OCD (age range: 7-18, 11 male, 21 female) and 32 sex- and age-matched healthy controls participated in the study. The tactile stimuli were mechanical vibrations (25 Hz) generated by a portable device (CM-4, Cortical Metrics). The stimuli were applied to the fingertips and the participants responded based on a two-site forced-choice task. The test battery consisted of the following experiments: simple/choice reaction time (stimulus amplitude: 300 μm, duration: 40 ms), dynamic detection threshold (amplitude ramp: 2 μm/s), amplitude discrimination (standard: 200 μm, duration: 0.5 s), and amplitude discrimination with single-site adaptation (adapting stimulus: 200 μm, duration: 1 s). OCD participants performed similarly to controls except in the amplitude discrimination experiments. Specifically, OCD participants had significantly higher (one-tailed t-test, $p = 0.012$) discrimination limens (113 μm) than those from the controls (84 μm). Adaptation caused a significant increase of the discrimination limen for both groups (paired t-test, both p's < 0.002), but this increase was almost identical (72 μm for OCD, 78 μm for controls). We did not find any sex-based differences except that the females had worse discrimination than males after adaptation in the control group (one-tailed t-test, $p = 0.006$). Preliminary multivariate analyses based on Mahalanobis distances and jackknife classification showed that 79% of the OCD participants could be allocated to the correct group (classification sensitivity). On the other hand, 43% of the controls were classified as normal (specificity). The psychophysical results are consistent with the reduced sensory gating hypothesis and suggest cortical hyperexcitability at suprathreshold tactile inputs in OCD. However, in order to achieve normal Weber fractions internally, the lower level (i.e. standard) stimulus should be weighed more than the comparison stimulus during amplitude discrimination. We are currently working to improve multivariate classification test by including clinical data.

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Poster

064. Thalamocortical Mechanisms

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Topic: D.09. Tactile/Somatosensory

Support: NSF Collaborative Research in Computational Neuroscience Grant IOS-1131948

NIH National Institute of Neurological Disorders and Stroke Grant 2R01-NS-048285

Title: Bottom-up sensory adaptation shifts the balance of thalamic burst/tonic firing

Authors: *C. SHEPHARD[1], C. WAIBLINGER[2,3], C. SCHWARZ[2,3], G. STANLEY[1]; [1]Wallace H Coulter Dept. of Biomedial Engin., Georgia Tech/Emory, Atlanta, GA; [2]Systems Neurophysiol., Werner Reichardt Ctr. for Integrative Neurosci., Tübingen, Germany; [3]Dept. of Cognitive Neurol., Hertie Inst. for Clin. Brain Research, Univ. of Tübingen, Tübingen, Germany

Abstract: Beyond acting as a simple relay from the periphery to cortex, the thalamus acts as a "gate" for the peripheral signals, controlling what does and does not get transmitted to cortex. Furthermore, this gating is dynamic, and can be influenced through both bottom-up sensory influence, and top-down mechanisms related to wakefulness and attention. In this work, we explored the bottom-up effect of stimulus adaptation on the encoding of features in the whisker thalamocortical circuit of the fentanyl-cocktail anesthetized rat using a classic signal-in-noise paradigm. Previous work has demonstrated that adaptation can lead to enhanced discriminability paired with reduced detectability, but the underlying mechanism is unknown. We hypothesize that bottom-up sensory adaptation shifts the gating of the thalamus from a burst encoding mechanism to a tonic encoding mechanism through depolarization of the thalamic membrane potential. In the context of the signal-in-noise paradigm, the level of the background stimulus "noise" adapts the pathway, effectively shifting thalamic neurons from burst to tonic firing for conveying information related to the embedded "signal". Preliminary experimental results suggest that thalamic cells (n=12) fire more burst spikes in response to "signals" presented in isolation than in noise and that this leads to a higher detectability, but a lower discriminability, as assessed using an ideal observer analysis of the thalamic unit spiking activity. We developed an integrate and fire neuron with an incorporated burst mechanism to systematically explore the

mechanisms underlying this shift from a burst to a tonic encoding scheme with increasing stimulus "noise" amplitudes. Consistent with the experimental findings, the model suggests that the "noise" is depolarizing the membrane potential of the simulated cell. Furthermore, a simple depolarization of the neuron will induce this shift and lead to a switch in the coding scheme suggesting that the depolarization of the thalamic neurons is one mechanism to alter information flow in the thalamocortical pathway. These experimental and computational findings are supported by previous results from our lab that suggest discrimination is enhanced in response to adapting stimulus contexts whereas detection is enhanced when information is presented in isolation.

Disclosures: **C. Shephard:** None. **C. Waiblinger:** None. **C. Schwarz:** None. **G. Stanley:** None.

Poster

064. Thalamocortical Mechanisms

Location: Halls A-C

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Program#/Poster#: 64.05/CC3

Topic: D.09. Tactile/Somatosensory

Title: Changes in sensorimotor cortex excitability caused by arm immobilization

Authors: ***Y. OKAMOTO**[1,4], S. YAMAMOTO[2], N. TAKESHITA[2], Y. UMEHARA[1,4], M. OSHIMA[1,6], M. MONMA[3], Y. KOHNO[5], K. NUMATA[2];
[2]Physical Therapy, [3]Radiological Sci., [1]Ibaraki Prefectural Univ. of Hlth. Sci., Ibaraki, Japan; [4]Rehabil., [5]Neurol., Ibaraki Prefectural Univ. of Hlth. Sci. Hosp., Ibaraki, Japan; [6]Rehabil., Jonan Hosp., Ibaraki, Japan

Abstract: It is well known that muscle weakness and atrophy is induced by disuse (e.g., by cast immobilization), but little is known about neural adaptation in sensorimotor cortex caused by limb immobilization. We employ transcranial magnetic stimulation (TMS) and somatosensory evoked potentials (SEPs) to investigate sensorimotor cortex excitability induced by 10-hour arm immobilization. Nineteen healthy right-handed subjects were separated into two experimental groups (TMS, 10 subjects; SEP, 9 subjects). In the TMS group, we used single-pulse TMS to study resting motor threshold (RMT) and motor evoked potentials (MEPs), and paired-pulse TMS to study short-interval intracortical inhibition (SICI) and intracortical facilitation (ICF). MEPs were recorded from the left and right first dorsal interosseous muscles. In the SEP group, SEPs were obtained by electrical stimulation of the median nerve and recorded bilaterally from

Fz, on sensory areas for the hands, on the C7 spinous process, and on Erb's point. In the TMS group, following single-pulse TMS, RMT significantly increased and MEP amplitude significantly decreased on the immobilized side, but no significant changes were observed contralateral to the immobilized side. Paired-pulse TMS caused no significant changes in SICI and ICF on either side. In the SEP group, the amplitude potential of the N30 component recorded from Fz upon stimulation of the constrained hand increased significantly, whereas no significant changes were observed for the other potentials. Ten hours of arm immobilization can lead to modulation of cortical processing of motor output and sensory input. Decreased primary motor cortex (M1) excitability occurred without changes in SICI and ICF, and increased N30 amplitude thought to derive from the supplementary motor area (SMA) reflected activated SMA. We assume that this phenomenon is regulated by cortico–basal ganglia circuits. However, the details remain unclear, and further study is required.

Disclosures: **Y. Okamoto:** None. **S. Yamamoto:** None. **N. Takeshita:** None. **Y. Umehara:** None. **M. Oshima:** None. **M. Monma:** None. **Y. Kohno:** None. **K. Numata:** None.

Poster

064. Thalamocortical Mechanisms

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ERC Advanced Grant (Arthur Konnerth)

Title: Rapid thalamocortical signal transfer through linear dendritic integration in layer 4 cortical neurons *in vivo*

Authors: *H. JIA, Z. VARGA, B. SAKMANN, A. KONNERTH;
Inst. For Neuroscience, Tech. Univ. Munich, Munich, Germany

Abstract: Mammalian cortical neurons compute sensory information that arrives through numerous synaptic inputs located on dendritic spines. A highly effective method to study

individual spine signaling and dendritic integration is two-photon Ca^{2+} imaging. However, due to technical difficulties in the imaging accessibility under *in vivo* conditions, Ca^{2+} imaging studies of dendritic spines had been largely restricted to superficial layers of the cortex, as well as the superficial dendritic compartments of deep-layer cortical neurons. Here we introduce an approach that allowed us to directly monitor single spine Ca^{2+} signals in layer 4 (L4) spiny stellate cells of the vibrissal mouse cortex *in vivo*, which allowed recordings of sensory stimulation-evoked Ca^{2+} transients in dendritic spines of L4 barrel cortical neurons at depths of up to 520 μm under the cortical surface. With the help of two-photon imaging guided navigation, we loaded single neurons in L4 with the Ca^{2+}-sensitive fluorescent indicator OGB1 by means of single-cell electroporation. Recordings were targeted in a subregion of the mouse vibrissal cortex corresponding to the C2 whisker, which was identified by intrinsic optical imaging. A LOTOS (low power temporal oversampling) method of two-photon Ca^{2+} imaging (Chen et al., Nature 2011; Varga et al., PNAS 2011; Hill et al., PNAS 2013) of dendritic spines was performed in depths ranging from 310 μm to 520 μm. Loose cell-attached recordings were also performed in combination with Ca^{2+} imaging of dendritic spines. By analyzing NMDA receptor-mediated Ca^{2+} signaling in spiny dendrites, we found that whisker stimulation produced large responses in spines but not in the parent dendrites. We identified stimulation-activated spines with short response latencies of about 10 ms, representing predominantly thalamo-cortical input sites. These short-latency responsive spines were denser at proximal dendritic regions comparing to distal regions. The amplitude of sensory-evoked spine Ca^{2+} transients was independent of the activity of neighboring spines, without evidence for cooperativity. Furthermore, we found that spine Ca^{2+} transients evoked by back-propagating APs (bAPs) summed linearly with sensory-evoked synaptic Ca^{2+} signals. Finally, the amplitudes of Ca^{2+} transients in both dendritic shafts and whisker-responsive spines increased linearly with respect to the number of APs evoked by whisker stimulation. Thus, taken together, our results identify in sensory information-receiving L4 cortical neurons a linear mode of dendritic integration that underlies the rapid and reliable transfer of peripheral signals to the cortical network.

Disclosures: **H. Jia:** None. **Z. Varga:** None. **B. Sakmann:** None. **A. Konnerth:** None.

Poster

064. Thalamocortical Mechanisms

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 64.07/CC5

Topic: D.09. Tactile/Somatosensory

Support: NIH, RO1 NS-050434

NIH, P50 MH086400

NIH, F32 NS-084763

DARPA-BAA-09-27

Title: The corticothalamic switch: controlling the thalamus with dynamic synapses

Authors: *S. R. CRANDALL, S. J. CRUIKSHANK, B. W. CONNORS;
Dept. of Neurosci., Brown Univ., Providence, RI

Abstract: Layer 6 corticothalamic cells provide massive excitatory input to thalamus. This "top-down" projection is thought to influence thalamic throughput of sensory signals by modulating the excitability and firing state of thalamic cells. Corticothalamic neurons monosynaptically excite thalamocortical cells, but also indirectly inhibit them by driving inhibitory cells of the thalamic reticular nucleus. Whether the overall impact of corticothalamic activity is to suppress or excite thalamic relay neurons remains unclear. Here we show that the influence of layer 6 on thalamic excitability is dynamic, with the excitatory-inhibitory balance shifting in an activity-dependent fashion. During low-frequency activity corticothalamic effects are mainly suppressive, whereas higher frequency activity (even short gamma-frequency bouts) converts the corticothalamic influence to enhancement. This shift depends upon the interactions of excitatory and inhibitory synaptic components of the CT circuit that have distinct forms of short-term synaptic plasticity. Clarifying these dynamic network mechanisms provides a fresh framework for interpreting the powerful but varied effects of corticothalamic activity on sensory processing.

Disclosures: **S.R. Crandall:** None. **S.J. Cruikshank:** None. **B.W. Connors:** None.

Poster

064. Thalamocortical Mechanisms

Location: Halls A-C

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Program#/Poster#: 64.08/CC6

Topic: D.09. Tactile/Somatosensory

Support: MB-UMBC Research and Innovation Partnership Seed Grant

Title: Roles of GABAA and GABAB receptors in regulating thalamic activity by the zona incerta: A computational study

Authors: *A. PARK[1], K. HOFFMAN[2], A. KELLER[1];
[1]Anat. & Neurobio., Univ. of Maryland, Baltimore, Baltimore, MD; [2]Mathematics and Statistics, Univ. of Maryland, Baltimore County, Baltimore, MD

Abstract: The posterior thalamic nucleus (PO) is a higher order nucleus heavily implicated in the processing of somatosensory information. We have previously shown in rodent models that activity in PO is tightly regulated by inhibitory inputs from a GABAergic nucleus known as the zona incerta (ZI). The level of ZI inhibition varies under both physiological and pathological conditions, leading to concomitant changes in PO activity. These changes are causally linked to a variety of phenomena from altered sensory perception to pathological pain. ZI regulation of PO is mediated by GABAA and GABAB receptors (GABAAR and GABABR) that differ in their binding kinetics and their electrophysiological properties, suggesting that each may have distinct roles in incerto-thalamic regulation. Thus, we developed a computational model to test this hypothesis. We created a two-cell Hodgkin-Huxley model representing PO and ZI with kinetically realistic GABAAR and GABABR mediated synapses. We simulated spontaneous and peripherally evoked firing in PO, and observed how these activities were affected by inhibition mediated by each receptor type. We show that spontaneous PO activity is preferentially regulated by GABABR mediated mechanisms, while evoked activity is preferentially regulated by GABAAR. We also show that modulation of ZI firing rate and synaptic GABA concentrations are effective means to regulate the incerto-thalamic circuit. Differential regulation of peripherally evoked and spontaneous activity by GABAAR and GABABR, respectively, represents an opportunity for the development of therapeutics, as different aspects of incerto-thalamic regulation and thus, thalamic function, can be targeted through manipulation of a specific class of GABAergic receptors. Thus, these findings may help provide interventions for pathologies of sensory processing such as altered states of arousal and chronic pain.

Disclosures: **A. Park:** None. **K. Hoffman:** None. **A. Keller:** None.

Poster

064. Thalamocortical Mechanisms

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Program#/Poster#: 64.09/CC7

Topic: D.09. Tactile/Somatosensory

Support: ISF 1160/11

ISF 1565/10

Minerva

DFG SFB 1089

Title: Cortical amplification dynamics of thalamic inputs in the barrel cortex

Authors: *K. COHEN-KASHI, B. MOHAR, Y. KATZ, I. LAMPL;
Weizmann Inst. of Sci., Rehovot, Israel

Abstract: Anatomical and electrophysiological studies have shown that thalamic inputs to layer 4 (L4) of sensory systems constitute only a small fraction of the total number of excitatory synaptic connections in this layer. Indeed, optogenetic inactivation of cortical firing demonstrated recently that only about a third of the sensory evoked excitatory current in L4 of the visual and auditory cortices can be accounted as thalamic input. In the barrel cortex, whisker stimulation evokes an early (50ms) excitatory response followed by a complete return to baseline and a delayed slow excitatory response that lasts up to few hundreds of milliseconds. The later response was hypothesized as having a role in the perception of the stimulus in behaving mice. Here, we evaluated the time course of cortical amplification of excitatory L4 cells response in the barrel cortex following whisker stimulation. To that end, we optogenetically silenced the cortex of lightly anesthetized GAD-ChR2 transgenic mice while intracellularly recording excitatory currents. While optogenetic silencing of cortical firing reduced the total early excitatory response to a single deflection of the primary whisker (PW) by ~53%, the late response was eliminated almost completely (~87%), indicating that it depends on cortical amplification. Furthermore, the fact that the excitatory current returned to baseline after the termination of the early component suggests the involvement of other layers or other cortical regions in the generation of the late response phase. The late response evoked by stimulation of adjacent whiskers (AW) was smaller compared to that evoked by the PW, indicating that cortical amplification is mostly column specific. Finally, we measured the contribution of thalamic versus intracortical inputs during repetitive whisker stimulation (10 stimuli, 20Hz). In a subset of cells (n = 10) which demonstrated repetitive response, cortical amplification increases during adaptation (relative thalamic input of 49.2±9.4% vs. 36.6±7.4% first vs. last five stimuli respectively), probably reflecting the contribution of the late cortical response. In summary, whisker stimulation evokes a fast early excitatory response followed later by a much slower excitatory response that reflects locally confined reverberating cortical activity. The late response may play an important function in amplifying thalamic inputs during adaptation and thereby increasing the throughput of information to the cortex.

Disclosures: **K. Cohen-Kashi:** None. **B. Mohar:** None. **Y. Katz:** None. **I. Lampl:** None.

Poster

064. Thalamocortical Mechanisms

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Topic: D.09. Tactile/Somatosensory

Support: DFG Grant GR3757/1-1

Boehringer Ingelheim Fonds

Max Planck Society

Title: Coding of active and passive whisker touches in mouse somatosensory thalamus

Authors: A. L. SUMSER, R. A. MEASE, B. SAKMANN, *A. GROH;
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Abstract: As somatosensory specialists, rats and mice rely on touch signals from their whiskers to sample the environment. Whisker touches can either be caused 1) actively, when rhythmically whisking against objects, or 2) passively, when whiskers are touched during non-whisking periods. While these touch modes activate the cortex differently [1], it is not well understood which neuronal mechanisms and circuits allow this discrimination. The somatosensory thalamus is a hub for the integration of ascending sensory pathways with corticothalamic inputs [2]. We hypothesized that passive versus active touch discrimination is performed the in thalamus, involving top-down cortical circuits referencing sensory inputs to behavior. To test this, we recorded spikes from location-recovered neurons in the ventral posterior medial (VPM) and posterior medial (POm) nuclei of the thalamus, regions which both receive massive cortical input. We compared spike responses in awake, head-fixed mice during 1) passive touches, induced by focal air puffs to the stationary whisker and 2) active touches, caused by the animal actively whisking against an object. Barrel cortex local field potentials (LFP were recorded to determine cortical activity states. In comparison to quiescence, spike rates increased approximately two-fold during free whisking and touch periods. In both VPM and POm, we found cells that responded to passive touches (air puffs) with short latencies (<10 ms). However, individual neurons responded very differently to active touches. In parallel, cortical state differed strongly between passive and active modes: Pronounced delta (1-4Hz) oscillations were only present during passive state [3], while during whisking, higher frequencies dominated, indicating the desynchronized brain state characteristic of sensory processing. While active and passive

touch may lead to the same receptor activation, our data suggest that thalamic responses encode these events differently and pass these differences on to the cortex. Whether this thalamic property arises from Top-Down cortical input is currently being investigated. [1] Sachidhanandam, S., Sreenivasan, V., Kyriakatos, A., Kremer, Y., & Petersen, C. C. H. (2013). Membrane potential correlates of sensory perception in mouse barrel cortex. *Nature Neuroscience*, 16(11), 1671-7. [2] Sherman, S. M., & Guillery, R. W. (1996). Functional organization of thalamocortical relays. *Journal of Neurophysiology* , 76(3), 1367-95. [3]Zagha, E., Casale, A. E., Sachdev, R. N. S., McGinley, M. J., & McCormick, D. a. (2013). Motor cortex feedback influences sensory processing by modulating network state. *Neuron*, *79*(3), 567-78.

Disclosures: **A.L. Sumser:** None. **R.A. Mease:** None. **B. Sakmann:** None. **A. Groh:** None.

Poster

064. Thalamocortical Mechanisms

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Support: NINDS RO1 NS069679

Klingenstein Fund for Neuroscience

Rita Allen Foundation

Title: *In vivo* dissection of L1 inputs in the Barrel cortex

Authors: *W. ZHANG[1], R. BRUNO[1,2];
[1]Neurobio. and Behavior, [2]Kavli Inst. for Brain Sci., Columbia Univ., New York, NY

Abstract: Layer 1 of the cerebral cortex is a largely acellular layer that consists mainly of long-range projection axons and apical dendrites of deeper pyramidal neurons. In the rodent barrel cortex, layer (L) 1 contains axons from both higher motor and sensory areas of the brain. Despite the abundance of synapses in L1 their actual contribution to sensory processing remains unknown. We investigated the impact of activating long-range axons on BC L2/3 pyramidal neurons. We focused on three main sources of BC-projecting synapses: the posterior medial nucleus of the thalamus (POm, the secondary somatosensory nucleus), the primary motor cortex (M1), and the secondary somatosensory cortex (S2). In each animal, we delivered the gene for channelrhodopsin (ChR2) to one of these three regions, and then photostimulated the ChR2-

positive axons in BC L1 while recording whole-cell recording from L2/3 cells *in vivo*. We found that while activation of POm axons elicits strong EPSPs in all recorded L2/3 cells, activation of M1 or S2 axons elicited small or no detectable responses. Only POM activation boosted sensory responses in L2/3 pyramidal neurons. We also found that under sedated and awake conditions, POM activation not only elicited a strong fast-onset EPSP in L2/3 neurons, but also a delayed persistent response. Pharmacological inactivation of POM abolished this persistent response but not the initial synaptic volley to L2/3. We conclude that the persistent response requires intrathalamic or thalamocortical circuits and cannot be mediated by specialized synaptic terminals or intracortical circuitry. This persistent activity may play a role in sensory processing.

Disclosures: **W. Zhang:** None. **R. Bruno:** None.

Poster

064. Thalamocortical Mechanisms

Location: Halls A-C

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Topic: D.09. Tactile/Somatosensory

Support: TUBITAK 113S901

BU BAP 13XP8

Title: Electrical microstimulation of hindpaw representation in rat SI cortex yields better detection probability compared to vibrotactile stimulation of the glabrous skin during operant conditioning

Authors: *I. DEVECIOGLU[1,2], B. GUCLU[1];
[1]Inst. of Biomed. Engin., Bogazici Univ., Istanbul, Turkey; [2]Biomed. Engin. Dept., Namik Kemal Univ., Tekirdag, Turkey

Abstract: We previously showed that rats usually can achieve accuracies >70% for detecting vibrotactile stimuli applied on the glabrous skin of the hindpaws. In this study we applied intracortical microstimulation (ICMS) within the hindpaw representation of SI cortices of three female Wistar albino rats which had performed poorly in a tactile detection task. Similar to the tactile detection task, the ICMS task required right/left lever press for detecting or not detecting the presence of ICMS. The tactile detection task involved sinusoidal displacement bursts with amplitude 120 μm, duration 0.5 s, and frequency 40 Hz. ICMS consisted of biphasic pulse pairs

(cathodic phase first, phase duration: 200 µs, amplitude: 300 µA, interpulse interval: 500 µs) delivered at 40 Hz with a duration of 0.5 s. Custom-made tungsten microelectrodes were implanted in SI (hind paw representation) and AgL (vibrissae representation in motor cortex). Multiunit receptive fields were mapped with von Frey hairs and by inducing muscle twitches. Electrical stimulation through SI electrode did not generate a muscle twitch; tactile stimulation of the skin did not elicit activity in AgL electrode. After implantation and mapping, rats recovered for 1-2 weeks. Each rat started the same training schedule and reached >90% accuracy in the visually-guided side-lever-press task. However, they performed at chance level (~50%) in the tactile detection task, even after 10-50 sessions (~100 trials/session). When tested with ICMS, two rats achieved >90% accuracy. The third rat did not want to perform the task with ICMS, i.e. did not press the trial-start lever. These results suggest that electrical microstimulation improve conditioning to neural activity elicited in cortical networks processing tactile information. This will be further studied in associative learning models with varying salience.

Disclosures: **I. Devecioglu:** None. **B. Guclu:** None.

Poster

064. Thalamocortical Mechanisms

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Topic: D.09. Tactile/Somatosensory

Support: R01NS075007

R90DA33463-02

Title: The role of retinoid-related orphan receptor beta in mouse primary somatosensory cortex barrel development and maintenance

Authors: ***T. D. LAUER**[1,2], Q. ZHANG[1,2], M. RUTLIN[3], S. NELSON[1,2];
[1]Biol., Brandeis Univ., Waltham, MA; [2]Natl. Ctr. for Behavioral Genomics, Waltham, MA;
[3]Columbia Univ., New York, NY

Abstract: The mouse whisker-barrel system has been used as a model for studying mammalian somatosensory circuit development and function for more than 40 years. Many genes and pathways have been shown to be important for formation of cortical barrels, yet relatively little is known about genetic control of barrel maintenance. We found that deletion of the nuclear

receptor Retinoid-related Orphan Receptor beta (RORb) causes progressive deterioration of barrels. In RORb knockouts (KOs), barrels are seen at P7, but have subtly reduced thalamocortical afferent (TCA) segregation, cortical neuronal clustering and cytochrome oxidase (CO) activity. This initial map degrades over time, and individual barrel structures are hardly distinguishable in adult RORb KOs. Transcriptional profiling from RORb+ cells in the barrel field was done to identify RORb-regulated genes that may control barrel formation and maintenance. RNAseq was performed on RORb-eGFP+ Layer 4 (L4) neurons sorted from KO and heterozygous animals (4 replicates). The initial analysis revealed 1246 differentially expressed genes with a false discovery rate (FDR) of 5%. Expression of receptors and signaling molecules previously found to be required for barrel formation were not significantly affected. However, mRNA levels of transcription factors (TFs) important for barrel formation were altered. Lmo4 had 56% increased expression (P=0.03), implying compensatory up-regulation. BTBD3, a TF needed for barrel cell dendritic polarization, was reduced by 29% but was not significant (P=0.06). A corresponding trend of reduced L4 dendritic asymmetry was evident from Golgi-Cox staining, but was also not significant (P=0.08, Kolmogorov-Smirnov test). Current efforts are underway that will determine which RORb-regulated genes are contributing the maintenance deficit in the RORb-loss of function animals. Additionally, RORa, RORb's closest paralog appears to be up-regulated at the protein level in the barrel field, suggesting RORa could be compensating for RORb loss of function. We are currently testing this hypothesis by generating double KO mice.

Disclosures: **T.D. Lauer:** None. **Q. Zhang:** None. **M. Rutlin:** None. **S. Nelson:** None.

Poster

064. Thalamocortical Mechanisms

Location: Halls A-C

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Program#/Poster#: 64.14/CC12

Topic: D.09. Tactile/Somatosensory

Title: Thalamo-cortical synchronization during sensory information processing in the vibrissal motor cortex in rats

Authors: ***A. MÚNERA**[1], M. NAVA-MESA[2], J. RAMÍREZ-LATORRE[2];
[1]Univ. Nacional De Colombia, Bogotá, Colombia; [2]Univ. del Rosario, Bogotá, Colombia

Abstract: The vibrissal sensorymotor system enables rodents to extract precise information about three-dimensional configurations in their immediate environment, generating motor

commands based on sensory input instantaneous changes; this mechanism requires continuous interactions between vibrissal motor cortex (vM1) and somatosensory thalamic nuclei (posteromedial, POM and ventroposteromedial, VPM). Bipolar stimulating whisker pad electrodes and vM1 and POM or VPM recording electrodes were implanted to anesthetized Wistar rats to evaluate these interactions. Single and paired whisker pad stimuli were administered while cortical and thalamic field potentials were recorded. Following single whisker pad stimuli, short-latency population spikes occurred simultaneously in vM1, POM, and VPM. Such spikes were followed by low frequency oscillations associated with long-lasting epochs of enhanced thalamo-cortical coherence. Following paired whisker pad stimulation population spikes became facilitated, while low frequency oscillations became depressed. Taken together such findings suggest strong thalamo-cortical coupling, as well as sustained intracortical inhibition, in vM1 during somatosensory information processing and motor command generation.

Disclosures: **A. Múnera:** None. **M. Nava-Mesa:** None. **J. Ramírez-Latorre:** None.

Poster

064. Thalamocortical Mechanisms

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Topic: D.09. Tactile/Somatosensory

Support: NIH Grant NS050437

Title: Thalamocortical feedforward inhibition by infragranular somatostatin-containing interneurons

Authors: *A. AGMON[1], H. HU[2];
[1]Dept Neurobiol & Anat, West Virginia Univ. Hlth. Sci. Ctr., Morgantown, WV; [2]West Virginia Univ., Morgantown, WV

Abstract: Inhibitory cortical interneurons are strongly activated by thalamocortical inputs and mediate disynaptic feedforward inhibition onto neighboring excitatory neurons, thereby curtailing their direct thalamocortical excitation and reducing spike dispersion. Most previous studies emphasize the role of parvalbumin (PV)-containing, fast-spiking (FS) interneurons in mediating feedforward thalamocortical inhibition. We previously reported (Tan et al 2008) that somatostatin-containing (SOM) interneurons may also be involved in feedforward inhibition, and

demonstrated that in response to high-frequency thalamocortical stimulation, infragranular FS interneurons are activated early but transiently due to depression of their thalamocortical EPSPs, while infragranular SOM interneurons are activated later but in a sustained fashion, due to facilitation of their EPSPs. Here we tested directly the relative contributions of FS and SOM interneurons to feedforward inhibition in infragranular excitatory (E) neurons, by recording their responses to trains of 10 thalamocortical stimuli at 10-40 Hz while suppressing firing of either SOM or FS interneurons optogenetically. Illuminating SOM interneurons in SOM-halorhodopsin mice hyperpolarized them by 8-25 mV and reduced their total spike count to 7-30% of control; illuminating FS interneurons in PV-archaerhodopsin mice hyperpolarized them by 4-7 mV and reduced their total spike count to ~60% of control. In E-neuron, thalamocortical stimulation elicited trains of either pure IPSPs or EPSP-IPSP sequences. Optogenetic suppression of FS interneuron reduced the first, and occasionally the second IPSP in the train, without significantly affecting later IPSPs. Total spike count in E cells, during and 1000 ms after the train, was increased by 1.6-fold at 40 Hz stimulation. In contrast, optogenetic SOM suppression most often left the first and second IPSPs unchanged, but strongly reduced, blocked or even reversed the later IPSPs in the train. In many E neurons as well as in some FS interneurons, SOM (but not FS) suppression elicited a slow depolarization during and following the train, triggering spikes and occasional “up states”. On average, SOM suppression increased the total spike count in E cells by 8-fold at 40 Hz stimulation. We conclude that SOM interneurons make a major contribution to feedforward inhibition of excitatory infragranular neurons during high-frequency thalamocortical volleys, and are critical for preventing over-excitation of infragranular excitatory neurons during sustained thalamocortical activity, such as may arise during exploratory behavior.

Disclosures: **A. Agmon:** None. **H. Hu:** None.

Poster

064. Thalamocortical Mechanisms

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Topic: D.09. Tactile/Somatosensory

Support: NIH DC008794

Title: Functional organization of the motor reticulothlamic input to VA/VL and CL neurons

Authors: ***Y.-W. LAM**[1], D. RHEE[2], S. SHERMAN[2];
[2]Neurobio., [1]Univ. of Chicago, CHICAGO, IL

Abstract: Most of our understanding of the organization of thalamus is based on studies of the sensory thalamic relays. To extend this, we investigated aspects of organization of other nuclei with a focus on the projection in mice from the GABAergic thalamic reticular nucleus (TRN) to the motor relays, the ventral lateral and ventral anterior nuclei (VA/VL), as well as the central lateral nuclei (CL). We used a slice preparation that preserves the connectivity between TRN and the thalamic nuclei of interest noted above. We used whole cell recordings in these nuclei and investigated the topography of their TRN input using laser scanning photostimulation. The pattern of these inputs was very similar to those described for the somatosensory thalamus. That is, TRN input to VA/VL neurons were spatially limited and topographically organized. Overall, the input zones, or footprints, were somewhat larger than those to the first order somatosensory nuclei, the ventral posterior lateral and ventral posterior medial (VPL and VPM), and in many cases, consisted of two separated footprints; in this respect, the pattern was more similar to the TRN input footprints to the higher order posterior medial nucleus (POm). The TRN input to CL was organized in a similar specific and topographic manner and interestingly, the TRN inputs of VA/VL and CL neurons were located in overlapping areas. As TRN is reported to be organized into functionally related segments, this result suggests that either CL neurons have motor related functions or the "motor" TRN in mice may actually consists of neurons with different functions. Overall, we conclude that the pattern of TRN inputs to these other thalamic nuclei, VA/VL and CL, is very similar to that described for sensory thalamic nuclei.

Disclosures: **Y. Lam:** None. **D. Rhee:** None. **S. Sherman:** None.

Poster

064. Thalamocortical Mechanisms

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Topic: D.09. Tactile/Somatosensory

Support: NIH grant NS061963

Whitehall foundation

Title: Layer 4 in area 4: Characteristic input-output connectivity of pyramidal neurons at the layer 3/5A border in the lateral agranular region of mouse motor cortex

Authors: *K. E. BORGES, N. YAMAWAKI, B. A. SUTER, X. LI, G. M. G. SHEPHERD; Physiology, Feinberg Sch. of Med., Northwestern Univ., Chicago, IL

Abstract: An enduring enigma about the functional organization of agranular cortical areas such as primary motor cortex (M1; area 4) is the absence of a distinct layer 4 (L4) and how this relates to the local and long-range synaptic circuits. We investigated the possibility that M1 possesses the hodological equivalent of L4 in the form of excitatory neurons at the L3/5A border with characteristic input-output circuits similar to those of L4 in sensory areas. Consistent with this idea, putative L4 neurons in M1 received excitatory input from both motor (VA/VL) and sensory (PO) thalamus, and sent excitatory output to L4 and L2/3 neurons, but received relatively little local input from L2/3. M1 L4 neurons were electrophysiologically diverse, but morphologically fairly uniform, with pyramidal-type apical and basal dendritic arbors and locally ramifying axons with branches extending into L2/3. Thus, M1 L4 neurons largely resemble their counterparts in sensory cortical areas in possessing a core set of characteristic local and long-range input and output connections. However, they differ from sensory L4 neurons in that they receive weak or no input from L6 neurons, and lack spiny stellate morphology. These results thus decrypt the circuit connections of L4 neurons in the lateral agranular area of M1, demonstrating a basic input-output organization resembling that of L4 neurons in other cortical areas.

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Poster

064. Thalamocortical Mechanisms

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Topic: D.09. Tactile/Somatosensory

Support: BNBFCRCNS 01GQ1113

Title: Thalamic encoding of kinematic whisker slips in the awake behaving rat

Authors: *C. WAIBLINGER[1,2], C. SCHWARZ[1,2], C. J. SHEPHARD[3], G. B. STANLEY[3]; [1]Werner Reichardt Ctr. for Integrative Neurosci., Tübingen, Germany; [2]Hertie Inst. for Clin. Brain Res., Tübingen, Germany; [3]Wallace H Coulter Dept. of Biomed. Engineering, Georgia Tech. and Emory, Atlanta, GA

Abstract: The rodent whisker-related somatosensory thalamus (VPm) controls and modifies ascending tactile signals destined for the barrel cortex. We set out to investigate which aspects of the signal is transmitted by VPm and used to generate the animal's percept. Further, we tested

whether this transmission is adaptive, i.e. is influenced by presenting different sensory environments. Our starting point was previous work on rodent vibrissae biomechanics and perception that strongly suggest that fast kinematic signatures (so called ‘slip-events’ lasting for ca. 10 ms) which change in number and waveform depending on the touched texture are preferentially used to generate the subject's tactile percept. Here, we trained head-fixed rats to detect precise whisker stimuli that mimic these natural signatures (tagged 'slip-like events') embedded in broadband noise. Analyzing reaction times we found that rats extract whisker slips of different shape and direction based on the amplitude relative to the background noise but largely ignore slip frequency. VPm multi units respond to the broadband noise but additionally extracted slip-like events by brisk bursts of spikes. When subjecting rats with slip-like events that switch direction at one point during the session, we observed that VPm multi units change their response properties on a times scale of tens of seconds or minutes - possibly relating to an adaptation process brought about by the changed sensory conditions. A more detailed investigation of single unit receptive field properties and information transmission using the broadband noise stimuli is under way. Our results so far, confirm and further elaborate the hypothesis that instantaneous events are decisive coding elements for whisker-related tactile perception.

Disclosures: **C. Waiblinger:** None. **C. Schwarz:** None. **C.J. Shephard:** None. **G.B. Stanley:** None.

Poster

064. Thalamocortical Mechanisms

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Program#/Poster#: 64.19/CC17

Topic: D.09. Tactile/Somatosensory

Title: The organization of the human thalamus estimated by large network functional connectivity

Authors: B. PASCUAL[1], M. HOLLENBECK[1], *J. C. MASDEU[2,1];
[1]Dept. of Neurol., Houston Methodist Hosp., Houston, TX; [2]Section on Integrative Neuroimaging, NIH, Washington, DC

Abstract: Introduction. The thalamus, a complex array of different nuclei, plays a major role in cortical activation. Data from non-human primates and from lesion studies suggest that the thalamus projects to the cortex in a highly organized fashion. However, the human thalamus is

highly evolved from non-human primates and lesion studies are limited by chance. Functional connectivity provides a novel approach to study the organization of the different thalamic nuclear groups. In order to clarify the functional connectivity of the left thalamus, we sought concordance between the resting-state BOLD signal in small nuclei of the left thalamus with a similar signal in the rest of the brain. We hypothesized that (1) different nuclei of the thalamus would be predominantly connected to different cortical and subcortical areas; and (2) from the pattern of cortical connectivity we could derive information on the functional relevance of each thalamic nuclear group. Methods. Using seed-based resting-state functional MRI data from 198 young adults, we examined cortico-subcortical correlations by means of a functional connectivity analysis. Fifty-seven seed regions of interest were projected along the anterior to posterior thalamus. Intrinsic functional connectivity MRI was used to extract low-frequency spontaneous BOLD fluctuations within those regions to calculate functional correlation maps. The topographical similarity of the resulting maps was calculated. Using these values, clustering analysis was performed to better understand the grouping of thalamo-cortical functional architecture within the thalamus. Results. Functional connectivity of the thalamus defined five major thalamic clusters: (1) Medial and anterior thalamic nuclear groups, which were positively correlated with the “limbic” and frontal lobes; (2) Ventral lateral nuclear group, correlated with basal ganglia and with precentral cortex; (3) Ventro-posterior and basal, which were correlated with postcentral structures, mostly in parietal lobe. (4) Dorsolateral and posterior nuclear groups, particularly the pulvinar, which were correlated with the precuneus; (5) Geniculate bodies, medial correlated with auditory cortex and lateral correlated with visual cortex. Many regions had cerebellar correlations, often larger with the contralateral cerebellar hemisphere. Conclusions. The functional connectivity of the thalamus is concordant with its known anatomic connectivity from studies in the monkey and from human lesion data. Refinement of this technique may render it even more useful to map the functional brain networks anchored in the thalamus

Disclosures: **B. Pascual:** None. **M. Hollenbeck:** None. **J.C. Masdeu:** None.

Poster

065. Generation of Motor Patterns

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 65.01/CC18

Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Support: NIH NINDS 1 R01 NS085006

Title: Bilateral symmetries in an inherently asymmetric motor pattern

Authors: *A. WENNING[1], B. J. NORRIS[2], R. L. CALABRESE[1];
[1]Biol., Emory Univ., ATLANTA, GA; [2]California State Univ., San Marcos, CA

Abstract: Bilateral symmetry is common to all vertebrates and many invertebrates, yet underlying asymmetries abound both in the body plan and in the nervous system. We were curious to what extent bilaterally organized central pattern generators (CPGs), the motor patterns they control, and the corresponding motor outputs were symmetric. We chose a system dedicated to one task to ask how side-to-side variations within one animal compared to the variations seen across animals. We chose leech heartbeat in which each CPG (one on each side) controls an ipsilateral ensemble of heart (HE) motor neurons, which in turn entrain the segmental sections of the ipsilateral heart tube. Though the layout is bilaterally symmetric, patterns are not. At any given time, the beat pattern, the motor pattern, and the temporal pattern of the premotor (HN) heart interneurons display a left/right asymmetry. Intersegmental coordination is rear-to-front on one side and synchronous on the other with switches between sides every 20 to 40 heartbeat cycles. Thus, both CPGs control, and both sides execute, both coordination modes – albeit not at the same time – and leech heartbeat switches between two patterns: left synchronous/right peristaltic and *vice versa*. We compared the side-to-side variations of the beat patterns, the motor pattern, the temporal pattern of the HN interneurons, and their synaptic weight to the HE neurons within one animal and across preparations. We focused on midbody segments 7 to 14 where the same HN interneurons control the HE motor neurons. In isolated ganglia chains we examined the intersegmental phase differences of these HN interneurons and their synaptic weights to the HE motor neurons. In intact (imaged) animals we quantified the heart constriction patterns. We found that the intersegmental phase differences of the HN interneurons, their synaptic weights in the HE neurons, and the heart constriction patterns differ on the two sides. While there was no preference for body side regarding synaptic weights and the temporal pattern of the CPG, the heartbeat pattern showed lateralization in the peristaltic mode. Within one animal, side-to-side variability in the parameters examined so far was similar to the variability seen across animals.

Disclosures: **A. Wenning:** None. **B.J. Norris:** None. **R.L. Calabrese:** None.

Poster

065. Generation of Motor Patterns

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Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Support: NIH Grant NINDS 1 R01 NS085006

Title: Do synaptic or intrinsic properties govern animal-to-animal variability in motor patterns?

Authors: *C. GUNAY[1], D. G. LAMB[2], R. L. CALABRESE[1];
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Abstract: Motor patterns are essential for life, and they are known to be robustly expressed even in the presence of large animal-to-animal variations in both synaptic and intrinsic properties of the neurons that produce them. This suggests that underlying mechanisms exist to preserve functional output motor patterns. Studying these mechanisms is more difficult in the complex motor networks of vertebrates. In invertebrates like the leech (Hirudo sp.), a complete network of premotor and motor neurons can be mapped and recorded. The well-studied leech heartbeat central pattern generator network is appropriate for studying correlates of animal-to-animal variability. Here we used recordings from 6 leeches of a complete input-output network composed of firing patterns from all premotor inhibitory interneurons, strengths of their synapses that impinge onto two bilateral pairs of output motor neurons, and those motor neurons' output firing patterns (Wright and Calabrese, J Neurosci, 31(48):17555-17571, 2011). Each individual leech had variations in input and output motor patterns and in synaptic strengths. Here, we are asking what motor neuron intrinsic properties are necessary to produce the target output patterns given the recorded input patterns and synaptic strengths. To answer this question, we have previously conducted an evolutionary parameter search to find the intrinsic properties represented by ion channel maximal conductance parameters of a Hodgkin-Huxley type, muticompartmental model of heart (HE) motor neurons leech ganglia 8 and 12 (Lamb and Calabrese, PLoS ONE 8(11): e79267, 2013). As a result of this search, we have found candidate models that can reproduce target output motor patterns of HE(8) and HE(12) motor neurons, or both (431 model instances) for one given input pattern. In the present work, we simulated these successful instances with inputs recorded from other leeches: premotor temporal pattern and corresponding synaptic weights. Because these synaptic weights have been recorded as relative values in isolation, they must be scaled by a coefficient to be used in a model motor network. We were able to find such coefficients for some of the 431 HE model instances to produce target output phases for 4 out of the 6 individual motor patterns. The question that remains is whether any synaptic configuration exists that can produce these remaining 2 output patterns. Furthermore, are there any general mechanisms that affect intrinsic properties that can allow a set of instances to match all 6 input patterns? These questions can be addressed by conducting further parameter searches of this model motor network and mining the results.

Disclosures: **C. Gunay:** None. **D.G. Lamb:** None. **R.L. Calabrese:** None.

Poster

065. Generation of Motor Patterns

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Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Support: NSF PHY-0750456 to GSC

NINDS 1 R01 NS085006 to RLC

Title: How can either stimulation or inhibition of the Na+/K+ pump current decrease the period of a central pattern generator?

Authors: *W. H. BARNETT[1], D. KUEH[2], R. L. CALABRESE[2], G. S. CYMBALYUK[1]; [1]Neurosci. Inst., Georgia State Univ., ATLANTA, GA; [2]Biol. Dept., Emory Univ., Atlanta, GA

Abstract: Growing evidence implicates the importance of Na+/K+ pump in central pattern generators (CPGs), which control rhythmic behavior such as locomotion [1]. The Na+/K+ pump has been shown to be a target for neuromodulation. In the leech heartbeat CPG, the neuropeptide myomodulin speeds up the period of the bursting pattern by increasing h-current and decreasing the Na+/K+ pump current in the heart interneurons (HNs) [2]. A role of the Na+/K+ pump current in the dynamics of HN neurons was indicated by experiments using the H+/Na+ antiporter monensin, which stimulates the pump by diffusively increasing the intracellular Na+ concentration. Interestingly, application of monensin decreases the period of a leech heartbeat half-center oscillator (HCO) in the presence of h-current. These results appear to be counterintuitive. How can either stimulation or inhibition of the Na+/K+ pump current decrease the period of a central pattern generator and what role does h-current play in this phenomenon? We developed a model of the HN neuron including intracellular Na+ concentration dynamics and the Na+/K+ pump current. We considered HCO and isolated HN neurons with and without h-current. HN neurons were isolated with bicuculline, and the h-current was blocked with Cs+. The model reproduced the key experimental results under all 12 experimental conditions. These results emphasize the role of the Na+/K+ pump current in the dynamics of the CPG neurons. The Na+/K+ pump dynamics directly affect HN neuron and HN-HCO bursting activity. Pump modulation provides an effective means for period control. The pump contributes to burst termination mechanism and governs interburst interval. Interaction between the pump current and the h-current creates a dynamic mechanism effectively controlling the interburst interval. References 1. Zhang HY, Sillar KT. Short-term memory of motor network performance via activity-dependent potentiation of Na+/K+ pump function. Curr Biol. 2012 Mar 20; 22 (6): 526-

31. 2. Tobin AE, Calabrese RL. Myomodulin increases Ih and inhibits the NA/K pump to modulate bursting in leech heart interneurons. J Neurophysiol. 2005 Dec; 94 (6): 3938-50.

Disclosures: **W.H. Barnett:** None. **D. Kueh:** None. **R.L. Calabrese:** None. **G.S. Cymbalyuk:** None.

Poster

065. Generation of Motor Patterns

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Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Support: NIH Grant NS085006

Title: Sensitivity of period to variations of maximal conductances in an HCO model

Authors: *A. DOLOC-MIHU, R. L. CALABRESE;
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Abstract: Robustness in response to developmental and environmental challenges is essential to the central pattern-generating networks (CPGs) that program rhythmic behaviors on all animals. Here, we used a half-center oscillator (HCO) (two mutually inhibitory neurons) model [1] that replicates the electrical activity (rhythmic alternating bursting of mutually inhibitory interneurons) of the leech heartbeat CPG under a variety of experimental conditions to determine the role of conductances in the robust maintenance of functional bursting activity. Precisely, we focused on the role of those parameters' changes which correspond to known neuromodulations such as the modulation of h current by myomodulin [2]. In our previous work [3], we had built a relational database (named HCO-db) of Hill's HCO [1] model instances. In the HCO model, an individual leech heart interneuron was represented as a single isopotential electrical compartment with Hodgkin and Huxley type intrinsic membrane and synaptic conductances. The HCO model has eight currents with voltage-dependent conductances including two types of inhibitory synaptic currents, spike mediated and graded. By varying a set of 8 key parameters (the leak reversal potential and maximal conductances of synaptic and several membrane currents) in all combinations possible (brute-force approach), we systematically explored the parameter space of this HCO model and analyzed more than 10 million of its simulated instances. We analyzed and classified within a group the simulated instances showing similar electrical activity, and we identified these groups which include instances showing burst characteristics (period and spike

frequency) similar to the animal. There were: 1,202,139 HCO instances; 99,066 realistic HCOs; 424 bursters, and 307 realistic bursters. Experimental studies have shown that period is a key attribute influenced by modulatory inputs variations in leech heart interneurons [2]. By querying our HCO-db database, we explored the sensitivity of period to changes in maximal conductance of the h current ($\bar{g}h$) for the above groups of instances. When $\bar{g}h$ was set to zero, no isolated neuron instances produced rhythmic burstss bur many instances of realistic HCO were observed. We also found that increasing the amount of $\bar{g}h$ monotonically decreases the period of the realistic HCOs, which confirms Hill's [1] results stated for a more restricted parametric space. References 1. Hill AAV, Lu J., Masino MA, Olsen H, Calabrese RL. J Comp. Neurosci 2001, 10:281-302. 2. Tobin AE, Calabrese RL: J Neurophysiol 2005, 94(6):3938-3950. 3. Doloc-Mihu A, Calabrese RL. J Biol Physics 2011, 37:263-283.

Disclosures: **A. Doloc-Mihu:** None. **R.L. Calabrese:** None.

Poster

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Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Support: NINDS 1 R01 NS085006 to RLC

NSF PHY-0750456 to GSC

Title: Stimulation of the Na^+/K^+ pump can accelerate rhythmic bursting in CPG neurons but requires the h-current

Authors: *D. KUEH[1], G. S. CYMBALYUK[2], R. L. CALABRESE[1];
[1]Dept. of Biol., Emory Univ., Atlanta, GA; [2]Neurosci. Inst., Georgia State Univ., Atlanta, GA

Abstract: The dynamics of various membrane and synaptic currents together shape the electrical activity of CPG neurons and networks that program motor output. Modulation of these currents can then regulate burst characteristics, e.g., period. The outward current generated by an electrogenic Na^+/K^+ pump is not often considered when exploring the bursting of CPG neurons, but can represent a target of modulation. To determine the effects of stimulating the Na^+/K^+ pump on bursting activity, we made simultaneous bilateral extracellular recordings from paired heart (HN) interneurons that form half-center oscillators in the leech heartbeat CPG. When the

pump activity of HN half-center oscillators was stimulated with monensin, a Na^+-H^+ antiporter, their burst period decreased significantly. This effect of monensin was not observed when the h-current was blocked by external Cs^+. To determine the effects of stimulated pump activity on single neurons, we pharmacologically isolated the HN neurons with bicuculline. When isolated in this way, the burst period decreased significantly. Application of monensin further decreased the burst period of these isolated HN neurons. Blocking the h-current in isolated HN neurons with Cs^+ increased their burst period dramatically, and subsequent application of monensin had no effect on burst period. However, monensin did eventually block all activity under these conditions and bursting could be restored after removal of Cs^+. Finally, inhibition of pump activity by the removal of external K^+, led to an initial decrease in burst period, but bursting was gradually and reversibly suppressed over time. Taken together, the stimulation of Na^+/K^+ pump activity in an HN half-center oscillator or in isolated HN neurons decreases the burst period, and this effect requires the presence of the h-current. Moreover, inhibition of pump activity initially speeds up rhythmic bursting activity as well, only to suppress all activity over time. Thus, the interaction of the electrogenic activity of the Na^+/K^+ pump with the h-current appears to play a significant role in the dynamics of bursting in the leech heartbeat CPG and perhaps in other rhythmically bursting neuronal networks as well.

Disclosures: **D. Kueh:** None. **G.S. Cymbalyuk:** None. **R.L. Calabrese:** None.

Poster

065. Generation of Motor Patterns

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Program#/Poster#: 65.06/CC23

Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Title: The effects of CsCl in the gastric and pyloric neurons in the stomatogastric ganglion of the lobster, Homarus americanus

Authors: *L. ZHU, A. I. SELVERSTON, J. AYERS;
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Abstract: Hyperpolarization-activated inward current (*Ih*) is an important pacemaker current that is known to regulate cellular excitability, post inhibitory rebound, and synaptic strength in the pyloric network in the stomatogastric ganglion (STG) in *Cancer borealis* and *Panulirus interruptus*. Schulz and Marder's research groups have profiled the ionic channel mRNAs in the STG in *Cancer borealis*, in which they've shown that the *Ih* channel mRNA (IH) is also

expressed in the gastric neurons, including GM, LG and LPG neurons. However, little is known about the role of *Ih* in gastric neurons in the STG. In our study, we investigated the role of *Ih* in rhythmic activity and cellular excitability in both gastric neurons (GM, MG, LG, LPG) and pyloric neurons (PD, VD, PY, LP) in *Homarus americanus.* Through determination of a dose response curve between 0.5mM and 500mM based on the rhythmic activities obtained from extracellular and whole cell current recordings, we found a dose of 100mM CsCl is necessary to achieve the strongest effects in blocking *Ih* in *Homarus*. At 100mM, the rhythmic activities of gastric neurons were abolished; the rhythmic activities of pyloric neurons were abolished or greatly reduced. Similarly, 300μM ZD7288 blocked gastric activity and most of pyloric activity. At 30mM CsCl, the slow oscillations (plateau potential) on all investigated gastric neurons were abolished, while the slow oscillations on all investigated pyloric neurons remained, indicating *Ih* concentration has differential effects on slow oscillations in the gastric and pyloric neurons examined. In addition, gastric neurons (GM, LPG) continued their spiking activities when the slow oscillations were abolished; however, pyloric neurons (VD, LP) lost spiking activities before the elimination of the slow oscillations. This indicates that the spiking activities are less sensitive to *Ih* concentration than the slow wave oscillations in the GM and LPG neurons, while the opposite relation applies to the VD and LP neurons. We are in the process of refining the differential sensitivities to *Ih* in different STG neurons. We performed an analysis of the changes in rhythmic properties at 30mM CsCl, including burst period, burst duration, and spikes per burst. Our results have shown that 1) Unblocking *Ih* induced temporary increase in the duty cycle of gastric neurons (GM, MG, LG) when compared to control levels (before the block), which indicates that *Ih* can increase the excitability of gastric neurons. 2) Blocking *Ih* increased the duty cycle of PY neurons, and reduced the spiking activities of VD neuron.

Disclosures: **L. Zhu:** None. **A.I. Selverston:** None. **J. Ayers:** None.

Poster

065. Generation of Motor Patterns

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Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Support: DFG SM 206/3-1

Title: Three different methods to identify transmitters of coordinating neurons in the crayfish swimmeret system

Authors: *A. C. SCHNEIDER[1], H. SEICHTER[1], S. NEUPERT[2], C. R. SMARANDACHE-WELLMANN[1];
[1]Zoological Institute, Animal Physiology, Emmy Noether Group, [2]Zoological Inst., Univ. of Cologne, Cologne, Germany

Abstract: Neural oscillators coordinated to produce meaningful behavior exist in a huge variety of animals, ranging from swimming and flying to walking and brain oscillations. We use the crayfish swimmeret system as a model to study coordination of neural oscillators because the mechanisms of coordinated motor output, especially neurons of the coordinating circuit, are identified on cellular level. Movement of the four pairs of swimmerets progresses in a metachronal wave from posterior to anterior with a stable phase lag of 25% between segments. Each swimmeret is controlled by one central pattern generator (CPG) located in each hemiganglion. The coordination of the CPGs is achieved by a coordinating circuit of exactly three neurons per hemiganglion: One ascending (ASC_E) and one descending (DSC) coordinating neuron, as well as one non-spiking Commissural Interneuron 1 (ComInt 1). The coordinating neurons ASC_E and DSC encode information about the activity state of their home ganglion and send it to the ComInt 1s of the other three ganglia, where the information, arriving with a gradient of synaptic strength, is decoded and integrated into the CPG. Each action potential of ASC_E and DSC causes a distinct and fast excitatory postsynaptic potential (EPSP) in ComInt 1. In our attempt to characterize the coordinating neurons we want to identify their putative transmitters. Because of the EPSP characteristics the transmitters of the coordinating neurons are supposedly of low molecular weight, like acetylcholine (ACh), serotonin (5-HT), or glutamate. For characterization we used (1) electrophysiological, (2) immunohistochemical, and (3) mass spectrometrical methods on an isolated preparation of the abdominal nerve cord. (1) In split-bath experiments we recorded intracellularly from ComInt 1 and perfused GABA and glutamate antagonists. As they did not block EPSPs in ComInt 1, we ruled them out as transmitters for ASC_E and DSC. (2) For the immunohistochemical experiments we stained ASC_E and DSC with intracellular dye injection and used antibodies against 5-HT in the respective ganglion. Results showed no colocalization of 5-HT immunoreactivity and the dye-filled coordinating neurons, also excluding this transmitter. (3) For the matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF), the coordinating neurons were dye injected and the thus visible soma or area of dendritic arborization isolated for analysis. We could identify ACh as putative transmitter of the coordinating neurons, present in samples of both the soma and area of dendritic arborization. Results further confirmed the absence of 5-HT in these neurons.

Disclosures: A.C. Schneider: None. **H. Seichter:** None. **S. Neupert:** None. **C.R. Smarandache-Wellmann:** None.

Poster

065. Generation of Motor Patterns

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UC Davis Chancellors Postdoctoral Fellowship

Title: Modeling encoding in identified coordinating neurons

Authors: *T. M. WRIGHT, JR, M. S. GOLDMAN, B. MULLONEY;
Neurobiology, Physiol. and Behavior, Univ. of California, Davis, Davis, CA

Abstract: Crayfish swim using four pairs of swimmerets on their abdomen that move in a periodic coordinated sequence. Each swimmeret is controlled by a local neural microcircuit that produces the patterned motor output that drives these movements. At each level of excitation, microcircuits in different segments have the same period but differ in phase. Although period changes in response to changes in excitation, these intersegmental phase differences do not change. Information required to coordinate these distributed microcircuits is encoded as bursts of spikes in identified coordinating neurons. A pair of coordinating neurons that project to targets in other segments originate in each microcircuit. Bursts of spikes in these neurons affect the phase and strength of motor output from the other microcircuits to which they project. As excitation changes, the periods and durations of bursts in these coordinating neurons change proportionately, but the numbers of spikes per burst do not change significantly. This invariance of intersegmental phase and spikes per burst suggests that encoding and decoding of coordinating information is modulated by changes in excitation that act either on synaptic currents, or on the neurons' intrinsic properties, or both. To describe the coordinating neurons' intrinsic encoding properties, we identified individual coordinating neurons in isolated ventral nerve cord preparations. We recorded their response to pairs of triangular ramp-currents whose periods and durations approximated the periodic synaptic currents these neurons receive in active preparations. Coordinating neurons exhibited two components of adaptation: a short-term

adaptation that causes spike hysteresis within a burst, and a longer-lasting adaptation that affects the second burst of the pair. To model these responses quantitatively, we constructed a mathematical model that captures the intrinsic excitability of these neurons. We tuned the model to capture the numbers and timing of spikes in response to different current durations and amplitudes, and also the intraburst and interburst components of adaptation. We show that the excitability of the model neuron can be tuned effectively by two parameters, and use the model to perform virtual experiments that show how excitability of coordinating neurons might be tuned by changing excitation.

Disclosures: **T.M. Wright:** None. **M.S. Goldman:** None. **B. Mulloney:** None.

Poster

065. Generation of Motor Patterns

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Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Support: SM 206/2-1

SM 206/3-1

Title: Robustness of coordination to temperature perturbation in a chain of microcircuits

Authors: *C. R. SMARANDACHE-WELLMANN, F. BLUMENTHAL;
Zoological Institute, Animal Physiology, Emmy Noether Group, Univ. of Cologne, Cologne, Germany

Abstract: Swimmerets are four pairs of limbs attached to the abdomen of crayfish, and are used for forward swimming. They are controlled and innervated by four of six abdominal ganglia. In each hemiganglion a neuronal circuit drives motor neurons which activate the limbs in alternating cycles of power-stroke (PS) and return-stroke movement. An identified coordinating circuit synchronizes these central pattern generators, so that a metachronal wave is present where the last segment starts the PS and the anterior modules follow with a latency of 0.25 phase. This synchronization is independent of the frequency of the rhythm and of sensory feedback. A different coordinating pattern was never observed in these crayfish. Here we wanted to test if temperature can change coordination. In normal conditions signal crayfish, Pacifastacus leniusculus, live in rivers where temperature ranges from 5°C (in wintertime) to 25°C (during

summertime). Therefore we used a similar temperature range to perturb the system. For these experiments we used animals which were kept at normal temperature (14°C, middle) and two sets of crayfish which were acclimated at extreme conditions: cold (4°C) and warm (25°C) for at least 6 weeks. All experiments were done on the isolated nervous system with extracellular recordings of all motor nerves responsible for swimmeret movements. We treated the nerve cord with saline having temperatures between 4 to 35°C. Here we tested if the nervous system's activity is stable towards temperature perturbations and if animals kept at extreme conditions reacted differently to sudden temperature changes. In all experiments, where the swimmeret rhythm was active, the coordinating pattern with a phase lag of 0.25 between PS bursts remained stable. On the other hand period, burst duration and PS burst strength decreased with increased temperatures. When we compared the different acclimated animals we observed very distinct effects. The coordinated rhythm of cold acclimated animals became unstable at around 15°C and broke down at high temperatures. In contrast warm acclimated crayfish had very slow or uncoordinated rhythms at low temperatures, which became stable at temperatures between 18°C to 30°C. Middle acclimated animals produced well coordinated rhythmic activity over a larger temperature range (4 to 25°C) than animals acclimated under extreme (cold or warm) conditions. When motor neurons were recorded intracellularly at the same time, their membrane potentials hyperpolarized during warm and depolarized in cold states. This change in membrane potential is probably due to a change in potassium flow according to Q10.

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Poster

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Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Support: Swedish research Council

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Title: Excitatory Hb9 interneurons involved in locomotor rhythm generation

Authors: *V. R. CALDEIRA, O. KIEHN, K. J. DOUGHERTY;
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Abstract: Rhythm generating neurons are ventrally-located, ipsilaterally-projecting, excitatory neurons in the thoracolumbar spinal cord. There is no known marker of rhythm generating neurons, making it difficult to identify and target them. However, recently, a subset of Shox2 interneurons was found to make up a fraction of the rhythm generating population (Dougherty et al., 2013). In searching for other rhythm generating neurons, we are focusing on the excitatory subsets of mixed-transmitter, known populations. One such population is the Hb9 interneurons (Hb9 INs). Although the homeobox gene Hb9 was initially characterized as a motor neuron (MN) marker (Thaler et al., 1999; Arber et al., 1999), the generation of an Hb9::GFP mouse line (Wichterle et al., 2002) revealed that, in addition to MNs, GFP is also expressed in both excitatory and inhibitory interneurons. A small population of excitatory Hb9 INs that retains Hb9 protein expression postnatally has undergone extensive electrophysiological characterization and has been postulated to be candidate for rhythm generating neurons in the mammalian spinal cord (Hinckley et al., 2005; Wilson et al., 2005). Nevertheless, the involvement of Hb9 INs in rhythm generation has never been directly tested. Here we show that Hb9::Cre leads to recombination in a mixed population of interneurons throughout the spinal cord. Transmitter phenotype and overlap with developmental markers was evaluated in Hb9::Cre; Rosa26::XFP cords using crosses with various Transmitter::XFP mice and antibody stainings. To target the glutamatergic Hb9 INs, we resorted to an intersectional genetics approach to selectively delete Vglut2 specifically from Hb9 neurons. In the resulting mutant mice (Hb9Cre-Vglut2Δ/Δ), glutamate is not released from Hb9 INs, thereby synaptically silencing the excitatory Hb9 population. Locomotor-like activity was evoked in *in vitro* spinal cords from these mice at P0 with combination of NMDA and 5-HT. We did not observe any difference in left-right and/or flexor-extensor phasing between mutants and controls, suggesting that excitatory Hb9 INs do not affect pattern generation. In controls, the mean locomotor frequencies increased with increasing NMDA concentrations. The frequencies of locomotor activity in the Hb9Cre-Vglut2Δ/Δ cords also increased with increasing NMDA concentration but were significantly lower than in controls. Thus, the blockage of synaptic output from glutamatergic Hb9 neurons results in a lower locomotor frequency and thereby suggests these interneurons play a role in rhythm generation.

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Poster

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Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

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Title: A cell-type catalog of the neurons in the stomatogastric ganglion of the Jonah crab Cancer borealis

Authors: *M.-L. GOERITZ, T. BROOKINGS, R. Z. YANG, A. C. SUTTON, E. MARDER; Biol., Brandeis Univ., WALTHAM, MA

Abstract: The networks in the stomatogastric ganglion (STG) of the Jonah crab *Cancer borealis* have been extensively studied as a model system for central pattern generation. The physiology of the underlying cells has been described in detail, but surprisingly few studies have addressed the morphology of these cells in this species. As we continue to learn about the intrinsic properties of STG neurons and their variance across animals, a systematic documentation and comparison of their morphology is needed. We assembled a morphological catalog of the 14 different cell types in the STG, using dye-fills and high-resolution confocal microscopy. We found cell type specific differences in overall appearance, branching pattern and branching complexity. We compared the architecture of the C. borealis STG with that of its distant relative, the Maine lobster *Homarus americanus*. The two species separated in the Paleozoic, but the stomatogastric neurons have largely retained their physiological identities. The overall shape of the STG in C borealis is flattened and the neuron somata are organized laterally around a shared neuropil in the center of the STG. This is different from the STG in the lobster, in which the somata are dispersed over the dorsal surface of the spindle-shaped ganglion. Despite these differences in appearance, we found several salient features conserved between the two species. Among these is the overall organization of the neurons, which consist of a large soma at the periphery of the ganglion, and a wide primary neurite that gives rise to several secondary branches or varying complexity, and one (or several) axons that leave the STG and connect to the musculature. In both species, neurons can exhibit dramatic changes in diameter at branch points, and both have hand-like structures of branch points with multiple offspring branches (Bucher et al 2007). However, we also found features that were specific to *C. borealis* STG neurons, such as a more circular cross-section of the processes, and elongated varicosities in small diameter processes, for example in the lateral gastric (LG) neuron. Supported by NIH grand MH 46742. References: Bucher D, Johnson CD, Marder EE. 2007. Neuronal morphology and neuropil structure in the stomatogastric ganglion of the lobster, Homarus americanus. J Comp Neurol 501:185-205.

Disclosures: **M. Goeritz:** None. **T. Brookings:** None. **R.Z. Yang:** None. **A.C. Sutton:** None. **E. Marder:** None.

Poster

065. Generation of Motor Patterns

Location: Halls A-C

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Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Support: Swedish research council

Karolinska Institutet

StratNeuro

Title: Endocannabinoids supplement a hardwired circuit to promote behavioral selection by switching between motoneuron pools in adult zebrafish

Authors: *J. SONG[1], K. AMPATZIS[2], J. AUSBORN[2], A. EL MANIRA[2];
[1]Karolinska Inst., Stockholm, Sweden; [2]Karolinska Institutet, Stockholm, Sweden

Abstract: Animals constantly make behavioral choices to move efficiently through the environment. When faced with a threat, animals make decisions in the midst of other ongoing behaviors through a context-dependent integration of sensory stimuli. The mechanisms underlying these behavioral selections in vertebrates are not well understood. In this study, we examine the neural underpinnings of the context-dependent priority of escape behavior over swimming by recapitulating these two motor behaviors and their interactions in an *in vitro* preparation of adult zebrafish. We show that the selection of escape over swimming is mediated by switching between fast and slow motoneuron pools. The fast motoneuron pool underlying escape is engaged via monosynaptic excitation. In contrast, the slow pool underlying swimming is disengaged via indirect inhibition that decouples these slow motoneurons from the premotor swim circuit. The onset of the escape and the associated inhibition of swimming activity are determined by a hardwired fast circuit. However, the threshold for initiation of escape and the extent of inhibition of swimming relies on endocannabinoid retrograde signaling. Thus, our results reveal a novel mechanism involving a hardwired circuit supplemented with endocannabinoid modulation that shifts between slow and fast motoneuron pools and hence mediates behavioral selection in vertebrates.

Disclosures: **J. Song:** None. **K. Ampatzis:** None. **J. Ausborn:** None. **A. El Manira:** None.

Poster

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Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Support: Swedish research council

Karolinska Institute

StratNeuro

Title: Functional organization and patterning of the V0 interneurons in adult zebrafish

Authors: R. BJÖRNFORS, J. AUSBORN, *A. EL MANIRA;
Karolinska Inst., Stockholm, Sweden

Abstract: Vertebrate locomotion is generated by spinal circuits consisting of ipsilateral interneurons that provide the excitatory drive and commissural interneurons underlying the mid-cycle inhibition. The identity of the different neuronal populations in terms of transcription factors is preserved across vertebrate species. In addition, these neuronal populations appear to play similar roles from fish to mammals. In adult zebrafish, it has been shown that the motor neurons and the V2a excitatory interneurons form three discrete microcircuits: slow, intermediate and fast with preferential connectivity and recruitment frequencies matching the muscle type they innervate. However, the pattern of organization and functional subdivision of the commissural V0 interneurons remains unclear. We sought to determine if the V0 population of spinal commissural interneurons conforms to a frequency dependent circuitry division or not. Our findings show that both glutamatergic and glycinergic V0 interneurons, can indeed be divided into three modules that are recruited at slow, intermediate and fast swimming frequencies. The slow neurons fire action potentials on every cycle throughout the swim episode. The intermediate neurons are recruited only at intermediate and high frequencies, but are silent at low locomotor frequencies. Neurons belonging to the slow and the intermediate modules have slightly more depolarized resting membrane potentials, lower rheobase, and generally higher phasic fluctuations in membrane potential during swimming. Upon current pulse injection, these neurons generally display either a tonic or bursting firing pattern of action potentials. The neurons of the fast module never fire action potentials during slow to intermediate locomotion but receive synaptic input in the form of membrane potential oscillations in phase with the

swimming frequency. They tend to have a more hyperpolarized membrane potential, higher rheobase and lower phasic membrane fluctuations during swimming. These neurons show a tonic or adaptive firing pattern of action potentials, although adaptive firing is prevalent. In summary, the V0 interneurons are divided into three functional modules that display specific synaptic and intrinsic properties that set their recruitment order during swimming. This modular division is in accordance with that of the motor neurons and the V2a interneurons.

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Poster

065. Generation of Motor Patterns

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Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Support: NSF grant PHY-0750456

Title: Mechanisms of emergent bursting activity in small neuronal networks

Authors: *J. F. CANNON[1], O. BURYLKO[2], W. H. BARNETT[2], G. S. CYMBALYUK[2]; [1]Biol., Georgia State Univ. Biol. Dept, Atlanta, GA; [2]Neurosci. Inst., Georgia State Univ., Atlanta, GA

Abstract: Bursting is the key regime of oscillatory networks controlling motor behaviors, Central Pattern Generators (CPGs). The endogenous dynamics of isolated CPG neurons are frequently found to be silent or spiking. We address an open question in neuroscience: how does bursting activity emerge in networks of non-bursting neurons? We present two mechanisms describing the emergence of bursting activity, one in a network of endogenously silent neurons and the other in a network of endogenously spiking neurons. The temporal properties of the transient responses of isolated silent and spiking neurons are organized by the cornerstone bifurcation. The networks presented here are constructed of neurons described by a 3D Hodgkin-Huxley style model which exhibits the cornerstone bifurcation. This bifurcation satisfies the criteria for both the Shilnikov blue sky catastrophe and the saddle-node bifurcation on invariant circle. The cornerstone bifurcation not only controls the burst duration (BD) and interburst interval (IBI) in bursting neurons but also determines the stereotypical transient responses of silent and spiking neurons. The mechanisms presented here are based on these responses. The first mechanism describes a half-center oscillator (HCO) consisting of two intrinsically silent,

mutually inhibitory neurons. The biophysical parameters of these two neurons were close to the critical values for the cornerstone bifurcation. This HCO showed anti-phase bursting activity. We found that if the half-activation voltage of a non-inactivating potassium current was systematically shifted towards the bifurcation value for the saddle-node bifurcation of periodic orbits, the BDs of both neurons increased in accordance with the inverse-square-root law and linearly depended on the spike number per burst. This new mechanism is different from release and escape. The second mechanism described the bursting activity of two intrinsically spiking, mutually excitatory neurons. The parameters of the neurons were also in vicinity of the cornerstone bifurcation. This network exhibited synchronized bursting. When the half-activation voltage of a hyperpolarization-activated current was systematically shifted to the bifurcation value for the saddle-node bifurcation of stationary states, IBIs of both neurons increased in accordance with the inverse-square-root law. The BDs of both neurons are controlled by the blue sky catastrophe bifurcation parameter which is the conductance of synaptic current. These mechanisms are generic and could govern the bursting regimes in rhythmic neuronal network such as central pattern generators.

Disclosures: **J.F. Cannon:** None. **O. Burylko:** None. **W.H. Barnett:** None. **G.S. Cymbalyuk:** None.

Poster

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Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Title: The modulation of transmission in the trigeminal nuclei and jaw opening reflex responses during superior laryngeal nerve stimulation

Authors: ***S. SAKAI**, K. TSUJI, J. MAGARA, T. TSUJIMURA, M. INOUE;
Dysphagia Rehabil., Niigata Univ., Niigata City/Niigata, Japan

Abstract: Introduction: The aim of the present study was to investigate the possible neuronal mechanisms for the modulation of jaw opening reflex (JOR) responses during superior laryngeal nerve stimulation (SLN) in anesthetized animals. Materials and Methods: Experiments were carried out on anesthetized rabbits with urethane. The inferior alveolar nerve (IAN) was stimulated to evoke the JOR in the digastric muscle. Current intensity was determined as 1.5 times (T) the threshold for evoking the JOR. In addition, single neurons responding to the IAN

stimulation were recorded in the trigeminal nuclei. To evoke the swallowing reflex, SLN was stimulated. Current intensity was determined as 1 to 4 T the threshold for evoking the swallowing reflex in 10 seconds. Peak to peak amplitude of JOR responses and activity of single neurons were compared between with and without SLN stimulation. Finally, the recording site in the brain stem was histologically identified. Results & Conclusion: 1. JOR was inhibited during SLN stimulation. 2. Responses of most neurons were inhibited during 2 or 4 T SLN stimulation. 3. Recording sites of the neurons were identified in the main sensory trigeminal nucleus and subnucleus oralis of the spinal trigeminal tract. 4. Current results suggest that low threshold evoked JOR is inhibited at the level of trigeminal interneurons in the JOR pathway during SLN stimulation. References: •Takako Fukuhara et al., 2011. Effects of electrical stimulation of the superior laryngeal nerve on the jaw-opening reflex. Brain Res. 1391: 44-53. •Aki Yamada et al., 2013. Effects of chewing and swallowing behavior on jaw opening reflex responses in freely feeding rabbits. Neurosci Lett. 535:73-7 •K.A. Olsson et al., 1986. Modulation of transmission in rostral trigeminal sensory nuclei during chewing. J Neurophysiol. 55(1): 56-75.

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Poster

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Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Support: NHLBI Diversity Supplement R01 HL104127

Title: Quantitative TRP mRNA expression in single Dbx1 neurons in the preBötzinger Complex of neonatal mice

Authors: *M. D. PICARDO, C. A. DEL NEGRO;
Applied Sci., Col. of William and Mary, Williamsburg, VA

Abstract: Inspiratory-related rhythm generated in the pre-Bötzinger complex (preBötC) of the medulla is carried out by a substantial subset of rhythmogenic neurons derived from a single genetic line expressing the transcription factor Dbx1. Electrophysiological characterization of the Dbx1-derived population shows that these neurons highly express Ca2+-activated non-selective cationic current (ICAN), which is hypothesized to generate the inspiratory drive potential.

However, the molecular identity of the channels that generate the inspiratory bursts have not been identified. Non-selective cation channels of the transient receptor potential (TRP) family are likely candidates to give rise to ICAN in the preBötC based on evidence from previous findings, but the molecular identity of the TRP channel(s) involved has not been ascertained. TRPM4, TRPM5,TRPC3, and TRPC7 mRNA have each been shown to be present in the preBötC. Whole cell recordings of preBötC neurons also show TRP-like physiology. In addition, TRPM4 and TRPM5 mRNA were detected in the preBötC. However, expression of TRP channels has not been documented in rhythmogenic preBötC neurons. Here we used quantitative polymerase chain reaction (qPCR) to investigate the expression of TRPM4, TRPM5, TRPC3 and TRPC7 mRNA in single Dbx1 neurons in the preBötC. mRNA extraction, cDNA synthesis and pre-amplification, and subsequent qPCR were performed on individual Dbx1 neurons that were isolated from newborn transgenic mouse slice preparations. TRP mRNA levels in the preBötC Dbx1 neurons were compared with those in preBötC non-Dbx1 neurons, as well as with the Dbx1 neurons in the amygdala. Data show that TRPM4 mRNA was detected in all preBötC Dbx1 neurons, whereas TRPM5 mRNA was expressed in ~15% of the samples only. Both TRPC3 and TRPC7 were detected in about half of the sampled preBötC Dbx1 neurons. Dbx1 neurons isolated from the amygdala did not express TRPM5 mRNA, and had significantly lower mRNA expression of the other TRP channels, compared to the preBötC Dbx1 neurons. Although mRNA levels do not always indicate that these TRP channels are present or functionally important, these qPCR data provide a valuable comparison of expression at the mRNA level. The consistent expression of TRPM4 mRNA in the rhythmogenic Dbx1 neurons could imply involvement of the TRPM4 channel in giving rise to ICAN and generating the inspiratory drive potential. These qPCR data may provide insight in understanding the ionic basis of inspiratory burst generation in Dbx1 neurons in the preBötC.

Disclosures: **M.D. Picardo:** None. **C.A. Del Negro:** None.

Poster

065. Generation of Motor Patterns

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Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Support: MH 46742

Title: Quantification of morphological differences from reconstructions of multiple types of stomatogastric neurons

Authors: *A. C. SUTTON[1,2], T. BROOKINGS[2], M. L. GOERITZ[2], E. MARDER[2];
[2]Volen Ctr. for Complex Systems, [1]Brandeis Univ., Waltham, MA

Abstract: Central pattern generators robustly maintain target output activity despite wide variations in molecular parameters such as mRNA expression and ion channel conductance. However, neuronal structure can also vary widely between animals, and the effects of these changes have not been examined in conjunction with physiology. The stomatogastric ganglion (STG) of the Jonah crab C. borealis is a central pattern generator that consists largely of motor neurons controlling rhythmic activity of stomach muscles. STG neurons have been shown to tune their mRNA levels of ion channels to achieve targeted activity (Turrigiano et al., 1994). It is possible that certain aspects of a neuron's morphology can serve as 'free parameters' for the neuron to reach its goal. However, it is not known which morphological features are variable candidates and which are not. To this end, we sought to quantitatively identify morphological characteristics of STG neurons to determine common or 'fixed' aspects of neurons, which should be similar in all neurons of a given type. Previously, qualitative examinations of morphology have suggested differences between certain STG neuronal subtypes. We imaged dye-filled STG neurons with a confocal microscope and used reconstructions to extract morphological features of the soma, dendritic arbor and dendritic field. Basic features of the soma such as volume, surface area and major axis were examined; similar aspects of the dendritic field were also examined, including volume and orientation in relation to soma. The dendritic tree was also studied, and among the analyses were branching behavior, bifurcation asymmetry, tortuosity, Sholl analysis and path length.

Disclosures: **A.C. Sutton:** None. **T. Brookings:** None. **M.L. Goeritz:** None. **E. Marder:** None.

Poster

065. Generation of Motor Patterns

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Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Support: NIH T32 Molecular Biology Training Grant

DoD CDMRP Hypothesis and Exploration Award No. SC090555

Title: Homeostatic maintenance of network synchrony: Compensatory mechanisms underlying restoration of synchronized bursting in variable motor neurons

Authors: *B. J. LANE, J. L. RANSDELL, S. S. NAIR, D. J. SCHULZ;
Univ. of Missouri - Columbia, Columbia, MO

Abstract: The electrically coupled Large Cell (LC) motor neurons in the Cardiac Ganglion (CG) of the crab *C. borealis* display synchronized rhythmic bursting during normal network output *in vitro*, even though the individual LCs have variable underlying electrical properties. We exposed LCs to treatments with either TEA or serotonin to change membrane conductances, and found that in both cases LCs desynchronize and then recover synchrony over time. Pharmacological blockade with TEA increases the number of spikes per burst and the duration of bursts, but these propertiesrevert towards baseline levels as synchrony is restored. Neuromodulation with 5-HT increases the number of spikes per burst and spike frequency, yet these outputs remain elevated as synchrony is restored. To determine the mechanism of restored synchrony in these conditions, we investigated two hypotheses: 1) Homeostatic changes in intrinsic excitability in each LC independently adjust output and converge on synchronous activity, and 2) Network-level properties such as electrical coupling are altered to restore synchrony. To test whether intrinsic excitability is altered in response to desynchronized activity, we performed two-electrode voltage clamps to determine how individual LC conductances are affected by each treatment, and how conductances change during the process of re-synchronization. When the K^+ current I_{HTK} is blocked with TEA, the K^+ current I_A increases, a potential mechanism for restored output. We found that serotonin increases the magnitude of the K^+ current I_A by approximately 30%, and are examining whether similar compensation occurs in other currents. To test whether increased electrical coupling is a potential mechanism to resynchronize LC bursting, we isolated pairs of LCs, induced disparate output with TEA, and applied dynamic clamp to add an artificial coupling conductance. We found that dynamic clamp was able to artificially re-synchronize LCs within a biologically realistic conductance range. We will determine whether a functional increase in coupling occurs between cells as they resynchronize, by desynchronizing LCs and measuring coupling coefficients between LCs during the process of re-synchronization. Our data so far suggest that both intrinsic excitability and electrical coupling strength are potential targets for homeostatic compensation that restores network synchrony in response to challenges such as neuromodulation.

Disclosures: **B.J. Lane:** None. **J.L. Ransdell:** None. **S.S. Nair:** None. **D.J. Schulz:** None.

Poster

065. Generation of Motor Patterns

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Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Title: Mechanisms of network synchrony arising from variable individual motor neurons of the crustacean cardiac ganglion

Authors: *S. S. NAIR[1], P. SAMARTH[1], B. LANE[2], D. J. SCHULZ[2];
[1]Electrical & Computer Engin., [2]Biol. Sci., Univ. Missouri-Columbia, COLUMBIA, MO

Abstract: The crab cardiac ganglion (CG) has five large motor cells (LCs) driven to burst by four small endogenous pacemaker cells (SCs). The CG is responsible for controlling the rhythmic contractions of the single-muscled crab heart. Our goal was to examine mechanisms of synchronization among the five LC motor neurons of the crab CG. We created a pool of model LCs using the biological current data and performed a sampling rejection technique to determine parameter sets that generated appropriate LC output characteristics. To be included in the model cell data set, a given LC model had to have passive properties within biological bounds and appropriate output responses to multiple current injection protocols consistent with experimental traces. Parameter sets obtained in this fashion were used to select model LCs for the network studies. A prediction using the single LC model was that the calcium current ICAN and the calcium-activated potassium current ISKKCa have to be in the ratio 1:0.83 for proper termination of the driver potential observed experimentally in isolated LC's in presence of tetraethylammonium (TEA). Also, the parameter set for model LCs revealed two strong inverse correlations: between leak and KA currents (r2=0.81) and between Nap and CaL currents (r2=0.69). We then developed a computational network model including the5 LCs and 4 SCs. This model was used to investigate the role of network parameters in maintaining synchronous output at both cellular and network levels. Biological results demonstrate that the variable properties of the individual motor neurons lead to loss of synchrony under conditions of neuromodulation and pharmacological blockade with channel blockers such as TEA. However, synchrony is dynamically restored following prolonged modulator exposure. Our goal was to use the model CG network to determine potential mechanisms underlying the loss, and restoration of, synchrony. We examined changes in intrinsic properties, synaptic drive, and electrical coupling to determine whether plasticity in these features could restore synchrony following model applications of neuromodulation and TEA.

Disclosures: **S.S. Nair:** None. **P. Samarth:** None. **B. Lane:** None. **D.J. Schulz:** None.

Poster

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Mairie de Paris Emergence

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Inserm Atip/Avenir

Fondation Bettencourt-Schueller

Title: Physiological recruitment of CSF-contacting neurons *in vivo*

Authors: *U. L. BOEHM[1,2,3,4], L. DJENOUNE[1,2,3,4,5], A. PRENDERGAST[1,2,3,4], S. NUNES-FIGUEIREDO[1,2,3,4], F. DEL BENE[6,7,8], C. WYART[1,4,3,2];
[1]Inst. Du Cerveau Et De La Moelle Épinière, Paris, France; [2]UPMC Univ. Paris 06, Paris, France; [3]Inserm UMR 1127, Paris, France; [4]CNRS UMR 7225, Paris, France; [5]Muséum Natl. d'Histoire Naturelle, Paris, France; [6]Inst. Curie, Paris, France; [7]CNRS UMR 3215, Paris, France; [8]Inserm U 934, Paris, France

Abstract: We investigate the function of GABAergic cerebrospinal fluid contacting neurons (CSF-cNs) in the spinal cord. These cells line the central canal and extend a brush of microvilli into the lumen but lack any obvious dendrites. They are conserved across many vertebrates and their atypical morphology makes them interesting candidates for sensory cells. The project aims to investigate the sensory roles and physiological recruitment of CSF-cNs. We use genetically encoded calcium sensors (GCaMP6f) to probe their activation in paralyzed and moving zebrafish larvae. Calcium imaging in paralyzed larvae revealed that CSF-cNs, unlike most other cells in the spinal cord, are not coordinately activated during fictive locomotion where no actual movement occurs. CSF-cNs express the ion channel Pkd2l1, which is suggested to be involved in responses to variations of pH and osmolarity. Accordingly, we show by combining two-photon calcium imaging and proton uncaging in the central canal that CSF-cNs respond to short exposures of acidic pH *in vivo*. We are currently testing other chemical cues that activate these cells. All together our work describes the physiological inputs of CSF-cNs suggesting that they can constitute a sensory feedback loop in the spinal cord. The role of Pkd2l1 in the responses of CSF-cNs to variation of pH is currently under investigation.

Disclosures: **U.L. Boehm:** None. **L. Djenoune:** None. **A. Prendergast:** None. **S. Nunes-Figueiredo:** None. **F. Del Bene:** None. **C. Wyart:** None.

Poster

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Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Support: NIH Grant R01 104127

Title: Synaptic depression influences inspiratory burst termination and post-inspiratory activity in the preBötzinger complex

Authors: *A. KOTTICK, C. A. DEL NEGRO;
Applied Sci., Col. of William and Mary, Williamsburg, VA

Abstract: The preBötzinger Complex (preBötC) of the ventrolateral medulla generates the inspiratory rhythm that drives breathing behavior. Recurrent synaptic excitation initiates inspiratory bursts, but whether excitatory synaptic mechanisms also play a role in burst termination and recovery during post-inspiration is not well understood. Using a neonatal mouse medullary slice preparation that spontaneously generates inspiratory activity and motor output, we tested the hypothesis that short-term synaptic depression influences inspiratory burst termination and post inspiratory activity. We used an excitatory light-gated ion channel, channelrhodopsin, to rapidly stimulate the preBötC throughout the respiratory cycle. We performed whole-cell recordings in the preBötC and measured a refractory period of ~2 seconds after endogenous inspiratory bursts that precluded optically evoked activity. We found no change in the amplitude distribution of spontaneous EPSPs before or after endogenous inspiratory bursts, suggesting that there is no postsynaptic modulation of synaptic transmission after endogenous inspiratory bursts. However, the duration of the refractory period was liable to extracellular calcium concentration, which is consistent with presynaptic modulation. These results provide the first experimental evidence that short-term synaptic depression in the preBötC influences inspiratory burst termination and post-inspiratory activity, which contrasts previous models that ascribed these functions to extrinsic sources of synaptic inhibition.

Disclosures: **A. Kottick:** None. **C.A. Del Negro:** None.

Poster

065. Generation of Motor Patterns

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Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Support: CONACYT CB-128392

CONACYT CB-153627

Title: Substitution of extracellular Ca2+ by Sr2+ prolongs inspiratory burst in preBötzinger Complex (preBötC) neurons. Implications for burst termination mechanisms

Authors: *C. MORGADO-VALLE[1], J. FERNANDEZ-RUIZ[2], L. LOPEZ-MERAZ[1], L. BELTRAN-PARRAZAL[1];
[1]Ctr. de Investigaciones Cerebrales, Univ. Veracruzana, Xalapa, Mexico; [2]Facultad de Medicina, Univ. Nacional Autonoma de Mexico, Mexico, DF, Mexico

Abstract: The role of Ca^{2+} in the mechanisms underlying the initiation and termination of the inspiratory burst in preBötC neurons is a matter of intense debate (Pace *et al*., 2007; Morgado-Valle *et al*., 2008; Mironov 2008; Del Negro et al., 2011; Beltran-Parrazal *et al*., 2012; Rybak *et al*., 2014). We aimed to test the effects of removing extracellular Ca^{2+} without affecting neurotransmission on burst initiation and termination. In a brainstem rhythmic slice we current-clamped preBötC neurons (V_m-60 mV) and bath applied ACSF with no Ca^{2+} and either 1.5 mM or 2.5 mM Sr^{2+} while recording integrated hypoglossal nerve (∫XIIn) activity as motor output. Substitution of extracellular Ca^{2+} by Sr^{2+} significantly increased the duration of inspiratory burst (from 653 ± 31 ms in control conditions to 982 ± 78 ms and 2048 ± 448 ms in 1.5 and 2.5 mM Sr^{2+} respectively); increased the decay time (by 60% and 290% for 1.5 and 2.5 mM Sr^{2+} respectively) and area (by 65% and 200% for 1.5 and 2.5 mM Sr^{2+} respectively) with respect to control conditions. At the systems level, substitution of extracellular Ca^{2+} by Sr^{2+} significantly increased ∫XIIn burst duration (by 30% for 1.5 or 2.5 mM Sr^{2+}), decreased ∫XIIn period (by 40% and 25% for 1.5 and 2.5 mM Sr^{2+} respectively) and had not effect on burst irregularity score. Substitution of Ca^{2+} by Sr^{2+} is the first reported ionic manipulation that significantly increases the duration of inspiratory burst. Sr^{2+} substitution of Ca^{2+} is a well-established method to desynchronize neurotransmitter release. Our findings suggest that the increase of inspiratory burst duration is determined by a presynaptic mechanism involving desynchronization of glutamate release within the network. We suggest that termination of inspiratory drive depends

on a presynaptic mechanism rather than in a postsynaptic mechanism involving extracellular Ca^{2+} for which Sr^{2+} cannot substitute.

Disclosures: **C. Morgado-Valle:** None. **L. Beltran-Parrazal:** None. **L. Lopez-Meraz:** None. **J. Fernandez-Ruiz:** None.

Poster

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Title: Multifunctional half-center oscillator controlling walking and paw-shake response in the cat

Authors: B. BONDY[1], A. N. KLISHKO[2], B. PRILUTSKY[2], *G. S. CYMBALYUK[1];
[1]The Neurosci. Inst., Georgia State Univ., ATLANTA, GA; [2]Sch. of Applied Physiology, Ctr. for Human Movement Studies, Georgia Inst. of Technol., Atlanta, GA

Abstract: Central pattern generators (CPGs) are neuronal networks controlling rhythmic motor behaviors such as flying, breathing, and walking. CPGs can produce characteristic rhythmic patterns even when deprived of sensory feedback. It is likely that CPGs can share neurons, forming multifunctional CPGs. It has been shown in several species that the same interneuron can be active during multiple behaviors [1]. Sensory information and modulatory tone would determine which pattern of activity and therefore, which behavior is produced. Here, we explore the idea that one set of neurons in a single multifunctional CPG could be intrinsically capable of producing multiple regimes of activity without changes in biophysical properties of its neurons and inter-neuronal connections. Such a multi-stable CPG would only require a transient

perturbation to switch between regimes. Multistability of a CPG arising from synaptic dynamics has been demonstrated in models of neuronal networks with depressing synapses [2]. We investigated whether two rhythmic cat behaviors, walking and paw shake, could be controlled by a single half-center oscillator (HCO) composed of two mutually inhibitory neurons. Is it possible for cellular dynamics to underlie multistability in a CPG? We have created a parsimonious HCO model with neurons containing two slow inward currents: a Na+ current (INaS), and a Ca++ current (ICaS) which inactivates slower and at more negative voltages than INaS. Two regimes of activity coexist in this model: a fast, 10 Hz paw shake regime with low spike frequency and high ICaS inactivation, and a slow 2 Hz walking regime, with high spike frequency and low ICaS inactivation. It is possible to switch between regimes with pulses of excitatory or inhibitory conductance. We also demonstrate that changes of certain conductances can lead to hysteresis of regimes, elucidating a role for neuromodulation in pattern switching. The multifunctional CPG model is also incorporated into a neuromechanical model of the cat hindlimbs in the AnimatLab environment [3] that reproduces realistic walking and paw-shake response. References 1. Berkowitz A, Roberts A, Soffe S (2010) Front Behav Neurosci 4 (36):1-18 2. Manor Y, Nadim F (2001) , J Neurosci 21 (23):9460-9470 3. Klishko A, Cofer D, Cymbalyuk G, Edwards D, and Prilutsky B (2012) BMC Neurosci 13:P70.

Disclosures: **B. Bondy:** None. **A.N. Klishko:** None. **B. Prilutsky:** None. **G.S. Cymbalyuk:** None.

Poster

065. Generation of Motor Patterns

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 65.24/DD5

Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Support: NSF Grant IOS-0818788

Title: Local spinal circuits dictate locomotor output in caudal stimuli-elicited startles of larval zebrafish

Authors: *Y.-C. LIU, M. E. HALE;
Organismal Biol. and Anat., Univ. of Chicago, Chicago, IL

Abstract: The startle response of zebrafish and goldfish has long been used as a model for understanding fundamentals of neural circuit organization and function. A major focus of study

has been on how variation in behavior arises at a circuit level. We've previously shown in larval zebrafish that stimulus direction can induce variations in axial bending behavior. Stimuli to the head result in an escape response called the C-start while a distinctly different S-start response may be triggered when the stimuli is directed at the tail. The most striking kinematic difference between these behaviors is that the body forms a "C" shaped bend during the C-start while during the S-start the body forms an "S" shaped bend. Neurons responsible for generating startles include a pair of Mauthner (M-) cells in the hindbrain that when fired initiates the behavior. When the left M-cell fires, it activates motoneurons in the spinal cord on the right side causing unilateral muscle contractions. It also excites commissural local (CoLo) neurons on the right side, which inhibits neuronal activity on the left side. While this model can explain the rapid C-shaped bend in C-starts, it does not allow for the caudal counter bend that is characteristic of the S-start. We have previously shown that in response to tail stimuli, both M-cells always fire during S-starts, while either one or both M-cells fire during C-starts. Involvement of M-cells in both caudal C- and S-starts suggest local variations in the spinal cord. We have found that primary motoneurons in the caudal spinal cord are often inhibited before the first burst of activity during tail-elicited startles, while rostral motoneurons are not, and this inhibition appears before the Mauthner spike. CoLo activity coincides with early motoneuron inhibition. These data taken together show that local inhibition caused by CoLos in the caudal spinal cord act as a switch that predetermines whether the M-cell command signal ultimately generates a C- or S-start to caudal stimulation. To investigate sensory inputs to the caudal spinal cord that may activate local CoLos, we conducted paired recordings between CoLos and Rohon-Beard (RB) cells. Preliminary data show RBs firing immediately after caudal stimuli, coincident with initial activity of CoLos in the same segment. Suprathreshold current injections in RBs result in PSPs in CoLos and may sometimes elicit spikes. These data suggest it is likely that RBs are the sensory cells responsible for local activation of inhibitory CoLos in the caudal cord that results in the counter bend of the S-start.

Disclosures: **Y. Liu:** None. **M.E. Hale:** None.

Poster

065. Generation of Motor Patterns

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 65.25/DD6

Topic: D.11. Vertebrate and Invertebrate Rhythmic Motor Pattern Generation

Support: NIH MH060605

Title: Phase maintenance requires precise matching of synaptic inputs and intrinsic properties

Authors: *H. ANWAR[1], F. NADIM[1,2];
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Abstract: Rhythmic motor activity often requires precisely timed output from each of the neurons in the underlying neural network. The generation of phasic patterns of neuronal activity arises from the synaptic interaction of the network neurons. Therefore, the relationship between intrinsic properties and the strength and dynamics of synaptic inputs must be maintained for the robust output. It is known that, in oscillatory networks, both ionic current levels and synaptic strength vary in the same cell type, yet, the phase relations of neurons are maintained. We examine the hypothesis that the strength and dynamics of synaptic inputs are tuned in each neuron to be the "best" input match for the intrinsic properties, and therefore to maintain constant activity phase across preparations. This hypothesis predicts that, across preparation, in a neuron type that produces a constant activity phase, 1) synaptic inputs are not correlated with the activity phase, 2) if synaptically isolated, different inputs would be required to produce a constant activity phase, and 3) the same input would produce different activity phases. We test this hypothesis in the pyloric network of the crab stomatogastric ganglion. The follower LP neuron receives synaptic input from the pyloric pacemakers which allows it to produce stable bursting activity. We measured synaptic currents in LP neurons across different preparations during ongoing pyloric oscillations and measured the parameters (rise, fall, amplitude, etc.) of the synaptic current with the bursting features (onset and end phase, ISI, etc.) of LP neurons across preparations. We found that both the synaptic parameters and the bursting features widely varied, but appeared to be uncorrelated, across preparations. We then blocked the synapses to isolate the LP neuron, which switched from bursting to tonic activity. We injected O-U noise current in the LP neurons with an expectation that certain levels of inputs would induce different kinds of bursts in each LP neuron. The burst-triggered-average of injected noise currents for different burst types (defined by # spikes/burst) showed that the same burst type in LP corresponded to different input types in different preparations. These results confirm the first two predictions of our hypothesis. We are currently examining whether the dynamic clamp injection of the same synaptic input into synaptically isolated LP neurons would produce different activity phases in different preparations. Together, these results would show that synaptic inputs in each preparation are tuned to match intrinsic properties and therefore maintain phase relationship of neurons across different preparations.

Disclosures: **H. Anwar:** None. **F. Nadim:** None.

Poster

066. Parkinson's Disease Models I

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 66.01/DD7

Topic: D.15. Basal Ganglia

Support: CIHR grant MOP13625

Title: The dorsal striatum is under diurnal control; an electrophysiological study comparing time of day and the effectiveness of a D2 antagonist

Authors: *A. FREDERICK, J. BOURGET-MURRAY, C. A. CHAPMAN, S. AMIR, R. COURTEMANCHE;
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Abstract: The circadian system influences both changes in behavior and alterations in physiology throughout the day. However, little is known about how the circadian system influences neural activity. Experiments in the dorsal striatum show robust circadian gene rhythms in medium spiny neurons and fluctuations in extracellular dopamine levels that peak later in the dark period, suggesting that basal ganglia function may be regulated in a diurnal manner. In order to explore this, we recorded local field potentials from pairs of electrodes in the medial and lateral dorsal striatum of urethane-anesthetized rats at four different times of day (1, 7, 13 and 19 hours after lights on in a 12h:12h light-dark cycle). Strength of oscillations and coherence were compared at baseline and after i.p. injection of the D2 antagonist raclopride. An inverse relationship in the presence of oscillations between 0-3 Hz and 3-8 Hz was observed at baseline, where the presence of 0-3 Hz activity decreased from around 95% of the time at one hour after lights on, to around 50% of the time at one hour after lights off. Furthermore, raclopride had the greatest effects one hour after lights off, where it decreased the occurrence of 3-8 Hz activity and increased 0-3 Hz activity by about 20%. 0-3 Hz coherence followed a similar trend as the oscillatory activity. Both comparisons within electrode pairs and between electrodes placed medially and laterally in the dorsal striatum showed decreased 0-3 Hz coherence at one hour after lights turned off that increased after raclopride administration. Comparisons with electrodes placed in the cerebellum demonstrated a similar modulation at 0-3 Hz, suggesting that the diurnal mechanisms that alter the drive of these oscillations under urethane anesthesia may be centrally located. From these results, we conclude that neural activity in the dorsal striatum is influenced diurnally. Since the peak time in raclopride response does not correspond to when extracellular dopamine is peaking, we propose that alterations in postsynaptic targets, such as changes in neurotransmitter receptors, are diurnally modulated and partially contribute to this modulation. This could have clinical implications in explaining why Parkinson’s disease patients experience diurnal fluctuations in motor symptoms and response to L-DOPA.

Disclosures: **A. Frederick:** None. **J. Bourget-Murray:** None. **C.A. Chapman:** None. **S. Amir:** None. **R. Courtemanche:** None.

Poster

066. Parkinson's Disease Models I

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 66.02/DD8

Topic: D.15. Basal Ganglia

Title: Functional MRI reveals paradoxical cerebral blood volume decreases in striatum during optogenetic stimulation of the striatonigral "direct" pathway

Authors: *D. ALBAUGH, G. STUBER, I. SHIH;
Univ. of North Carolina At Chapel Hill, Chapel Hill, NC

Abstract: Introduction. The striatonigral “direct” pathway of the basal ganglia is well-known for its role in the facilitation of movement, and recent work suggests that stimulation of this pathway is therapeutic in Parkinson’s disease. However, the broader neural networks modulated by direct pathway stimulation remain to be described, which may potentially uncover novel therapeutic stimulation targets for movement disorders. Here, we discuss our preliminary investigations using optogenetic stimulation coupled with a functional magnetic resonance imaging (fMRI) readout to evaluate the global brain response profile to selective direct pathway stimulation. Methods. Wild-type rats received ChannelRhodopsin-2 viral infusions (AAV5, CamKIIa promotor) targeting the dorsolateral striatum, and optical fibers for stimulation of direct pathway projections were placed directly above the substantia nigra pars reticulata. This approach allows us to specifically stimulate the direct pathway without requiring opsin expression selectivity in striatal cell bodies. Following a period of at least 8 weeks to allow for sufficient opsin expression, rats were sedated for fMRI using a 9.4T Bruker system. Changes in cerebral blood volume (CBV) were measured in response to direct pathway optical stimulation at multiple frequencies (10, 20,30, and 40Hz) and compared to within-scan baseline measurements. Results. Direct pathway stimulation resulted in robust, time-locked CBV decreases in the striatum. These responses were frequency-dependent, with the largest CBV decreases occurring at 40Hz. No CBV responses were noted in the substantia nigra pars reticulata or any other region examined. Discussion. It is not readily apparent why direct pathway stimulation would result in striatal CBV decreases, which are traditionally associated with regional silencing in brain activity. However, our group has previously shown that increases in striatal activity can result in

paradoxical BOLD or CBV signal decreases. Thus, we hypothesize that the obtained CBV response reflects antidromic stimulation of direct pathway striatal neurons. Future work will determine behavioral and whole-brain functional connectivity correlates of striatonigral pathway stimulation.

Disclosures: **D. Albaugh:** None. **G. Stuber:** None. **I. Shih:** None.

Poster

066. Parkinson's Disease Models I

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 66.03/DD9

Topic: D.15. Basal Ganglia

Support: NINDS Intramural Research Program

Title: Excessive synchronization in basal ganglia-thalamocortical circuits in the hemiparkinsonian rat during bradykinesia and dyskinesia

Authors: ***K. B. DUPRE**, A. J. MCCOY, E. BRAZHNIK, C. DELAVILLE, A. V. CRUZ, N. NOVIKOV, C. P. DODGE, C. M. GERBER, C. HATCH, M. A. COHEN, D. S. KURUP, J. R. WALTERS;
NIH NINDS, BETHESDA, MD

Abstract: The hemiparkinsonian rat is commonly used as a model for Parkinson's disease (PD). However, in addition to being relevant to PD, this preparation provides the opportunity to explore the properties of brain circuits as they become recruited into a sustained, excessively synchronized and oscillatory state. Synchronized activity associated with bradykinesia emerges in the dopamine (DA) cell-lesioned hemisphere over the first week after unilateral injection of 6-hydroxydopamine into the medial forebrain bundle. Chronic recordings in the motor cortex (MCx), striatum, globus pallidus, subthalamic nucleus (STN), substantia nigra pars reticulata (SNpr) and ventral medial thalamus (VM) have shown sustained alterations in LFP spectral peaks with increased power and coherence in the 30-35 Hz range during walking on a circular treadmill. Interestingly, the levels of spiking entrainment to the 30-35 Hz LFP activity varies widely across these nuclei, with respect to the proportion of cells with significant spike-LFP phase-locking and its relative increase following DA cell lesion. Increases in phase-locking were greater in the STN, SNpr, and VM and lowest in the striatum. The sequence of spike timing in the MCx, STN, SNpr, and VM nuclei was assessed by comparing the phase relationship of each

spike train to a common oscillation, in this case the MCx LFP. The temporal relationships between the phase-locked spikes support a sequential entrainment of activity from MCx-STN/SNpr-VM-MCx, consistent with the anatomical connections within this circuit. A different synchronized state can also be seen during L-dopa-induced dyskinesia. Synchronized activity, referred to as 'finely tuned gamma,' with varying dominant frequencies in the 70-120 Hz range, is present in LFPs recorded from the MCx, VM and striatum. This activity is not prominent in the STN and SNpr power spectra, however significant coherence in this range is observed between these structures and the MCx. Notably, the peak frequency of this band varies with rat strain, with a lower peak frequency occurring in Sprague Dawley compared to Long-Evans rats. Surprisingly, spike-LFP phase-locking in the MCx significantly decreases as high gamma oscillations increase in LFP power. Collectively, these results highlight the fact that LFP is not necessarily a reliable marker for synchronized spiking or spike timing in a given brain area, as prominent spectral LFP peaks may not be accompanied by spike-LFP phase-locking. It remains to be determined whether highly synchronized LFP oscillatory activity in a given circuit may disrupt information flow in ways other than through local synchronization of spiking activity.

Disclosures: **K.B. Dupre:** None. **A.J. McCoy:** None. **E. Brazhnik:** None. **C. Delaville:** None. **A.V. Cruz:** None. **N. Novikov:** None. **C.P. Dodge:** None. **C.M. Gerber:** None. **C. Hatch:** None. **M.A. Cohen:** None. **D.S. Kurup:** None. **J.R. Walters:** None.

Poster

066. Parkinson's Disease Models I

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 66.04/DD10

Topic: D.15. Basal Ganglia

Support: NINDS Intramural Research Program

Title: Dopaminergic modulation of 45-55 Hz low gamma power in the medial prefrontal cortex and the subthalamic nucleus in a behaving rat model of Parkinson's disease

Authors: *C. DELAVILLE, A. J. MCCOY, J. R. WALTERS;
NIH NINDS, Bethesda, MD

Abstract: The role of dopamine (DA) in modulating activity in motor cortex (mCx) and associated subcortical circuits has been a subject of recent interest. Studies have shown dramatic changes in oscillatory activity in these circuits in animal models of Parkinson's disease (PD) and

PD patients. These changes are particularly evident in the hemiparkinsonian rat during treadmill walking (Delaville et al., 2014). On the other hand, dysfunction of the medial prefrontal cortex (mPFC) and the subthalamic nucleus (STN) is thought to lead to non-motor symptoms such as anxiety, attention, depression and other cognitive impairments often observed in PD. Here, we focused on neuronal activity in the mPFC and its relationship with STN activity in normal and hemiparkinsonian rats during rest and treadmill walking. Simultaneous recordings of spike and LFP data were obtained from electrode bundles implanted in mPFC, STN, and mCx before and over a 3 week period after unilateral 6-hydroxydopamine-induced DA cell lesion, mimicking severe PD. In control rats, spectral power in the 45-55 Hz frequency range was greater in the mPFC, STN and mCx during walking compared to inattentive rest. However, the mean peak frequency of low gamma power in the mCx (46 Hz) was lower than in the STN and the mPFC (51 Hz). The presence of significant 45-55 Hz LFP coherence between the mPFC and STN, in contrast to the lack thereof between mCx and STN and between mCx and mPFC, argues that the source of the low gamma activity observed in the mCx is different from that in the STN and mPFC. In the days following DA depletion, 45-55 Hz LFP power decreased in both the mPFC and STN as did the coherence between these 2 structures. However, three weeks after severe DA depletion, the 45-55 Hz power in the mPFC and STN had returned to original levels, implying a non-DA compensatory mechanism in the establishment of this gamma oscillation. Interestingly, neither apomorphine, a DA agonist, nor L-dopa, a DA precursor, increased gamma power in the mPFC or STN at day 7 and day 21 after DA depletion. On the contrary, these treatments induced a decrease in 45-55 Hz power and mPFC-STN coherence. Additionally, no modulation of the 45-55 Hz LFP amplitude was observed in conjunction with the cycle of forepaw stepping during treadmill walking, implying that this low gamma oscillation is related to general activity but not directly connected to repetitive movements involved in stepping. These data suggest that the 45-55 Hz power in the mPFC and STN is modulated by activity state and changes in DA receptor stimulation as well as non-DA mechanisms which, over time, compensate for DA loss.

Disclosures: **C. Delaville:** None. **A.J. McCoy:** None. **J.R. Walters:** None.

Poster

066. Parkinson's Disease Models I

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 66.05/DD11

Topic: D.15. Basal Ganglia

Support: NS045962

Title: Effects of NMDA/AMPA receptor blockade on abnormal striatal neuronal activity in parkinsonian monkeys

Authors: *A. SINGH[1], K. BURKE[1], J. WHITHEAR[1], B. DYAVARSHETTY[1], S. TRAYNELIS[2], S. PAPA[1,3];
[1]Yerkes Natl. Primate Res. Center, Emory University,, Atlanta, GA; [2]Dept. of Pharmacol., [3]Dept. of Neurol., Emory Univ. Sch. of Med., Atlanta, GA

Abstract: In non-human primate models of advanced parkinsonism, medium spiny neurons (MSNs) are markedly hyperactive and often exhibit reversal of levodopa-induced firing rate changes ("inversion of dopamine responses") in correlation with levodopa-induced dyskinesias (Liang et al., 2008). Hyperfunction of striatal glutamate signaling is thought to play a primary role in the mechanisms of dyskinesias. However, the impact of glutamatergic transmission on abnormal MSN responses to dopamine has not been studied. The electrophysiological effects of striatal NMDA or AMPA receptor antagonism were studied in four awake, behaving, parkinsonian rhesus monkeys. The competitive NMDA antagonist LY235959 or AMPA antagonist NBQX was delivered by microinjection at the site of extracellular recordings in the striatum of monkeys followed by systemic levodopa administration (s.c.) during the recording session. The doses of antagonist were determined on the basis of *in vitro* tests for selectivity of receptor binding and *in vivo* tests of magnitude of firing frequency reduction. Behavioral effects of the antagonists were also evaluated with systemic administration. We found that the reduction of MSN baseline activity via local microinjection of LY235959 or NBQX completely abolished the abnormal inversions of firing rate changes induced by dopamine inputs. Comparisons with the vehicle alone as control confirmed the specific effect of the local drug microinjection. These NMDA/AMPA antagonists also reduced dyskinesias following systemic injections, demonstrating correlated behavioral effects in the same animals that exhibited physiological effects. These results indicate that the ionotropic glutamate transmission primarily controls the MSN activity in the parkinsonian state. This has profound implications for the striatal pathology developed in advanced PD that is associated with abnormal responses to dopamine.

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Poster

066. Parkinson's Disease Models I

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

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Topic: D.15. Basal Ganglia

Support: BFU2012-37907

SAF2008-03118-E

SAF39875-C02-01

Eranet-Neuron

CiberNed CB06/05/0006

Departamento Salud, Gobierno de Navarra

Title: Detection of cannabinoid receptors CB1 and CB2 within basal ganglia output neurons in macaques. Changes following experimental parkinsonism

Authors: *S. SIERRA SAN NICOLAS[1,2], A. J. RICO[1,2], I. G. DOPESO-REYES[1,2], E. RODA[1], E. MARTINEZ-PINILLA[1], A. VAZQUEZ[5], J. L. LABANDEIRA-GARCIA[6,3], R. FRANCO[7,4], J. L. LANCIEGO[1,2];
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Abstract: Although type 1 cannabinoid receptors (CB1Rs) are expressed abundantly throughout the brain, the presence of type 2 cannabinoid receptors (CB2Rs) in neurons is still somewhat controversial. Taking advantage of newly-designed CB1R and CB2R mRNA riboprobes, we demonstrate by PCR and *in situ* hybridization that transcripts for both cannabinoid receptors are present within labeled pallidothalamic-projecting neurons of control and MPTP-treated macaques, whereas the expression is markedly reduced in dyskinetic animals. Moreover, an *in situ* proximity ligation assay was used to qualitatively assess the presence of CB1Rs and CB2Rs, as well as CB1R-CB2R heteromers within basal ganglia output neurons in all animal groups (control, parkinsonian and dyskinetic macaques). A marked reduction in the number of CB1Rs, CB2Rs and CB1R-CB2R heteromers was found in dyskinetic animals, mimicking the observed reduction in CB1R and CB2R mRNA expression levels. Cannabinoid receptors were only found in neuronal cell bodies, with a complete absence of receptors in both pallidothalamic and striatopallidal axon terminals. This cellular localization suggests that the functional role is independent of cannabinoid receptor-mediated control of neurotransmitter release at nerve terminals.

Disclosures: **S. Sierra San Nicolas:** None. **A.J. Rico:** None. **I.G. Dopeso-Reyes:** None. **E. Roda:** None. **E. Martinez-Pinilla:** None. **A. Vazquez:** None. **J.L. Labandeira-Garcia:** None. **R. Franco:** None. **J.L. Lanciego:** None.

Poster

066. Parkinson's Disease Models I

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Support: BFU2012-37907

SAF2008-03118-E

SAF39875-C02-01

Eranet-Neuron

CiberNed CB06/05/0006

Departamento Salud, Gobierno de Navarra

Title: Detection of CB1-GPR55 receptor heteromeric complexes within identified subtypes of striatal neurons in monkeys

Authors: *A. J. RICO[1,2], I. G. DOPESO-REYES[1,2], S. SIERRA-SAN NICOLAS[1,2], E. RODA[1,2], M. LANZ[1], D. SUCUNZA[1], D. PIGNATARO[1,2], E. MARTINEZ-PINILLA[1], R. FRANCO[4,3], J. L. LANCIEGO[1,2];
[1]FIMA, Pamplona, Spain; [2]Neurosciences, CiberNed, Pamplona, Spain; [3]Neurosciences, CiberNed, Barcelona, Spain; [4]Biochem. and Mol. Biol., Univ. of Barcelona, Barcelona, Spain

Abstract: Endocannabinoids are neuromodulators acting on specific CB1 and CB2 receptors, and these receptors represent potential therapeutic targets for neurodegenerative diseases. Cannabinoid ligands also regulate the activity of GPR55, another cannabinoid receptor that has been recently ‘deorphanized’. Our working hypothesis is that the pleiotropic signaling of CB1, CB2 and GPR55 receptors is due to heteromers formed between these receptors and that heteromers may be feasible targets for Parkinson’s disease. The aim of the present paper was to characterize cannabinoid CB1-GPR55 receptor heteromers in the basal ganglia input nuclei. Here

the *in situ* proximity ligation assay (PLA) was used to detect the presence of receptor heteromeric complexes within different types of striatal neurons, comprising both projection neurons and interneurons. Striatal medium-sized spiny neurons (MSNs) giving rise to either the direct and indirect basal ganglia pathways were identified following the retrograde transport of biotinylated dextran amine (BDA). BDA was injected into either the external or the internal subdivisions of the primate globus pallidus (GPe and GPi, respectively). Next, triple immunofluorescent stains were carried out to visualize (i) BDA-labeled neurons, (ii) CB1-GPR55 heteromers and (iii) either parvalbumin- or ChAT-positive striatal interneurons. Obtained results showed the presence of CB1-GPR55 heteromeric complexes within both types of projection neurons as well as in parvalbumin-positive interneurons, whereas cholinergic interneurons lacked these types of receptor heteromers. CB1-GPR55 receptor heteromers were found in both the neuronal membrane as well as in intracellular locations. These cellular/subcellular locations suggest a functional role for CB1-GPR55 receptor heteromers independent from cannabinoid receptor-mediated control of neurotransmitter release at axon terminals.

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Poster

066. Parkinson's Disease Models I

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Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 66.08/DD14

Topic: D.15. Basal Ganglia

Support: Supported by NS045962

Title: Synchronized oscillatory activity in the striatum of parkinsonian monkeys

Authors: *G. M. JEYARAJ[1,2], A. SINGH[2], J. S. WHITHEAR[2], S. M. PAPA[2];
[2]Yerkes Natl. Primate Res. Ctr., [1]Emory Univ., Atlanta, GA

Abstract: Dopamine depletion in Parkinson’s disease (PD) has been associated with synchronized oscillatory activity at 13-30 Hz (beta band) in the cortico-basal ganglia network. This activity was found in cortex, globus pallidus, and subthalamic nucleus in rodent and primate models, and patients. However, it remains unclear how nigrostriatal dopamine loss leads to

modulate oscillations in the striatum that may cause motor disability in PD. We studied the local field potentials (LFPs) in MPTP-treated non-human primates that had stable, chronic parkinsonism of moderate to severe degree as a primate model of advanced PD. LFPs were recorded in cortex, striatum and globus pallidus during the "off" state (baseline parkinsonian disability). We observed oscillations in the low-beta band frequencies (13-20 Hz) in cortex, striatum, and globus pallidus internus (GPi). High coherence of oscillation in the low-beta band was seen in cortex-striatum and striatum-GPi. In cortex, striatum and GPi, there also was a significant relative power in the alpha band (7-12 Hz), which in the striatum and GPi was more robust than to the low-beta band. No substantial oscillatory activity in higher frequency bands was observed. These results suggest that the striatum and its downstream circuits have a complex pattern of oscillatory activity including alpha and low-beta frequencies with possible contributions to pathological motor behavior. Further studies may elucidate the function of particular basal ganglia oscillations in PD.

Disclosures: **G.M. Jeyaraj:** None. **A. Singh:** None. **J.S. Whithear:** None. **S.M. Papa:** None.

Poster

066. Parkinson's Disease Models I

Location: Halls A-C

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Program#/Poster#: 66.09/DD15

Topic: D.15. Basal Ganglia

Support: NS045962

Title: Profoundly altered striatal MSN activity in Parkinson's disease patients

Authors: ***S. M. PAPA**[1], A. SINGH[1], K. MEWES[1], R. GROSS[2], M. DELONG[1];
[1]Neurol., [2]Dept. of Neurosurg., Emory Univ., ATLANTA, GA

Abstract: Loss of striatal dopamine modulation in Parkinson's disease (PD) presumably leads to abnormal discharges of striatal output neurons (medium spiny neurons, MSNs) based on recordings in animal models. But the status of the MSN activity in the human disease remains unknown. We examined the MSN firing in patients undergoing deep brain stimulation (DBS) surgery. We analyzed data obtained during electrophysiologic mapping in PD patients (n = 11), along with dystonia (n = 5) and essential tremor (n = 10; ET) patients for comparison. Striatal MSN activity was also recorded in awake, behaving, parkinsonian and normal monkeys (n = 2). Strict criteria were applied for MSN classification. The mean firing rate of MSNs was

significantly higher in PD (32 ± 9 Hz) than dystonia (9.5 ± 4 Hz) and ET (< 3 Hz). Similar to PD patients, the mean firing rate of MSNs was significantly higher in the parkinsonian monkey (23 ± 1.3 Hz) than the normal monkey (2.13 ± 0.2 Hz). In PD, a larger fraction of striatal neurons exhibited burst activity and burst firing rate was also significantly elevated. These findings are aligned with previous data from parkinsonian monkeys (Liang et al., 2008), challenging the classic functional model of PD. The primary role of glutamatergic hyperactivity in these striatal changes suggests that manipulating the glutamate signaling is critical for improved responses to dopamine replacement. The less increased frequencies in dystonia suggest that high MSN activity specifically correlates with PD, but also that there may be grounds for the frequently alluded Dystonia-PD continuum. The very low firing rates in ET resemble MSN activity in normal monkeys, suggesting a parallel with normal humans. These findings demonstrate profound alterations of the MSN discharge in patients with PD. Further analyses of clinical correlates of the MSN changes may help understand their pathophysiological significance.

Disclosures: **S.M. Papa:** None. **A. Singh:** None. **K. Mewes:** None. **R. Gross:** None. **M. DeLong:** None.

Poster

066. Parkinson's Disease Models I

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 66.10/DD16

Topic: D.15. Basal Ganglia

Support: EPSRC doctoral training grand

Title: Phase offset of entrained cortical inputs influences selectivity in a neural model of the basal ganglia

Authors: *Z. FOUNTAS, M. SHANAHAN;
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Abstract: Low-frequency oscillatory activity have been the target of extensive research both in cortical structures and in the basal ganglia (BG), due to numerous reports of associations with brain disorders and the normal functioning of the brain. Whereas a number of computational models of the BG investigate these phenomena, these models tend to focus on intrinsic oscillatory mechanisms, neglecting evidence that points to the cortex as the origin of this oscillatory behaviour. In this study we constructed a neural model of the BG circuitry and used it

to investigate the relationship of wave properties of entrained cortical inputs, dopamine and the steady-state selectivity of the BG, i.e. their effectiveness as an action selection device. Our simulations indicated a significant impact of the phase offset between entrained cortical signals with different amplitudes. In particular, we found that in specific low frequencies, and when the phase of a strong input signal precedes in time the phase of a second, weaker signal, the effectiveness of the BG varies depending on the magnitude of this offset, while in high bands, this effect disappears. Furthermore, we investigated the effect of dopamine on the process of selection which was found to modulate the frequency spectrum of the previous effects. Interestingly, the critical areas of our results match with the frequency bands that are enhanced in Parkinson's disease and suppressed by medication. Numerous further associations can be observed including the entrainment between the prefrontal cortex and hippocampus and various parkinsonian symptoms.

Disclosures: **Z. Fountas:** None. **M. Shanahan:** None.

Poster

066. Parkinson's Disease Models I

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 66.11/DD17

Topic: D.15. Basal Ganglia

Support: Dystonia Medical Research Foundation

Canadian Institutes of Health Research Operating Grant MOP 62917

Title: The nature of the connection between internal global pallidus and primary motor cortex in human

Authors: ***Z. NI**[1], S. KIM[1], N. PHIELIPP[1], S. GHOSH[1], C. GUNRAJ[1], A. M. LOZANO[2], M. HODAIE[2], R. CHEN[1];
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Abstract: Internal global pallidus (GPi) is the main output nucleus of basal ganglia. Deep brain stimulation (DBS) of the GPi is an effective treatment for dystonia, but its mechanisms of action remains poorly understood. The nature and time course of the connections between GPi and motor cortical areas in humans are not known. We examine the connectivity between GPi and

primary motor cortex (M1) in cervical dystonia patients with bilateral GPi DBS implanted. Ten patients were studied. DBS on one side was set at the lowest frequency of 3 Hz with the clinically used contact and voltage. The contralateral DBS was turned off. Three experiments were performed. The cortical evoked potential induced by DBS was recorded with 64-channel electroencephalograms in Exp. 1. Exp. 2 examined the time course of the effects of DBS on M1 excitability with transcranial magnetic stimulation (TMS). GPi DBS was delivered followed by TMS at interstimulus intervals (ISIs) from 1 to 300 ms. Three different TMS current directions (posterior-anterior, anterior-posterior and lateral-medial) were tested because they activate different cortical circuits. In addition, DBS frequency of 10 Hz was compared to 3 Hz. The effects of different DBS voltages and contact locations were also tested. Exp. 3 tested the hypothesis that cortical plasticity can be induced by an interventional protocol with repetitive pairing of DBS GPi and TMS at specific intervals. Intervention with 180 pairs of GPi DBS and TMS in different ISIs were delivered. Motor evoked potential (MEP) after interventions was compared to that before interventions. We found that GPi DBS produced cortical evoked potential in motor areas with two peaks at latencies of 9.8 ± 1.7 and 25.4 ± 2.1 ms. GPi DBS facilitated MEP produced by TMS with anterior-posterior and posterior-anterior currents at ISI coincident with early peak of evoked potential (~ 10 ms) and inhibited MEP at the later peak (~ 25 ms), whereas DBS had no effect on the MEP induced by lateral-medially directed current. DBS at 3 and 10 Hz showed similar results. Both the early facilitation and later inhibition increased with higher DBS voltages and decreased when DBS was applied at contacts not used for clinical benefit. Long term potentiation-like effect with increased MEP after the intervention was found when DBS and TMS were paired at ISI around later inhibitory phase (~25 ms). Pairing of DBS and TMS at ISI around the early facilitatory phase and that with stimulations delivered in revered order (TMS delivered ~25 ms before DBS) did not produce MEP facilitation. We conclude that GPi interconnects with M1 and the connectivity between two brain areas may be related to the clinical benefit of GPi DBS in dystonia.

Disclosures: **Z. Ni:** None. **S. Kim:** None. **N. Phielipp:** None. **S. Ghosh:** None. **C. Gunraj:** None. **A.M. Lozano:** None. **M. Hodaie:** None. **R. Chen:** None.

Poster

066. Parkinson's Disease Models I

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 66.12/DD18

Topic: G.07. Data Analysis and Statistics

Support: NIH

NSF

Title: Pathway-based genome-wide association analysis of Parkinson's disease

Authors: *M. ZHANG[1], V. PUNGPAPONG[2], D. ZHANG[1];
[1]Statistics, Purdue Univ., West Lafayette, IN; [2]Statistics, Chulalongkorn Business Sch., Bangkok, Thailand

Abstract: We proposed an empirical Bayes variable selection framework to combine pathway information in genome-wide association study (GWAS) analysis, and applied it to the Parkinson's disease GWAS data obtained from the National Center for Biotechnology Information database of genotypes and phenotypes (NCBI dbGaP). Unlike Bayesian variable selection methods that rely on computation-intensive Markov chain Monte Carlo algorithms, we proposed an iterated conditional modes/medians algorithm to implement an empirical Bayes variable selection. First, iterated conditional modes are utilized to optimize values of the hyperparameters so as to implement the empirical Bayes method. Second, iterated conditional medians are used to estimate the model parameters and therefore implement the variable selection function. In addition to the advantages of Bayesian inference, such as natural incorporation of known pathway and other biological information in the statistical model, our proposed method enjoys fast computation, increased statistical power of the analysis, and improved estimation of the model parameters. Extensive computer simulation studies show the more efficient computation and superior performance of our proposed approach, and we got some interesting results from the Parkinson's disease data analysis.

Disclosures: **M. Zhang:** None. **V. Pungpapong:** None. **D. Zhang:** None.

Poster

066. Parkinson's Disease Models I

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 66.13/DD19

Topic: D.15. Basal Ganglia

Title: Comparison of progression rate between neural and non-neural rigidity components in parkinson's disease

Authors: *R. XIA[1], D. POWELL[2], Z.-H. MAO[3];
[1]Dept of Physical Therapy, Univ. of St. Mary, Leavenworth, KS; [2]Dept. of Physical Therapy, Campbell Univ., Buies Creek, NC; [3]Dept. of Electrical and Computer Engin. and Dept. of Bioengineering, Univ. of of Pittsburgh, Pittsburgh, PA

Abstract: Parkinson's disease (PD) is a progressive neurodegenerative movement disorder and clinically characterized by rigidity, bradykinesia, tremor and postural instability. Recent imaging studies have shown that rigidity progresses faster than other PD symptoms. Rigidity is defined as a uniform increase in resistance to passive movement throughout an entire range of motion and is accounted by increased reflex responses, referred to as neural component, as well as altered mechanical properties, termed as non-neural component. The purpose of this study was to examine and compare the rate of progression between the neural and non-neural rigidity components over a course of 12 months. Six subjects with idiopathic PD participated in a study protocol including two testing sessions with an interval of approximately 12 months. Torque resistance of the more affected wrist joint was measured during passive flexion and extension movements in patterns of pseudorandom binary sequences. To quantify the neural and non-neural contributions to rigidity, a parallel-cascaded system identification technique was applied. The rate of progression was assessed by calculating the absolute change from the first to the second test (i.e., $T_2 - T_1$) and a percentage of change between the two tests [i.e., $(T_2 - T_1)/T_1$] for both rigidity components. A paired *T*-test was applied to compare the rate of progression between the two rigidity components. Results showed that non-neural rigidity appeared to progress faster than neural rigidity for both absolute (0.055 +/- 0.012 cf. 0.042 +/- 0.006 Nm; P = 0.467) and relative changes (178.9% +/- 57.4% cf. 43.8% +/- 12.4%; P = 0.027). Our finding shows that non-neural rigidity component or myoplasticity of muscle fibers progresses at a faster rate as rigidity became worsening over time. A larger sample size is warranted to confirm this conclusion.

Disclosures: **R. Xia:** None. **D. Powell:** None. **Z. Mao:** None.

Poster

067. Posture and Gait: Kinematics; Muscle Activity; Exercise and Fatigue; and Biomechanics I

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 67.01/DD20

Topic: D.16. Posture and Gait

Support: AHA Grant 14PRE18870084

NIH Grant T32HD057845

Title: A novel method for describing impaired muscle activation phasing during pedaling post-stroke

Authors: *C. H. MULLENS[1], D. A. BROWN[2];
[1]Physical Therapy and Human Movement Sci., Northwestern Univ., Chicago, IL; [2]Physical Therapy, Univ. of Alabama Birmingham, Birmingham, AL

Abstract: Stroke is a major cause of locomotor disability, with 795,000 people having a stroke yearly and 50% of ischemic stroke survivors showing lasting impairment. One major element underlying this impairment is inappropriate muscle phasing, in which a muscle is active at a phase in the locomotor cycle where it is typically inactive, or vice versa. It is difficult to analyze EMG recordings of this inappropriate phasing, however, because paretic muscle activation often shows reduced amplitude and inconsistent bursting characteristics. Prior work using pedaling (an established, well-constrained model to investigate muscle phasing) has used methods such as comparing activation within 30- or 90-degree regions of the pedaling cycle, or calculating the center of the 90-degree window of greatest muscle activity. These methods have disadvantages, and are imprecise or do not detect changes outside the window of interest. We propose a method of phase estimation based on cross-correlation, and present preliminary results describing cycle-to-cycle variability of phasing in both the paretic and non-paretic vastus medialis (VM) muscle during pedaling. We recorded EMG during motorized pedaling at 40rpm in the VM, a uniarticular knee extensor with prolonged activation post-stroke during walking and pedaling. We compared filtered EMG during individual cycles in paretic (P) and non-paretic (NP) legs against an average activation from all cycles of the non-paretic leg, by circular cross-correlation, and determined angle of greatest correlation. This provided an estimated activation phase shift for individual cycles. Based on preliminary data from two post-stroke subjects (S1 & S2), activation shifts of individual cycles relative to the NP average cycle were normally distributed in both P and NP (based on a Lilliefors test). In both subjects, several P cycles were more delayed than any cycle of NP (11% and 34% of cycles), and average VM activation in P was shifted significantly later (Wilcoxon $p<0.001$) than NP activation (mean shift of 14.6°±9.0° for S1 and 18.9°±14.9° for S2). In both subjects, the relative activation phasing of many cycles in the P leg was similar to those of the NP leg. Surprisingly, some activation shifts of the P leg were even phase advanced relative to the NP average. This preliminary work showed that there is considerable variability in muscle activity phasing during a kinematically-constrained, bilateral, cyclical movement task, and that even when overall average behavior appears delayed, we observed some subset of behavior that was equivalent between the nonparetic and paretic leg.

Disclosures: **C.H. Mullens:** None. **D.A. Brown:** None.

Poster

067. Posture and Gait: Kinematics; Muscle Activity; Exercise and Fatigue; and Biomechanics I

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 67.02/DD21

Topic: D.16. Posture and Gait

Support: Heart and Stroke Foundation of B.C. and Yukon

Title: Association between anticipatory hamstrings activation, pelvic displacement and centre of pressure excursion during unilateral arm raise perturbations in standing

Authors: *K. J. MILLER, C. K. COCHRANE, T. I. IVANOVA, J. GARLAND;
Physical Therapy, Univ. of British Columbia, Vancouver, BC, Canada

Abstract: An experimental paradigm commonly employed to investigate anticipatory postural adjustments (APA) to internal perturbations in standing is the performance of rapid unilateral arm movements in the sagittal plane. In healthy individuals, the APA is associated with feedforward activation of the hamstring (HAM) muscles and an initial posterior shift in centre of pressure (CoP) on the ipsilateral (IPSI) side (Mochizuki et al. 2004). The purpose of the study was to explore the relationships between the initial HAM activity and anterior-posterior (AP) movement of the CoP and pelvis. We hypothesized that the IPSI HAM activation was serving to control the position of the pelvis, thereby minimizing postural sway during the arm movement. Ten healthy right hand dominant participants stood with their feet on two adjacent floor-mounted force platforms and performed 10 trials raising one arm "as fast as possible" to shoulder height. This was repeated for the other arm (order randomized). Kinematics of the pelvic position (10 motion analysis cameras), CoP and electromyography (EMG) data were collected. Arm acceleration was measured using an accelerometer secured on the hand. Total pelvic displacement, derived from the peak anterior and posterior displacement of the anterior superior iliac spine (ASIS) markers, and the corresponding total CoP excursion in the AP direction were measured in relation to the baseline (taken 500ms prior to initiation of arm movement). Associations between the timing of IPSI HAM onset relative to arm movement initiation, IPSI HAM area (normalized to baseline), total IPSI pelvic displacement, total IPSI AP CoP, and arm acceleration were examined. Average arm acceleration varied between participants from 36.3 – 85.7 m/s^2, with no significant difference between arms. Partial correlations (controlling for arm acceleration) revealed a moderately strong relationship between HAM onset and total pelvic displacement (r = 0.41) and a moderately strong relationship (r = -0.40) between HAM area and total pelvic displacement. That is, when there was a more feedforward and larger amplitude

HAM burst, there was less displacement of the pelvis. However, the relationships between AP CoP excursion and HAM onset (r = 0.27), HAM area (r = -0.17) and pelvic displacement (r = -0.22) were relatively weaker. These findings suggest that anticipatory HAM activation contributes to control of the pelvic position during unilateral arm movements. However, the relationships found between HAM activation and AP CoP excursion, and between pelvic displacement and AP CoP excursions were less robust, highlighting the multi-factorial nature of postural control.

Disclosures: **K.J. Miller:** None. **C.K. Cochrane:** None. **T.I. Ivanova:** None. **J. Garland:** None.

Poster

067. Posture and Gait: Kinematics; Muscle Activity; Exercise and Fatigue; and Biomechanics I

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 67.03/DD22

Topic: D.16. Posture and Gait

Support: NSERC

CFI

Title: The influence of attentional resources on the ability to generate compensatory arm reactions in young and older adults

Authors: J. LAING, *C. TOKUNO;
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Abstract: Dual task paradigms have previously been used to demonstrate that attentional resources are required to generate lower limb postural responses for reactive balance control. The purpose of this study was to extend these findings by examining whether the performance of a concurrent cognitive task also affects an individual's ability to produce compensatory arm reactions in response to an unexpected loss of balance. Twenty young adults (18-30 years) and 16 older adults (63 years or older) participated in this study. At the beginning of each trial, participants stood quietly on a moveable platform without (low attentional requirement) or with the presence of a concurrent cognitive task. The cognitive task required participants to count backwards by 2's (moderate attentional requirement) or by 7's (high attentional requirement). At

a random time during each trial, the moveable platform was rapidly translated in either the forward or backward direction. In response to each support surface translation, participants were required to recover their balance as quickly as possible without stepping. The ability to generate compensatory arm reactions was quantified through the measurement of electromyographic (EMG) onset latencies and amplitudes from the shoulder muscles (i.e., anterior, lateral, and posterior deltoids). Results indicate that across all experimental conditions, shoulder EMG activity occurred 11 ms (8.2%) later and 74.6% larger in older compared to young adults. However, the attentional requirements of the cognitive task did not significantly alter EMG onset latencies or EMG amplitudes for both young and older adults ($p=0.125$-0.406). Since compensatory arm reactions were not affected by the availability of attentional resources, this suggests that postural responses involving the upper limbs incorporate different neural processes than those of the lower limbs. Furthermore, contrary to lower limb postural responses, older adults do not appear to require greater attentional resources in order to generate compensatory arm reactions.

Disclosures: **J. Laing:** None. **C. Tokuno:** None.

Poster

067. Posture and Gait: Kinematics; Muscle Activity; Exercise and Fatigue; and Biomechanics I

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 67.04/DD23

Topic: D.16. Posture and Gait

Title: Tremor-amplitude and signal-complexity are affected by handgun aiming technique in both experienced and novice shooters

Authors: ***K. J. KELLERAN**[1], S. MORRISON[2], D. M. RUSSELL[2];
[1]Old Dominion Univ., Newport News, VA; [2]Physical Therapy and Athletic Training, Old Dominion Univ., Norfolk, VA

Abstract: In aiming at a target, humans produce small fluctuations in posture, referred to as postural tremor. The dynamics of tremor are influenced by the limb measured and by whether the limb is supported or not. However, research has not yet established whether different arm postures impact postural tremor. This study sought to compare handgun fluctuations during different shooting postures, of: (1) Two handed versus one handed grip, and (2) Straight elbow versus bent elbow. In addition, the impact of skill level on tremor was examined. 15 experienced

and 15 novice shooters volunteered. Participants stood 21 feet from a target and aimed a weighted mock handgun for 10 sec, with an accelerometer affixed near the gun barrel. Participants performed five trials per posture. The amplitude of the signal was computed by the root mean square (RMS), while the signal complexity or irregularity was quantified by Approximate Entropy (ApEn), of the vertical acceleration signal. Two groups, three hand grips (double, right, left), and two arm positions (bent, straight) were analyzed in a 2x3x2 mixed design ANOVA. The double-hand grip significantly decreased tremor amplitude, but increased signal complexity, when compared to either single-hand grip (Double: 0.017 +0.004 G, ApEn 1.194+0.125, Right: 0.022 +0.006 G, ApEn 1.059+0.129, Left: 0.022 +0.005 G, ApEn 1.028+0.114). The bent arm position also significantly reduced tremor amplitude, with no significant difference in tremor complexity, when compared to the straight arm position (Bent: 0.019 +0.005 G, ApEn 1.086+0.142, Straight: 0.022 +0.006 G, ApEn 1.101+.142). Tremor amplitude was not affected by group, however more experienced shooters revealed significantly greater complexity in the tremor signal (0.021 +0.006 G, ApEn 1.136+0.143) when compared to novice shooters (0.021 +0.006 G, ApEn 1.051+0.128). Rather than having an additive effect, using two hands reduced the magnitude fluctuations at the gun. Allowing motion at the elbow also dissipated the fluctuations at the gun. The advantage of a reduction in amplitude were achieved while increasing signal complexity with two hands, but not for bent elbows. Experience failed to impact the amplitude of tremor, however experienced shooters produced a more a complex tremor profile.

Disclosures: **K.J. Kelleran:** None. **S. Morrison:** None. **D.M. Russell:** None.

Poster

067. Posture and Gait: Kinematics; Muscle Activity; Exercise and Fatigue; and Biomechanics I

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 67.05/DD24

Topic: D.16. Posture and Gait

Support: Cerebral Palsy International Research Foundation

Title: Robotic pelvis perturbation during sitting astride improves balance and walking in children with cerebral palsy

Authors: *M. WU[1], J. KIM[2], D. JAEBLER-SPIRA[2], P. ARORA[2];
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Abstract: Children with Cerebral palsy (CP) typically present with compromised balance control. Impairments in balance control limit their walking capacity and negatively impact their daily activities. As a result, one of the major goals of rehabilitation in children with CP is to improve their balance, particularly dynamic balance. Hippotherapy has been used in clinics to improve balance in children with CP for several decades. However, this beneficial therapy is often not available for a majority of patients because of limited access to horses, weather conditions, and the relatively high cost due to the need for multiple professional staff during a hippotherapy session. As a result, there is a need to develop novel robotic systems to make this type of therapy more widely available for children with CP. In this study, we tested whether providing a controlled 3D perturbation at the pelvis during sitting astride through a cable-driven robotic system will improve dynamic balance and gait in children with CP. We hypothesize that adding a perturbation force to the pelvis during sitting astride will increase the amount of active postural stabilization and trigger reactive postural responses in children with CP. We expect that children with CP will exhibit an increase in muscle activity of trunk flexors and extensors in response to the perturbation load with a subsequent improvement in balance and walking following robotic pelvis perturbation training. Three children with CP were recruited to participate in this study in two test sessions with one week interval in between. In session 1, subjects were fitted with an overhead harness while sitting astride on the robotic horse. A controlled sine wave force was applied to the horse saddle in the anterior-posterior direction through the cable-driven actuators. The frequency of loading was 1Hz and lasted for 15-20 minutes. Standing balance and overground gait speed were assessed before and after robotic horse training. Muscle activity from trunk and leg muscles were recorded during the test. In session 2, a similar protocol was used but a controlled perturbation force was applied in the mediolateral direction. Results indicated that standing balance improved after one session of pelvis perturbation training using the cable-driven robot. In addition, self-selected overground gait speed also slightly increased after training, suggesting a potential transfer from robotic hippotherapy to overground walking in children with CP. The results from this study may be used to develop a training paradigm for improving dynamic balance and walking function in children with CP.

Disclosures: **M. Wu:** None. **J. Kim:** None. **D. Jaebler-Spira:** None. **P. Arora:** None.

Poster

067. Posture and Gait: Kinematics; Muscle Activity; Exercise and Fatigue; and Biomechanics I

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 67.06/DD25

Topic: D.16. Posture and Gait

Title: Quantification of gait status through an iPod wireless gyroscope application

Authors: *T. J. MASTROIANNI[1], R. LEMOYNE[2];
[1]Cognition Engin., Pittsburgh, PA; [2]Northern Arizona Univ., Flagstaff, AZ

Abstract: Traditional gait analysis systems are limited to a clinical setting. Wireless and wearable devices enable the capacity to quantify a subject's gait features in a potentially autonomous environment. For example, the wireless accelerometer has been successfully tested and evaluated in the context of a subject's preference, such as a homebound setting. The gyroscope is sensor capable of measuring rate of rotation, as opposed to the accelerometer that measures acceleration. An iPod software application enables the capacity to measure the gyroscope signal as a wireless platform. The data file of the gyroscope signal may be conveyed by wireless connectivity to the Internet as an email attachment. The iPod can be mounted to the subject at a specified and convenient mounting position for gait analysis. The iPod wireless gyroscope application successfully demonstrates the capacity to quantify gait features at a location of the subject's preference.

Disclosures: **T.J. Mastroianni:** None. **R. LeMoyne:** None.

Poster

067. Posture and Gait: Kinematics; Muscle Activity; Exercise and Fatigue; and Biomechanics I

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 67.07/DD26

Topic: D.16. Posture and Gait

Support: VH Henry Fund

UW Graduate School

Title: Bifurcation in lower limb muscle coordination frequency content during quiet standing reflects structure of neuromuscular control

Authors: *K. G. GRUBEN[1], W. L. BOEHM[2];
[1]Kinesiology & Biomed. Engin., Univ. Wisconsin, MADISON, WI; [2]Biomed. Engin., Univ. of Wisconsin - Madison, Madison, WI

Abstract: Human standing requires coordination of muscles crossing multiple joints, which, together with the skeleton, produce force at the foot-ground interface (F). This study analyzed the time course of F to understand the coordinated control used to remain balanced. The focus on F stems from its involvement in producing torque about the center-of-mass (CM), driving angular motion of the body as a whole. To date, research has focused on the location of the center-of-pressure (CP) of F. This neglects the direction of F despite its equally important role in the torque of F about CM. Using a custom force platform with high-precision horizontal force sensors to enable accurate measurement of F direction, we recorded F during quiet human standing. We estimated the horizontal CM location using double integrated horizontal F divided by mass. We expressed F in a reference frame attached to the CM. As CP shifted anterior/posterior to a vertical line through CM, we found that F direction changed systematically with CP. That systematic relationship was well characterized as having an intersection point of F lines-of-action located near the CM. Secondary analysis revealed that shifts in CP included low frequency changes as well as much quicker shifts. The slow changes were of larger amplitude and dominated the relationship, exhibiting the intersection near the CM. At higher frequencies, smaller amplitude changes in CP and F direction occurred that were also well described as having an intersection point. That point was located in a region below the knee joint. A F vector pattern of intersecting near a fixed point in space is the product of coordination among multiple muscles spanning multiple joints. F vectors from multiple limb postures intersecting have been previously reported in seated isometric leg presses, for isolated CP shifts while standing, and while walking in both the observed F and in the modified F (that which remains after accounting for CP shift with respect to the foot). In the seated task, and in the modified force of walking, the intersection point was near the CM just as we observed for the low frequency standing behavior in the present study. The higher frequency behavior with the intersection point below the knee is qualitatively similar to that observed for isolated CP shifts in standing. Thus, we describe the force output of two control modes that appear to regulate upright posture during standing. Those modes resemble control observed in other tasks and thus may represent control strategies that humans use to across tasks for postural stabilization.

Disclosures: **K.G. Gruben:** None. **W.L. Boehm:** None.

Poster

067. Posture and Gait: Kinematics; Muscle Activity; Exercise and Fatigue; and Biomechanics I

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 67.08/DD27

Topic: D.16. Posture and Gait

Support: NSF EFRI-1137229

Title: Expertise in balance is mediated by a shared set of muscle synergies that generalize across motor behaviors

Authors: *A. B. SAWERS[1,2], L. H. TING[1,2];
[1]Emory Univ., Atlanta, GA; [2]Georgia Inst. of Technol., Atlanta, GA

Abstract: Here we sought to characterize patterns of muscle activity across differences in balance proficiency. Prior research has shown that trained experts use common kinematic patterns to perform skilled and routine grasping behaviors. Furthermore, we have shown that common muscle patterns, or muscle synergies, are shared between overground walking and sub-cortically mediated reactive balance responses. Here we examined whether the same muscle synergies would also be used when performing a more challenging walking balance activity, and whether the degree of sharing is associated with motor expertise. We hypothesize that expertise in balance proficiency is mediated by an increase in the sharing of subcortically encoded muscle synergies across motor behaviors. To test this hypothesis we recruited four ballet dancers (experts), unimpaired adults (novices), and transtibial amputees (TTA). Balance proficiency was assessed using a narrow balance beam walking task. Non-negative matrix factorization was used to extract muscle synergies from EMG signals of 16 leg and trunk muscles on the right side. Muscle synergies during beam walking were compared to those for overground walking and reactive balance. Experts demonstrated substantially better walking balance proficiency, quantified by the distance walked on the narrow beam (experts: 32.6 m, novices: 21.3 m, TTA: 16.9 m, $p < 0.016$). There was no difference in the number of muscle synergies for overground walking (experts: 6.25 ± 0.5, novices: 7.0 ± 1.4, TTA: 5.5 ± 1.3) or beam walking (experts: 6.25 ± 0.96, novices: 5.5 ± 1.3, TTA: 5.25 ± 0.96) within ($p > 0.05$) or between ($p > 0.05$) cohorts. However, experts shared a greater percentage of muscle synergies (60% ± 15%) between narrow beam and overground walking compared to novices (25% ± 21%) or TTA (20% ± 19%). This suggests that the muscle coordination patterns used by experts for beam walking were more

similar to those used for overground walking than in novices or TTA. Experts also shared more synergies between narrow beam walking and brainstem-mediated reactive balance (40% ± 8%) than novices (28% ± 8%) or TTA (19% ± 11%), implying greater reliance on subcortically encoded muscle synergies for beam walking. These results provide initial evidence that motor expertise in balance is mediated by a set of versatile rather than task-specific muscle synergies that generalize across motor behaviors. Moreover, consistent with findings of greater automaticity of motor performance in experts these muscle synergies likely reflect greater input from subcortical versus cortical pathways for motor control.

Disclosures: **A.B. Sawers:** None. **L.H. Ting:** None.

Poster

067. Posture and Gait: Kinematics; Muscle Activity; Exercise and Fatigue; and Biomechanics I

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 67.09/DD28

Topic: D.16. Posture and Gait

Support: Research was sponsored by the Army Research Laboratory and was accomplished under Cooperative Agreement Number WN911NF-10-2-0022.

Title: Cortical responses before and after physically demanding locomotor tasks

Authors: ***J. R. LUKOS**[1], J. BRADFORD[2], A. RIES[1], K. OIE[1], D. FERRIS[2];
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Abstract: Studies investigating performance in cognitive tasks prior to and succeeding physical activity have resulted in mixed findings. Cognitive performance after physical exhaustion is known to result in impoverished mental abilities (Frey et al., 1997). However, other lines of research suggest that moderate physical activity heightens cognitive-motor responses (e.g., Pesce et al., 2009). To date, the majority of studies investigating the effect of locomotor activity on cognitive performance have been restricted to behavioral outcomes. Additionally, those studies that have looked at cognitive performance changes after physical activity focused on cycling tasks. Thus, the effect of sustained locomotor tasks on electrocortical processing remains unclear. The goal of this study was to investigate the effect of moderate and demanding locomotor tasks on cognitive performance. To do this, we recorded high-density

electroencephalography while healthy young subjects performed a continuous cognitive vigilance task (visual oddball discrimination) in a stationary seated position before and after walking at 1 m/s for one hour. Subjects performed two levels of locomotor activity: carrying an unloaded backpack (unloaded condition) and carrying a backpack with 40% of their body weight added (loaded condition). Based on previous observations of increased P300 amplitude following cycling exercise (Magnie et al., 2000), we hypothesized that the cortical responses to visual stimuli would be affected by locomotor activity. Specifically, the amplitude of the P300 response after walking would increase compared to baseline measures and that the magnitude of these changes would vary based on subjects' level of exertion. Statistical analysis of 10 subjects showed no significant differences in P300 amplitude over the posterior medial cortical midline (~channel Pz) in response to oddball targets when comparing pre- to post- walking trials. Also, no statistical differences were present between loaded and unloaded conditions. Our results suggest that unlike acute, intense cycling exercises, sustained physical exertion of carrying a heavy backpack during walking has minimal after effects on electrocortical event-related potentials. Further analyses of other cortical regions and neurophysiological measures, such as frequency-spectral modulations, functional connectivity analyses, and cortico-muscular coherence, may provide additional insight into the effects of sustained locomotor activity on cognitive processing.

Disclosures: **J.R. Lukos:** None. **K. Oie:** None. **A. Ries:** None. **J. Bradford:** None. **D. Ferris:** None.

Poster

067. Posture and Gait: Kinematics; Muscle Activity; Exercise and Fatigue; and Biomechanics I

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 67.10/DD29

Topic: D.16. Posture and Gait

Title: The effect of structured auditory stimulation on movement variability and associated cortical involvement

Authors: ***S. J. HARRISON**[1], M. L. HOUGH[2], N. STERGIOU[2];
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Abstract: Movement variability in many rhythmic behaviors often exhibits characteristic temporal structures. In gait and tapping, stride to stride, and beat to beat variations are marked by long-term correlations in time. Motivated by evidence suggesting that changes in the temporal structure of movement variability often accompanies pathology, we explore the possibility manipulating the temporal structure of movement using variants of structured auditory stimuli to which participants in our experiments intentionally coordinated. Stimuli comprised of a rhythmic metronomic beat with varying temporal noise structures (e.g. white, pink, brown) were investigated in both tapping and locomotion tasks. Our results show that the stimuli were effective at changing the variability structure of the observed movement patterns. We also report an accompanying analyses of cortical involvement measured through functional near-infrared spectroscopy.

Disclosures: **S.J. Harrison:** None. **M.L. Hough:** None. **N. Stergiou:** None.

Poster

067. Posture and Gait: Kinematics; Muscle Activity; Exercise and Fatigue; and Biomechanics I

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 67.11/DD30

Topic: D.16. Posture and Gait

Title: Assessement of centre of pressure measures on one-leg stance task in subjects with chronic low back pain

Authors: *L. A. STURION[1], L. D. LOPES[1], M. R. OLIVEIRA[1], T. K. COSTA[1], M. G. CALDERON[1], C. G. MACEDO[1,2], R. A. DA SILVA[1];
[1]UNOPAR, LONDRINA, Brazil; [2]UEL, Londrina, Brazil

Abstract: Introduction: An unstable trunk could contribute to lumbar strain injury and subsequently to chronic low back pain. Few studies investigated the effects of postural control, with regard to stability, in subjects that relate mechanical back pain unknown. **Objective:** The purpose of this study was to assess the postural control in subjects with chronic low back pain. **Methods:** Ten chronic low back pain and 10 healthy subjects participated in this study. The subjects performed three trials of one-legged stance task on a force-platform during 30 s (30 s of rest between each trial), with a standardized protocol (without shoes, open eyes regarding target at eye level from 1.5 m of front, arms parallel to trunk). The mean across three trials was retained for analysis, while different balance parameters were computed: center of pressure area (COP);

and mean Velocity of COP in both the anteroposterior and mediolateral directions of movement. **Results:** Chronic low back pain subjects presented poor postural control than control (P <0.01) for mean velocity of COP variable (anteroposterior: 2.75±0.35 vs 1.97±0.19; and mediolateral: 2.96 ± 0.29 vs 2.16±0.15, respectively). Between-groups differences was still reached for sway area of COP variable *(P* = 0.079). **Conclusions:** Chronic low back pain subjects present poor postural control during one-leg stance balance task. These results have implications for rehabilitation programs for balance improvement in subjects with chronic low back pain.

Disclosures: L.A. Sturion: None. **L.D. Lopes:** None. **M.R. Oliveira:** None. **T.K. Costa:** None. **M.G. Calderon:** None. **C.G. Macedo:** None. **R.A. Da silva:** None.

Poster

067. Posture and Gait: Kinematics; Muscle Activity; Exercise and Fatigue; and Biomechanics I

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 67.12/DD31

Topic: D.16. Posture and Gait

Title: Effect of a sustained load on trunk during postural control measures in healthy subjects

Authors: *E. R. SANTOS[1], L. D. LOPES[1], F. B. PIRES[1], L. A. STURION[1], M. R. OLIVEIRA[1], C. F. AMORIM[2], K. P. FERNANDES[1], R. A. DA SILVA JR[1]; [1]UNOPAR, LONDRINA, Brazil; [2]UNICID, SÃO PAULO, Brazil

Abstract: Introduction: Postural changes with sustained loads as in works lifting load, can lead trunk unstable, spine stress and overload, and subsequently to low back pain. Objective: Asses the effect of a sustained external load on trunk during postural control measures in healthy subjects. Methods: Ten healthy subjects was recruited and performed randomly two experimental tasks on a force platform: 1) bipedal support with open eyes and arms along the body WITHOUT CHARGE (WOC); 2) bipedal support with open eyes and arms along the body WITH CHARGE (WC), corresponding to 10% of body weight, both the tasks were maintained at- 1 minute (with 3 minutes of rest between them). The variables stabilography analyzed were: sway area of Centre of pressure (A-COP), velocity of COP in anteroposterior (VEL-AP) and medial-lateral (ML-VEL) directions. Results: Significant differences between tasks (p <0.05) were found for all COP variables. The mean value for WOC task was 0.9 ± 0.4 cm² and WC of 3.11 ± 3.2 cm ² (221% higher) for A-COP. In VEL AP, the mean value was of 0.66 ± 0.1 cm/s for WOC, while in WC of 0.96 ± 0.3 cm/s(30% higher, p ≤ 0.01). In VEL-ML the differences were of 0.53 ± 0.1 cm/s

for WOC and of 0.67 ± 0.1 cm/s for WC (14% higher, $p \leq 0.01$). Conclusion: Negative effect on postural control was found in healthy subjects when sustaining a load on trunk at 10% of body weight.

Disclosures: **E.R. Santos:** None. **L.D. Lopes:** None. **F.B. Pires:** None. **L.A. Sturion:** None. **M.R. Oliveira:** None. **C.F. Amorim:** None. **K.P. Fernandes:** None. **R.A. da Silva Jr:** None.

Poster

067. Posture and Gait: Kinematics; Muscle Activity; Exercise and Fatigue; and Biomechanics I

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 67.13/DD32

Topic: D.16. Posture and Gait

Support: NIH Grant HD048741

Title: Strategic learning neither interferes with nor substitutes for split-belt adaptive learning in walking

Authors: *A. LONG[1], R. ROEMMICH[2], A. BASTIAN[2];
[1]Biomed. Engin., [2]Neurosci., Johns Hopkins Univ., Baltimore, MD

Abstract: Walking rehabilitation ideally makes use of any beneficial interactions between different motor learning mechanisms, such as strategic and adaptive learning. Strategic learning requires a person to explicitly think about how to walk in response to instruction such as "place your foot here." Adaptive learning is a process in which a person learns implicitly from motor errors when a perturbation is introduced. However, little is known about how these two mechanisms interact or if one type of learning can be substituted for the other. We used a split-belt treadmill and a real-time virtual visual environment to compare these learning mechanisms in healthy young adults. To investigate adaptive learning, subjects walked on the split-belt treadmill with one belt moving faster than the other. Subjects learn to place the foot on the fast belt further in front of their body and store a new walking pattern once the belts return to the same speeds. To investigate strategic learning, we provided visual feedback of the subject's foot placement in relation to a visual target on a screen and told them to “step on the target.” We studied people learning from these two methods in isolation and combination. The ADAPTATION group adapted to a split-belt treadmill perturbation gradually introduced but

with no visual feedback. The STRATEGIC + ADAPTATION group performed the same walking protocol (gradually split-belt speeds) but was provided visual feedback of foot placement and targets throughout the entire adaptation period. Visual feedback indicated where to step to adapt, and was easily followed by the subjects. We observed similar amounts of learning and after-effects in both of these experimental groups, demonstrating that strategic learning neither enhanced nor interfered with adaptation. To test whether strategic learning could substitute for adaptive learning, a separate STRATEGIC group was provided with foot placement feedback and targets, but with both belts at the same speed. Subjects easily followed the movement of the target window and reached a final state similar to the adaptation groups. However, we did not observe any after-effect when the feedback was removed. Overall, we find that strategic learning does not interfere with adaptation when both mechanisms are engaged to produce the same motor pattern, and that strategic learning does not substitute for adaptation. These results are important for walking rehabilitation because they suggest that adaptation produces unique benefits for learning new patterns. Supported by NIH HD048741.

Disclosures: **A. Long:** None. **R. Roemmich:** None. **A. Bastian:** None.

Poster

067. Posture and Gait: Kinematics; Muscle Activity; Exercise and Fatigue; and Biomechanics I

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 67.14/EE1

Topic: D.16. Posture and Gait

Support: NIH HD048741

Title: A dual-learning paradigm can simultaneously train multiple characteristics of walking

Authors: ***M. STATTON**[1,2], A. TOLIVER[1,2], A. J. BASTIAN[3,2];
[1]Johns Hopkins Univ., Baltimore, MD; [2]Kennedy Krieger Inst., Baltimore, MD; [3]Neurosci., Johns Hopkins Univ. Sch. of Med., Baltimore, MD

Abstract: Walking is a complex motor pattern that is often impaired after damage to the nervous system. Cerebral stroke survivors, for example, frequently exhibit deficits in their walking pattern including step length asymmetry. We have previously used a split-belt treadmill paradigm to produce adaptive changes and robust aftereffects in interlimb coordination (i.e. step length symmetry), which has proved useful for training stroke survivors to correct this movement

deficit (Reisman et al., 2005; 2007). However, disruption of a global behavior like walking is typically exhibited as a combination of multiple local abnormalities rather than a single deficit. Stroke survivors, in addition to step length asymmetry, also demonstrate deficits in intralimb characteristics of the walking pattern such as decreased paretic knee flexion, resulting in a "circumducted" swing pattern, which are not influenced by split-belt training (Reisman et al., 2005). Here we asked whether a dual-learning paradigm can be used to simultaneously adapt subjects' step lengths and knee flexion patterns, and how this compares to subjects adapting each component separately. Young, healthy adults walked on a split-belt treadmill with the "fast" and "slow" belt speeds set at 1.5 m/s and 0.5 m/s respectively, while watching a real-time visual display which gave feedback on the amount of knee flexion of each leg. Subjects were instructed to match the feedback of both knees while walking - indicating symmetric knee flexion. During adaptation, the gain of the knee flexion display was reduced on the "slow" leg in order to drive the subject to learn to increase it during training. Control groups either adapted knee angle only via visual feedback (no split-belt), or adapted step symmetry only via split-belt walking (no visual feedback). Preliminary results show that split-belt adaptation alone and knee angle adaptation alone led to changes in step symmetry and knee angle symmetry, respectively. When both were adapted simultaneously, both step symmetry and knee angle symmetry were learned at a similar rate and showed similar after-effects post-adaptation compared with controls. Our results demonstrate that we can simultaneously change two features of walking, and these changes appear to be independent - adapting knee flexion does not influence the rate or amount of step symmetry adaptation, and vice versa. Importantly, this suggests that we can be successful in simultaneously training more than one deficit during long-term rehabilitation treatments.

Disclosures: **M. Statton:** None. **A. Toliver:** None. **A.J. Bastian:** None.

Poster

067. Posture and Gait: Kinematics; Muscle Activity; Exercise and Fatigue; and Biomechanics I

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 67.15/EE2

Topic: D.16. Posture and Gait

Support: NIH Grant HD048741

Title: Two ways to save: Different perturbation dynamics lead to savings during split-belt treadmill walking

Authors: *R. T. ROEMMICH[1,2], A. J. BASTIAN[1,2];
[1]Motion Analysis Lab., Kennedy Krieger Inst., Baltimore, MD; [2]Neurosci., Johns Hopkins Univ., Baltimore, MD

Abstract: "Savings" refers to the observation that relearning is faster than initial motor learning. Thus, some elements of prior learning are stored, recalled, and used to advantage during relearning. Savings occurs across many types of motor learning paradigms, ranging from eye-blink conditioning to reaching and walking adaptation. However, the initial learning conditions that enable faster relearning remain controversial. Here we consider split-belt walking adaptation, and ask if savings depends on 1) a memory of the perturbation that drives learning or 2) a memory of the adapted motor pattern? We collected kinematic data from young adults (n=60) as they walked on a split-belt treadmill. All participants 1) adapted walking to a split-belt perturbation during which one belt moved twice as fast as the other (Adaptation 1, belt speeds 1.4 and 0.7 m/s), 2) underwent a washout period with the belts moving at tied speeds (De-adaptation, belt speeds both 0.7 m/s), and 3) adapted walking again during a second period of split-belt walking (Adaptation 2). Adaptation 2 was structured identically across groups (belt speeds 1.4 and 0.7 m/s for 10 minutes). However, we altered Adaptation 1 and De-adaptation among groups to investigate how dynamics of adaptation and washout affect savings. First, we compared savings after ten minutes of gradual versus abrupt adaptation (both groups underwent identical abrupt 10-minute De-adaptation periods). We observed savings following abrupt adaptation, but no savings after gradual adaptation. Next, we investigated savings after extending the time at plateau (i.e., full adaptation) during gradual adaptation such that the participants here received ten minutes of exposure to the full perturbation (matching the abrupt adaptation group). This also resulted in savings following an abrupt 10-minute De-adaptation period, though to a lesser degree than savings from an abrupt perturbation. Last, we tested whether savings was observed if we introduced the perturbation abruptly, but limited the time at plateau. Savings occurred after short, 2-minute abrupt adaptation, but to a lesser degree than the 10-minute abrupt adaptation. In sum, we found that a memory of the perturbation and a memory of the adapted motor pattern both contribute to savings in a relearning paradigm. Thus, abrupt adaptation or gradual adaptation with extended time at plateau resulted in savings. These findings demonstrate that savings can be achieved via independent behavioral mechanisms, which may also be useful when considering how to improve locomotor learning within the context of rehabilitation.
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Disclosures: **R.T. Roemmich:** None. **A.J. Bastian:** None.

Poster

067. Posture and Gait: Kinematics; Muscle Activity; Exercise and Fatigue; and Biomechanics I

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 67.16/EE3

Topic: D.16. Posture and Gait

Support: Grant-in-Aid for JSPS Fellows

Title: Mode-dependent control of human walking and running revealed by limited transfer of adaptation across the gaits with different velocities

Authors: *T. OGAWA[1,2], N. KAWASHIMA[3], H. OBATA[4], K. KANOSUE[1], K. NAKAZAWA[4];
[1]Fac. of Sport Sciences, Waseda Univ., Tokorozawa , Saitama, Japan; [2]Japan Society for the Promotion of Sci., Tokyo, Japan; [3]Res. Institute, Natl. Rehabil. Ctr. Persons with Disabilities, Tokorozawa, Saitama, Japan; [4]The Univ. of Tokyo, Tokyo, Japan

Abstract: We have previously demonstrated a specificity in the neural functional networks underlying human walking and running based on the degree of transfer in newly-acquired movement patterns across the gaits. In the current study, we further investigated possible influence of gait velocity (reflecting relative difficulty for executing each gait) on the degree transfer. Sixteen healthy volunteers participated in the experiment. Walk-to-run transition speed (dividing the relative ease/difficulty of walking and running) was 1.68 ± 0.18 m s-1. Subjects participated in two different experiments in random order where in one experiment they adapted to walk and in another to run on a split-belt treadmill driven asymmetrically at 2.0 and 1.0 m s-1 in velocities. To address the degree of adaptation and transfer, six catch trial periods (10 seconds each) to either walk or run on symmetrically-driven belts were interspersed during the adaptation periods. The speed of the treadmill during the catch trials (walking and running) were 0.75, 1.50, and 2.25 m s-1, and were selected to reflect the relative ease to walk (0.75 m s-1), both walk and run (1.50 m s-1), and run (2.25 m s-1). Degree of adaptation and transfer were assessed by calculating asymmetry in the peak values of the ground reaction force in the anterior braking component. The results clearly demonstrated that adaptation to split-belt walking and running resulted in emergence of aftereffect in all the velocities in the respective gait (walk after adapting to walk and run after adapting to run, respectively). On the other hand, the transfer of the adaptation across gaits occurred only partially for both directions (walk to run and run to walk). That is, the emergence of the aftereffect was always greater in walking after adapting to walk and in running after adapting to run despite the relative ease to walk and run at speeds lower and higher than the walk-to-run transition speed. These results suggest that the two major forms of locomotion in humans, walking and running are perceived as being controlled by the central nervous system as a function of different gait modes and are not necessarily dependent on given gait speeds.

Disclosures: **T. Ogawa:** None. **N. Kawashima:** None. **H. Obata:** None. **K. Kanosue:** None. **K. Nakazawa:** None.

Poster

067. Posture and Gait: Kinematics; Muscle Activity; Exercise and Fatigue; and Biomechanics I

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 67.17/EE4

Topic: D.16. Posture and Gait

Title: Contrasting two strategies accomplishing split-belt treadmill adaptation in humans

Authors: *H. YOKOYAMA[1], T. OGAWA[2], K. NAKAZAWA[1], N. KAWASHIMA[3];
[1]The Univ. of Tokyo, Tokyo, Japan; [2]Fac. of Sport Sciences, Waseda Univ., Saitama, Japan;
[3]Natl. Rehabil. Ctr. for Persons with Disabilities, Saitama, Japan

Abstract: Split-belt treadmill walking has been extensively utilized as a useful model to reveal an adaptability of human bipedal locomotion. Previous studies have clearly identified different types of locomotor adaptation, such as, reactive and predictive controls, and each of those distinct control pattern might be accomplished at different level in central nervous system. In the present study, in order to know how these two types of control pattern contribute to achieve locomotor adaptation, we designed unique experimental conditions, those are "temporal symmetry" and "spatial symmetry" adaptation conditions. While former pattern preserve symmetric rhythm with the guide of auditory cue, latter pattern would preserve symmetric limb motion by accomplishing with the guide of asymmetrically-supplied auditory cue during walking on split belt treadmill. We hypothesized that temporal symmetry condition would facilitate to involve automatically-induced reactive control pattern, and spatial symmetry condition would involve predictive forward control. 15 healthy subjects were asked to walk on split-belt treadmill in three different conditions, (1) temporal and (2) spatial symmetric, and (3) ordinary pattern of split-belt adaptation, on a different day. Three dimensional motion analysis and ground reaction forces measurements were conducted during and after split-belt treadmill walking, and then, general spatiotemporal gait parameters and following parameters were calculated; center of oscillation, phase shift of lower limbs, and braking/propulsive force. The results demonstrated that characteristics of adaptation and the extent of after effect showed differences between temporal and spatial symmetry conditions, for example, anterior component of the GRF (braking force) showed a clear pattern of adaptation and subsequent aftereffects in temporal symmetry but

not in spatial symmetry condition, and the ordinary pattern of split belt adaptation was an intermediate type between contrasting two types. We will discuss about mechanism underlying obtained results from the aspects; (1) contribution of each predictive and reactive control and (2) relevance between cortical modulation and spinal pattern generating network underlying split-belt adaptation.

Disclosures: **H. Yokoyama:** None. **T. Ogawa:** None. **K. Nakazawa:** None. **N. Kawashima:** None.

Poster

067. Posture and Gait: Kinematics; Muscle Activity; Exercise and Fatigue; and Biomechanics I

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 67.18/EE5

Topic: D.16. Posture and Gait

Support: NWO VICI 453-08-004

ZonMw 50-50310-9

Title: Optimising filtering parameters for a 3D motion analysis system

Authors: ***J. B. SMEETS**, P. J. BEEK, S. SCHREVEN;
Human Movement Sci., VU Univ. Amsterdam, Amsterdam, Netherlands

Abstract: In the analysis of movement data it is common practice to use a low-pass filter in order to reduce measurement noise. However, the choice of a cut-off frequency is typically rather arbitrary. Here, we propose to use rigid marker clusters to determine the dynamic precision of a given 3D motion analysis system, and to use this precision as criterion to find the optimal cut-off frequency for filtering the data. We tested this method using a model-based approach in a situation in which measurement noise is a serious concern: the registration of the kinematics of swimming using a video-based motion analysis system. For the model data we found that under some conditions, filtering the data with a single cut-off frequency of 6 Hz decreased the accuracy of the reconstruction of the kinematics compared to using the unfiltered data. If the cut-off frequency was used that yielded optimal dynamic precision, then the accuracy improved by 29% compared to using raw data irrespective of the cluster position, close to the optimal accuracy

improvement of 30%. We conclude that 3D motion analysis systems can be made more accurate by optimising the cut-off frequency used in filtering the data with regard to their precision.

Disclosures: **J.B. Smeets:** None. **P.J. Beek:** None. **S. Schreven:** None.

Poster

067. Posture and Gait: Kinematics; Muscle Activity; Exercise and Fatigue; and Biomechanics I

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 67.19/EE6

Topic: D.16. Posture and Gait

Support: CSU Provost Summer 2013 Undergraduate Research Award

Title: Analysis of the turning behavior of the African clawed toad (Xenopus laevis)

Authors: ***R. DEAN**, R. ANDRIKANICH, A. KIFER, S. POLAND, S. SREDNIAWA, M. BARR, S. ADAMS;
Cleveland State Univ., CLEVELAND, OH

Abstract: African clawed toads, which remain aquatic as adults, turn rapidly and accurately towards the origin of surface waves created by prey. Here, we describe basic features of this turn; we focus on the initial direction of body movement and its relation to leg action and hip, knee and ankle movements, characterized respectively as pushing/pulling or extension/flexion/passive return. Stimulus direction determines which of two alternative patterns are performed. Rostral stimuli elicit immediate forward body movement as one or both legs extend and push the body forward. Body rotation and turn angle are adjusted by differential thrust, resulting from stronger extension of leg joints contralateral to the stimulus. Thus, for rostral stimuli, the most frequent pattern utilizes bilateral hip, knee, and ankle extension, which become less bilaterally symmetrical as the stimulus is further from the midline axis until one leg is essentially passive during the turn. This is true whether the toad lunges to strike at close targets or just swims to approach distant ones. In contrast, more lateral and caudal stimuli elicit initial backward movement of the body as one or both hips flex, knees may extend, and legs pull forward. During this pull, joint movements again vary by turn angle and, together with the hip flexion, initiate body rotation. Turning usually continues with a sweeping push by the contralateral leg, resulting from hip and ankle extension and variable knee flexion. As expected, movement patterns for stimuli on the left and right sides are mirror images. Although the most common pattern changes

in characteristic ways depending upon stimulus and turn angles, turns are not truly stereotyped and different combinations of joint movement may be used for similar stimulus angles. Further, patterns change when toads are close to a boundary and modify their turn to clear this obstruction. Patterns also vary among individual toads. Some toads begin almost all turns except lunges by pulling backward to begin rotation. Other toads respond with a forward push for stimuli angles as large as 90° to the side. This behavior is particularly true for the occasional toad that, for unknown reasons, develops a resting posture with abnormally extended legs. For these toads, turning appears less efficient and reaching their final heading may require additional kicks that provide further course correction.

Disclosures: **R. Dean:** None. **R. Andrikanich:** None. **A. Kifer:** None. **S. Poland:** None. **S. Sredniawa:** None. **M. Barr:** None. **S. Adams:** None.

Poster

068. Posture and Gait: Aging and Stroke

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 68.01/EE7

Topic: D.16. Posture and Gait

Support: R21AG037123

Title: The impact of walking-induced fatigue on falls risk and balance in older adults

Authors: ***S. MORRISON**[1], S. R. COLBERG[2], H. K. PARSON[3], S. NEUMANN[3], R. HANDEL[3], E. VINIK[3], J. PAULSON[2], A. I. VINIK[3];
[1]Physical Therapy, [2]Old Dominion Univ., Norfolk, VA; [3]Eastern Virginia Med. Sch., Norfolk, VA

Abstract: For older adults, falls are a serious health problem with over 30% of people over 65 suffering a fall at least once a year. A key component in fall prevention is identifying those predictive variables which lead to falls. An important component often overlooked in the assessment of predictive factors is how these variables change as a function of performing activities of daily living like walking. Although this would appear to be an obvious consideration as most falls occur during movement, the majority of fall risk assessments are performed under resting conditions. The aim of this project was to assess the impact of incline walking at a moderate pace on falls risk, leg strength, simple reaction time and balance in adults. Forty five healthy individuals participated in this study. Subjects were divided into three age groups (Group

1: 30-39 yrs; Group 2: 60-69 yrs; Group 3: 70-79 yrs). Reaction time, leg strength, standing balance and falls risk was assessed prior to and following a period of incline walking on an automated treadmill. Falls risk was assessed using the Physiological Profile Assessment (PPA). For the walking task, three 8-minute trials were performed. Fatigue during walking was elicited by increasing the treadmill incline in increments of 2^0 (from level) every two minutes to a maximum of 8^0. As expected, the results revealed significant age differences in reaction time, strength, balance and overall falls risk prior to performing the walking task. Interestingly, walking had a differential effect on the young and older individuals. Following this activity, both older groups exhibited significantly slower reaction times coupled with diminished leg strength, greater sway and increased falls risk. However, for the young group, the short bout of activity resulted in faster reaction times, increased strength with no subsequent changes in falls risk or sway measures. The general improvements seen in many of the dependent measures within the young group following walking point to the possibility that this activity served as a warm-up, leading to improved post-walking performance. In contrast, older adults exhibited declines in all of the specified fall-related metrics following walking, indicating that this activity had a detrimental impact on their ability to maintain optimal balance. This finding is of particular importance given that, for many older adults, falls occur when they are moving and/or fatigued. The findings of this study demonstrate that a more comprehensive understanding of the problem of falls in older adults can be gained from assessing functional properties related to balance and strength both at rest and following activity.

Disclosures: **S. Morrison:** None. **S.R. Colberg:** None. **H.K. Parson:** None. **S. Neumann:** None. **R. Handel:** None. **E. Vinik:** None. **J. Paulson:** None. **A.I. Vinik:** None.

Poster

068. Posture and Gait: Aging and Stroke

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 68.02/EE8

Topic: D.16. Posture and Gait

Support: UNO UCRCA

UNO GRACA

Title: As risk of falls increases in the elderly, standing postural control shows stronger long-range correlations

Authors: *T. J. RAND, M. MUKHERJEE;
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Abstract: Objective: Aging is associated with changes in physical function, such as standing posture, that lead to an increased risk of falling. However, within the aged population, it is not clear how variations in the risk of falls may be associated with postural control. This research investigates postural control in early and late aging and considers overall movement as well as the time-dependent structure of the movement. **Methods:** 34 participants were separated into three groups: young (Y; 19 – 35; n = 10), early aging (EA; 60 – 75; n = 12), and late aging (LA; 76+; n = 12). Each participant stood on a force platform for 90 seconds in three different conditions: normal standing, eyes closed, and sway referenced visual surround. The center of pressure (COP) was recorded and analyzed in the anteroposterior (AP) direction and the mediolateral (ML) direction. The COP values were used to calculate range, root mean square, and long-range correlations (using detrended fluctuation analysis; DFA) of postural sway. The EA and LA group also performed the timed up and go (TUG) test, a functional test related to fall risk, and the Falls Risk for Older People – Community Setting (Frop-Com), a fall risk questionnaire. A 3x3 mixed model ANOVA compared COP variables and an independent samples t-test compared fall-risk measures. **Results:** Comparisons between groups revealed differences between the Y and LA groups for several variables. The LA group had an increased ML range ($P = .019$) and an increased ML DFA ($P = .042$). However, there were no differences between the Y and EA groups, or the EA and LA groups. When comparing the LA and EA groups for measures of fall risk the LA group demonstrated higher scores on the Frop–Com ($P = .005$) and longer time on the TUG ($P < .001$). **Conclusions:** These results demonstrate an increase in overall ML sway for the LA group however such sway patterns are very regular with low variability. Such postural control is constrained with reduced ability to respond to environmental perturbations. This is also demonstrated in an increased fall-risk in this group. Therefore, within an aged group there is deterioration of postural control. Specifically in terms of flexibility within the temporal patterns of ML sway.

Disclosures: **T.J. Rand:** None. **M. Mukherjee:** None.

Poster

068. Posture and Gait: Aging and Stroke

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 68.03/EE9

Topic: D.16. Posture and Gait

Support: NIA P30 2P30AG028747

Title: Variability in grasp response and the relationship with response time to balance perturbations in older adults at high compared to low risk of falling

Authors: B. JOHNSON[1], R. CREATH[1], R. NEFF[2], *K. P. WESTLAKE[3];
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Abstract: Protective arm responses are one strategy that can effectively lessen the potential for a hip fracture and other major injuries in older adults at risk of falling. While a successfully executed response is both rapid and accurate, it is unknown how these variables are affected with age and how they differ between older individuals at high compared to low fall risk. The objective of this study was to determine differences in reach to grasp balance recovery strategies and associated movement time in older adults who fall compared to those who do not fall and to younger adults. Twenty older adults and 10 young adults participated (25 +/-2yrs). Each older adult was categorized into a high fall risk (n=10, mean age 69 +/- 4yrs) or low fall risk (n=10, 69 +/- 5yrs) group. Participants stood on a translatable lateral surface that was used to induce balance perturbations. The feet were restrained and a rail was placed to the right and left of each subject. For each perturbation, subjects were instructed to grab one rail to regain stability. Four conditions were administered: (1) Predictable: subjects were told the direction of perturbation; (2) Unpredictable: subjects were unaware of the direction; (3) Unpredictable with minimally loaded working memory; and (4) Unpredictable with moderately loaded working memory. Results indicated significant differences in the grasping strategy between the groups for all 4 conditions. Cut off percentages for grasping to the rail opposite the direction of platform translation were >90% for the young group, 70-80% for the older low risk group, and 50-60% for the high-risk group. Differences were also identified between groups for the grasp accuracy of conditions 3-4 with 95-100% accurate and complete grasp in the young group, 90-95% for the older low risk group, and 70-80% for the high-risk group. We conclude that older individuals, and in particular older individuals at high risk of falling, demonstrate greater variability in reach to grasp strategy and accuracy than young adults in response to balance perturbations. Future research will examine additional neuromechanical factors underlying this variability.

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Poster

068. Posture and Gait: Aging and Stroke

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 68.04/EE10

Topic: D.16. Posture and Gait

Support: Grant-in-Aid for Young Scientists (B) (25870656)

Title: Effects of attentional dispersion on sensory-motor processing of anticipatory postural control during unilateral arm abduction in the elderly

Authors: *C. YAGUCHI[1], K. FUJIWARA[2];
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Abstract: The ability of attentional dispersion is one of the factors affecting the deterioration of equilibrium function in the elderly. In this study, we systematically investigated the effects of attentional dispersion during unilateral arm abduction on sensory-motor processing and anticipatory postural control in the elderly. Subjects were 16 healthy elderly persons. A visual cue signal (S1) was presented for 100 ms around the centrally located fixation point. At 1 s after S1 onset, a visual imperative stimulus (6°×6° checkerboard) was presented (S2). Interval between S1s was 3 s. S2 comprised target and non-target stimuli presented at the position (left, right or center) indicated by S1. The distance from the fixation position to left or right S2 and presentation duration of S2 were determined for each subject (6°-9°, 150-250 ms, respectively). If S1 simultaneously indicated three positions, S2 was presented unpredictably in one of the three positions with the equal probability. As results, subjects covertly focused attention on the position (attentional focusing) or divided attention for three positions (attentional dispersion). The order of each S1 and S2 and the presentation positions of S2 were random. In response to the target S2, regardless of the presentation position, subjects abducted their right arm at maximum speed. The reaction time of middle deltoid (MD) to target S2 onset, onset time of postural muscles with respect to MD onset, and the following components of event-related brain potentials were measured. P1-N1, N2 and P3 components were analyzed as indices of the sensory, perceptual and cognitive processing of S2, respectively. The late component of contingent negative variation (CNV) was used to evaluate motor preparation before S2 and anticipatory attention directed to S2. No significant effects of attentional dispersion were found on the amplitude or latency of P1, N1 and P3. N2 and late CNV amplitudes were significantly smaller by attentional dispersion than by attentional focusing ($p < 0.05$). MD reaction time was significantly longer and onset time of postural muscles was significantly later by attentional dispersion than by attentional focusing ($p < 0.05$). These results suggest that the elderly persons would pay a lot of attention to sensory processing even in attentional dispersion condition. Therefore, the attentional allocation on the following higher processing, such as the discrimination or motor preparation would be insufficient by attentional dispersion. These

changes of the sensory-motor processing by attentional dispersion may relate to the delay of MD reaction time and onset time of postural muscles.

Disclosures: **C. Yaguchi:** None. **K. Fujiwara:** None.

Poster

068. Posture and Gait: Aging and Stroke

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 68.05/EE11

Topic: D.16. Posture and Gait

Title: Management of different types of exercise for postural control in elderly: A randomized controlled trial

Authors: ***M. R. OLIVEIRA**[1], R. DA SILVA[2], C. SOUZA[2], M. GUARIDO[2], J. DASCAL[3], D. TEIXEIRA[3];
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Abstract: Introduction: Different types of exercise are indicated for the elderly to prevent functional capacities due to aging and increase the balance. Objectives: This study aimed to evaluate the effect of three different types of exercises (mini trampoline, aquatic gymnastic and general floor gymnastics) on postural control measures in elderly. Methods: 39 physically independent elderly women, mean age 69 ± 4 years, were randomly assigned to three intervention groups: 1) mini trampoline (MT, n= 11), 2) aquatic gymnastic (AG, n= 15), e 3) general floor gymnastics (GG, n= 13). Each group performed physical training including cardiorespiratory, muscular strength and endurance, flexibility and sensory-motor exercises at 12 weeks. To determine the effects of each intervention group, five tasks were used to evaluate postural control in different balance tasks on a force platform: two-legged stand with eyes open and closed; semi-tandem stand with eyes open and closed and one-legged stand. During the tasks, Centre of Pressure (COP) measures were computed and analyzed from mean across three trials (with 30 s of rest between them) in each balance task. Results: Postural control in all tasks was significantly improved ($P < 0.05$), after management of three modalities of exercises employed, with effect size in mean for aquatic exercise reaching 0,63 characterized medium effect. Conclusions: These results support that mini trampoline, aquatic and general gymnastics are efficient to improve the balance in elderly women after 12 weeks training.

Disclosures: **M.R. Oliveira:** None. **R. da Silva:** None. **C. Souza:** None. **M. Guarido:** None. **J. Dascal:** None. **D. Teixeira:** None.

Poster

068. Posture and Gait: Aging and Stroke

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 68.06/EE12

Topic: D.16. Posture and Gait

Title: Relationship between foot posture measures and force platform parameters during two balance tasks in older and young subjects

Authors: *R. A. DA SILVA JR[1], C. E. CARVALHO[2], A. W. GIL[3], M. R. OLIVEIRA[4], J. A. NASCIMENTO[4], D. A. A. P. OLIVEIRA[4];
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Abstract: Introduction: Different clinical methods are used to assess the dimensions of foot in order to increase the clinical relevance decisions for posture, balance and deformity of foot in older. **Objectives:** To compare age differences on anthropometric posture measures of foot and balance parameters and determine the relationship between them. **Methods:** Sixty-eight older (mean age 68 yrs) and 42 adults (in mean age 21 yrs) participated of this study. Foot posture was tested across four domains: 1) hallux flexion and extension range of motion (ROM) using goniometer, 2) height navicular and 3) length of the foot both using pachymeter, and 4) foot print: width of fore foot, arch index and hallux valgus. Balance was tested in two conditions on a force platform: bipodal at 60-s trials; and unipodal at 30-s trials (with bref rest across trials). Sway ellipse area of centre of pressure (COP) and sway COP velocity in anteroposterior and mediolateral directions were computed. **Results:** Older shown significant (P<0.01) poor balance than adults only in unipodal condition (COP area 9.97 vs 7.72 cm^2). Older presented significantly (P<0.05) low hallux mobility and high values of the width of fore foot and transverse arch index than adults. The correlations between all foot posture and COP parameters varied, across groups, of weak to moderate (r -0.01 to -.046). Low mobility of hallux was significantly related to higher COP values in older. **Conclusions:** These results have any clinical implications for balance and foot posture assessments.

Disclosures: **R.A. da Silva Jr:** None. **C.E. Carvalho:** None. **A.W. Gil:** None. **M.R. Oliveira:** None. **D.A.A.P. Oliveira:** None. **J.A. Nascimento:** None.

Poster

068. Posture and Gait: Aging and Stroke

Location: Halls A-C

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Program#/Poster#: 68.07/EE13

Topic: D.16. Posture and Gait

Support: NIH Grant P30 AG024827, AG 021885

Title: Small movement errors during split-belt locomotor adaptation do not increase the generalization of learning to natural walking in older adults

Authors: H. M. HARKER[1], C. J. SOMBRIC[1], P. J. SPARTO[2], *G. TORRES-OVIEDO[1]; [1]Dept. of Bioengineering, [2]Dept. of Physical Therapy, Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Several studies have investigated mechanisms mediating the generalization of robotic-assisted motor learning to real-life situations (e.g., Torres-Oviedo and Bastian 2012). However, little is known about how these mechanisms are affected by natural aging processes. To bridge this gap, we studied the generalization of motor learning in older adults, and whether it could be manipulated. We know young and old subjects learn a new walking pattern when one leg moves faster than the other on a split-belt treadmill (Reisman, et al. 2005, Bruijn et al. 2012). Interestingly, young subjects learn a more generalized pattern when they experience small errors on the treadmill that are closer to those they experience naturally (Torres-Oviedo, et al. 2012). Here, we investigated whether a similar effect would be observed in older adults. To this end, we compared the generalization of split-belt adaptation effects to over ground walking when two groups (n=8, each) of older subjects (75 ± 5 y.o.) experienced small or large errors during adaptation. Both groups walked over ground and on the treadmill with both belts moving at the same speed for a 'baseline' condition. Then, subjects walked on the split-belt treadmill while the belts changed from a 1:1 to a 2:1 speed ratio either abruptly (large-errors group) or gradually (small-errors group). Directly following these adaptation periods, both groups walked over ground once again. Kinematic data were recorded throughout the experiment to characterize movement patterns. The amount that each group adapted was assessed by finding the steady-state value (i.e. last 40 strides averaged) of the recorded movement pattern during adaptation. Generalization was measured as the difference between the movement pattern observed at the beginning of over ground walking post-adaptation (i.e., first 5 strides averaged) and that

observed during baseline over ground walking. We found that older adults were able to reach similar steady-state at the end of split-belt walking regardless of the size of the errors they experienced during split-belt walking ($p = 0.29$). While we observed large transfer of the learned motor patterns to over ground walking in both groups, it was not significantly different ($p=0.61$). Our results suggest that the generalization of learning in older adults, unlike in young subjects, is not limited by the large errors experienced during adaptation. This could possibly be because older adults naturally experience large errors during their daily walking movements. Our results are promising because they suggest that movement patterns learned on a treadmill could carry-over to natural movements in older clinical populations.

Disclosures: **H.M. Harker:** None. **C.J. Sombric:** None. **P.J. Sparto:** None. **G. Torres-Oviedo:** None.

Poster

068. Posture and Gait: Aging and Stroke

Location: Halls A-C

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Topic: D.16. Posture and Gait

Support: NIH P30 AG024827, AG 021885

Title: You only get better with age: Age predisposes one to learn new movements slower but to carryover what is learned more to novel situations

Authors: *C. J. SOMBRIC[1], H. M. HARKER[1], P. J. SPARTO[2], G. TORRES-OVIEDO[1]; [1]Dept. of Bioengineering, [2]Dept. of Physical Therapy, Univ. of Pittsburgh, Pittsburgh, PA

Abstract: How does aging affect our ability to learn new movements and generalize them to different situations? We have shown that after split-belt walking (i.e., two legs moving at different speeds) young subjects learn a locomotor pattern with spatial and temporal features that are different from normal gait (Malone et al. 2012). Interestingly, young subjects disengage the locomotor pattern learned on the split-belt treadmill when walking overground (Torres-Oviedo and Bastian 2012). Here we investigated if this capacity is maintained with healthy aging. We further tested whether subjects' ability to switch actions in a cognitive task is correlated with their ability to switch locomotor patterns. Older (76±3 yo, n=8) and younger (27±8 yo, n=8) adults walked on a split-belt treadmill (2:1 belt ratio). Then, all subjects walked on the treadmill when the two belts moved at the same speeds. We quantified learning by computing 1) the

steady-state movements reached during split-belt walking, 2) the rate at which these steady-states were achieved, and 3) the adaptation effects after split-belt walking (i.e., aftereffect) on the treadmill. To assess generalization both groups walked overground after split-belt walking and we calculated the initial aftereffects (first 5 steps) overground. Lastly, we assessed subjects' ability to switch actions with a task in which they had to match two objects based on their shape or color. We found that while older adults learned the split-belt pattern at a slower rate, both groups reached similar spatial ($p=0.35$) and temporal ($p=0.09$) steady states during adaptation. Both groups also learned the same amount, as shown by similar spatial ($p=0.75$) and temporal ($p=0.72$) aftereffects on the treadmill. Moreover, older adults generalized more of the learned movements than young adults in the temporal domain ($p=0.02$), but not in the spatial domain ($p=0.48$). Lastly, while there were trends, the performance in the cognitive switching task was not a predictor of the generalization in the spatial ($p=0.082$) and temporal domain ($p=0.077$). This indicates that the perseverance of treadmill movements in the overground context is not mediated by the same neural mechanisms underlying the perseverance of action selection in the cognitive domain. These results suggest that age related cerebral changes may not affect the centers enabling the learning of new motor patterns, but may affect those underlying the switching of motor patterns across contexts. Therefore, treadmill-assisted rehabilitation would be more effective in older vs. younger clinical populations because older adults will apply more of what they learn on a treadmill to natural overground walking.

Disclosures: **C.J. Sombric:** None. **H.M. Harker:** None. **P.J. Sparto:** None. **G. Torres-Oviedo:** None.

Poster

068. Posture and Gait: Aging and Stroke

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 68.09/EE15

Topic: D.16. Posture and Gait

Title: Adaptation changes in dynamic postural control and contingent negative variation during repeated transient forward translation in the elderly

Authors: *M. MAEKAWA[1], K. FUJIWARA[2], N. KIYOTA[3], C. YAGUCHI[4];
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Abstract: Adaptation changes in postural control and contingent negative variation (CNV) for the elderly were investigated during repeated forward floor translation. Fifteen healthy elderly persons, living in the suburban area of Kanazawa City, Japan, underwent backward postural disturbance by a forward-floor translation (S2) 2 s after an auditory warning signal (S1). A set with 20 trials was repeated until a negative peak of late CNV was recognized in the 600-ms period before S2, and the last set was defined as the final set. Electroencephalograms, center of foot pressure in the anteroposterior direction (CoPap), and electromyograms of postural muscles were analyzed. CoPap displacement generated by the floor translation was significantly decreased until the twelfth trial in the first set, and mean CoPap displacement was smaller in the second and final sets than in the first set. The mean displacement was significantly smaller in the final set than the previous set. A late CNV with a negative peak was not recognized in the first and second sets. However, most subjects (13/15) showed a negative peak by the fourth set, when the late CNV started to increase negatively from about 1,000 ms after S1 and peaked at about 300 ms before S2. At about 160 ms before the CNV peak, the CoPap forward shift started. The increase in timing of the gastrocnemius activity related to the CoPap shift was significantly correlated with the CNV peak timing ($r = 0.64$). After S2, peak amplitudes of the anterior postural muscles were significantly decreased in the final set compared to the first set. It was demonstrated that even for the elderly, with so many repetitions of postural disturbance, a late CNV with a negative peak was recognized, leading to accurate postural preparation. This suggests the improvement of frontal lobe function (e.g., anticipatory attention and motor preparation) in the elderly.

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Poster

068. Posture and Gait: Aging and Stroke

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 68.10/EE16

Topic: D.16. Posture and Gait

Title: Elderly gait dynamics and the effects of environmental constraints

Authors: *E. G. JAMES;
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Abstract: Elderly gait has been found to be characterized by altered stride time dynamics during treadmill walking (Hausdorff et al., 1997). Prior theoretical work has shown that environmental

constraints can alter the organization of motor behavior (Newell, 1986). The present study examined the effects of different levels of environmental constraints on gait in the elderly during treadmill walking. Seven elderly (age 65 - 70 years) and 8 young (age 18-25 years) adults walked at their preferred speed for 10 minutes on a treadmill with lengths of 1 and 1.5 m. The *SD* and long-range correlations (using Detrended Fluctuation Analysis) in stride time were examined to determine possible age and treadmill length effects and interactions. There were no significant differences in the *SD* of stride time. Consistent with prior research (Hausdorff et al., 1997) the elderly had significantly lower stride time Detrended Fluctuation Analysis α-exponents when walking on a 1.5 m long treadmill ($p = 0.009$). However, when walking on a treadmill of 1 m length the elderly stride time α-exponents increased to a level that was not significantly different than those of the young adults ($p = 0.398$). The elderly also had a higher *SD* of stride position on the treadmill than the young adults when walking on the 1.5 m treadmill length ($p = 0.033$) but not the 1 m length ($p = 0.165$). A decrease in the treadmill length led to a decrease in the stride position α-exponents for the elderly ($p = 0.001$). These results indicated that constraining the treadmill length reversed the age-associated change in stride dynamics. Future research is needed to determine whether this alteration in gait dynamics is retained after long-term gait training.

Disclosures: E.G. James: None.

Poster

068. Posture and Gait: Aging and Stroke

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

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Topic: D.16. Posture and Gait

Support: AHA Scientific Development Grant

Title: Falls-risk post-stroke: Examining contributions from paretic versus non-paretic limbs to unexpected forward gait slips

Authors: *T. S. BHATT[1], T. KAJROLKAR[2];
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Abstract: Balance and gait-related impairments resulting from post-stroke hemiparesis are the main contributors to the high annual incidence of falls in community-dwelling stroke survivors,

especially from environmental perturbations. Previous evidence has demonstrated the importance of reactive stepping strategies in re-establishing one's dynamic stability and preventing perturbation-induced falling. While evidence from stance perturbation studies have demonstrated the preference of non-paretic limb for reactive stepping in people with hemiparetic stroke such preference could be biased as the perturbation was induced simultaneously under both limbs. This study aimed to examine the effect of paretic side versus non-paretic side gait slips on slip outcome, dynamic stability and compensatory stepping response. ***Methods:*** Twenty people with chronic (> 6 months) hemiparetic stroke (57$\pm$ 6 yrs) participated in the study. Low-friction platforms were used to induce an unexpected finite (24 cm) forward slip either under the paretic (n=10) or non-paretic (n=10) limbs while walking in a safety harness. Kinematic data was recorded using an 8-camera passive marker system. Slip outcome (including the incidence of falls and balance loss), dynamic stability (based on the center-of-mass position and velocity) and compensatory step strategy (aborted versus recovery) and kinematics were computed. ***Results:*** There was an equal incidence of falls (50%) between the nonparetic-slip and paretic-slip groups. While the paretic-slip group demonstrated a majority (90%) of recovery stepping responses (a forward step with the non-paretic limb), the non-paretic-slip group however, demonstrated an aborted stepping response (unloading of the paretic limb followed by its immediate re-loading) for 70% of trials. As a consequence, the dynamic stability at contralateral liftoff of the stepping limb was significantly lower for the nonparetic-slip group with a correspondingly greater slip displacement and velocity than the paretic-slip group. ***Conclusion:*** Both the paretic and non-paretic-slip limbs contributed equally to fall-risk on the unexpected finite slip. An aborted stepping response executed by the paretic limb during the nonparetic-slip could lead to fatal vertical falls, especially from infinite slips. Similarly, an unsuccessful recovery step executed by the nonparetic limb during the paretic-slip can increase fall-risk. Intervention strategies targeted towards improving reactive stepping and fall-risk prevention should focus on both paretic and nonparetic limb training.

Disclosures: **T.S. Bhatt:** None. **T. Kajrolkar:** None.

Poster

068. Posture and Gait: Aging and Stroke

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Topic: D.16. Posture and Gait

Support: AHA Grant SDG12200001

Title: Long-term retention of locomotor adaptation following short-term training in people with stroke

Authors: D. TAN, *E. V. VASUDEVAN;
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Abstract: Hemiparesis is a frequent and debilitating consequence of stroke. Lower limb hemiparesis is associated with asymmetric walking patterns, called hemiparetic gait. Training on a split-belt treadmill, which has two belts that can drive each leg at a different speed, can immediately improve hemiparetic gait following a single training session[1], but these effects are assumed to be short-lasting unless training occurs over an extended period[2]. Recent evidence from our lab, however, suggests that neurologically intact adults can retain a long-term memory of split-belt adaptation after only 1 or 2 training sessions[3]. Our objective in the present study was to determine whether stroke affects the ability to consolidate a memory of split-belt adaptation and retain it long-term. The study took place over 3 visits: an initial session ("Day 1") and two subsequent sessions 24 hours later ("Day 2") and 4 weeks later ("1 Month"). At each visit, baseline interlimb coordination was first assessed on an over ground walkway and then on the split-belt treadmill with the belts running the same speed ("tied-belts" at 0.5 m/s). Participants were then adapted to the split-belt treadmill (0.5:1.0 m/s) for 16 min. Near the end of adaptation, changes in walking coordination were briefly assessed on the treadmill (tied-belts) and over the ground. Between visits, participants were told to go about their normal activities, including walking. As in previous studies[1,4], we found that people with stroke were capable of adapting and storing locomotor aftereffects within testing sessions. We also found evidence that they could store a memory of split-belt adaptation long-term - partial aftereffects were still evident during baseline treadmill walking on Day 2 and 1 Month. In contrast to controls, however, people with stroke did not show "savings" - they did not re-adapt faster to split-belts on Day 2 and 1 Month, compared to Day 1. This may reflect an impaired ability to learn and refine specific walking strategies to suit different environments. This ability may be critical for community ambulation, where it is important to anticipate environmental changes and alter gait accordingly. Overall, these results suggest that short-term training can result in long-term retention of locomotor aftereffects post-stroke; however, learning and remembering strategies to adapt to split-belts may be more impaired. 1. Reisman*, et al. Neurorehabil Neural Repair* **23**, 735-744 (2009). 2. Reisman*, et al. Neurorehabil Neural Repair* **27**, 460-468 (2013). 3. Vasudevan & German. *Society for Neuroscience Annual Meeting* **749.04** (2013). 4. Savin*, et al. Neurorehabil Neural Repair* **27**, 24-34 (2013).

Disclosures: D. Tan: None. **E.V. Vasudevan:** None.

Poster

068. Posture and Gait: Aging and Stroke

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 68.13/EE19

Topic: D.16. Posture and Gait

Support: CAPES Grand

Title: All Nintendo Wii Fit games are useful for rehabilitation of all patients with chronic stroke? A study based on learning principles

Authors: *M. E. PIEMONTE, C. S. MIRANDA, T. P. OLIVEIRA, J. X. M. GOUVEIA, D. B. PEREZ, A. E. TEIXEIRA, A. L. COSTA;
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Abstract: Background: Motor rehabilitation can be characterized as a process of 'relearning' how to move to respond satisfactorily to the demands of daily living, and is based on the premise that training leads to improved performance both in terms of acquiring new skills and adapting or refining previously acquired skills. Thus, knowledge on the effectiveness of the motor learning process in patients with stroke is pivotal for planning more effective rehabilitation strategies. In the absence of a clear picture of the changes during the learning process in stroke, and consequent lack of definitive guidelines for devising more effective rehabilitation strategies, it is important to investigate the learning potential of patients with stroke by applying new therapeutic strategies and validating their utility. Objectives: To evaluate the learning, retention and transfer of performance improvements after Nintendo Wii FitTM training in patients with Stroke. Participants: Fourteen chronic stroke patients, with a mean age of 51.42 ± 10.21 years, 8 men and 6 women, mean time poststroke, 4.47± 4.06 years; 60% left hemiparesis and a minimum score of 24 on the Mini-Mental State Examination. Procedures: Evaluation - Both groups were evaluated before the first session of training, 48 hours and 7 days after training. As learning and retention measures were used the game's scores. In order to determine the transfer of learning was used the scores in three force plate tests. Quantitative postural control assessment was conducted using three subtests: limits of stability (LOS), walk across (WA) and step up/over (SUO) of the NeuroComTM Balance Master according to standard protocol. Training - Five attempts were done per game: the first and the last was considered as performance measure. Results: The results evidenced a significant improvement in 3 of the 5 games analyzed. LOS analyses showed that endpoint excursion to paretic side improved significantly post training. There were significant improvement in the speed, and step length. SUO analyses showed significant increases in movement time difference and impact index difference. There was a correlation between global cognitive abilities, but not motor abilities, with the learning in the games. Conclusions: The patients with chronic stroke were able to improve their performance in Nintendo Wii FitTM games according to the demands of games and of the preserved cognitive

capacities. Thus, it is very important to consider these issues to elect this kind of training with therapeutics purposes.

Disclosures: **M.E. Piemonte:** None. **C.S. Miranda:** None. **T.P. Oliveira:** None. **J.X.M. Gouveia:** None. **D.B. Perez:** None. **A.E. Teixeira:** None. **A.L. Costa:** None.

Poster

068. Posture and Gait: Aging and Stroke

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 68.14/EE20

Topic: D.16. Posture and Gait

Support: Fapesp 2012/19943-0

Title: Effects of target uncertainty during arm reaching movements in upright position in stroke individuals

Authors: ***S. M. FREITAS**, C. A. LIMA, A. S. BALDAN, S. R. ALOUCHE;
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Abstract: Many daily living activities involve movements of the upper limbs during upright standing (e.g., reaching a glass in a cabinet) which depend on the adequate control of reaching the object (focal movement) and on the generation of appropriate postural adjustments. Individuals post-stroke have functional limitations in using their upper limbs to reach an objet during standing. It is unknown, however, if they use different strategies to reach a target in an upright stance when the target position is uncertain. Therefore, the aim of the present study was to investigate the effects of the final target position uncertainty on the balance and focal movement (performed with the limb ipsilateral to the lesion) of post-stroke individuals. Fifteen individuals with hemiparesis (eight on the right and seven on the left side) and eight healthy individuals in upright position (with each foot in an individual force plate) performed reaching movements towards a target shown in the center of a monitor, placed at a distance of 115% of the upper limb's length. The focal movement and postural adjustments were evaluated in certain (target final position was known) and uncertain (target could move to an upper or lower position after the participant started to move) conditions. Joint kinematic of the focal movement (shoulder, elbow and wrist) and postural adjustments (hip, knee and ankle) in the sagittal plane were recorded. Center of Pressure (CP) displacement and joint angles were evaluated 150 ms before and during the movement. The movement onset time was longer in uncertain than in

certain condition; however, the movement time was similar between conditions. Before and during the movement, participants with hemiparesis showed larger CP displacements in the paretic side than in the non-paretic side while the CP displacements were symmetrical for healthy individuals. In the certain condition, the focal e postural joint amplitude was smaller than in the uncertain condition. Participants used mainly the hip joint, regardless the task condition, except those with left hemiparesis that presented greater amplitude at the ankle joint in the uncertain compared to certain condition. Overall, the uncertainty in target position during reaching in an upright position influences the amplitude of joint excursions for both focal and postural movements; however, they do not affect the CP displacement. In addition, the results suggest that stroke individuals, in particular those with left hemiparesis, use different strategies to maintain the postural balance but not to perform the focal movements during the uncertain condition.

Disclosures: **S.M. Freitas:** None. **C.A. Lima:** None. **A.S. Baldan:** None. **S.R. Alouche:** None.

Poster

068. Posture and Gait: Aging and Stroke

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 68.15/EE21

Topic: D.16. Posture and Gait

Support: NIH Grant 1R21HD058267 (Wu)

Title: Using swing resistance and assistance to improve gait asymmetry in patients post stroke

Authors: *S.-C. YEN[1], B. SCHMIT[2], M. WU[3];
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Abstract: Patients post stroke often demonstrate step length asymmetry. For those who demonstrate a shorter step length in the affected leg, applying either swing resistance or swing assistance to that leg may help improve symmetry. In principle, swing resistance can augment errors in step length, initiate patients' error correction process, and induce aftereffects consisting of an increased step length following removal of the resistance. On the other hand, swing assistance can directly pull the affected leg forward and result in an increase in step length. However, because such assistance deviates the leg kinematics away from the status quo, patients could treat it as an error perturbation and in turn correct such error by reducing the amplitude of

leg swing, leading to a decrease in step length and worse asymmetry. The purpose of this study was to clarify how patients post stroke adapt to swing resistance and assistance applied to the affected leg during walking. We recruited 9 subjects with chronic stroke to participate in this study; all of them demonstrated shorter step length in the affected leg. They participated in two treadmill adaptation sessions, one for swing resistance and the other for swing assistance. Subjects showed error correction during adaptation to swing resistance and produced an aftereffect consisting of improved step length symmetry. On the other hand, subjects showed improvement in step length symmetry when walking with swing assistance, although the improvement did not sustain following the removal of the assistance. Subjects did not correct swing assistance during adaptation, suggesting that the injured central nervous system may use healthy gait state prior to stroke as a reference to determine movement error rather than using the current gait state. Overall, the results suggest that swing resistance and swing assistance may engage different motor learning pathways for correcting gait asymmetry.

Disclosures: **S. Yen:** None. **M. Wu:** None. **B. Schmit:** None.

Poster

069. Posture and Gait: Injury and Disease

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 69.01/EE22

Topic: D.16. Posture and Gait

Support: NIA Grant P30-AG028747

Title: Reactive versus volitional balance training to prevent falls: Pilot study preliminary results

Authors: *D. N. SAVIN JR[1], J. BARTON[2], M. W. ROGERS[3];
[1]Dept Physical Therapy & Rehabil., Univ. Maryland Baltimore, BALTIMORE, MD; [2]Neurol., [3]Dept. Physical Therapy & Rehabil. Sci., Univ. of Maryland Baltimore, Baltimore, MD

Abstract: Background: Neuromotor deficits with advancing age and leading to impaired balance control may precipitate falls and loss of mobility. Falls generally occur in one of two ways: unexpectedly, in reaction to unexpected events (e.g. a slip or trip), or more predictively during a planned voluntary movement such as while reaching. Current neurorehabilitation interventions to enhance balance and prevent falls focus mainly on training planned (expected) voluntary actions (volitional balance control), with little attention directed at improving reactive balance control to unexpected disturbances. Objective: To compare volitional versus reactive balance training to

enhance reactive balance control in older adults at risk for falls. We hypothesized that reactive balance training would result in greater improvements in reactive balance control compared to volitional balance training in fall-risk older adults. Methods: Participants with a history of ≥ 1 fall in the past year and no other significant neurological or orthopedic conditions were enrolled and randomized to either a reactive or voluntary balance training group. All training sessions occurred twice a week for eight weeks with each session lasting 45-50 minutes. Reactive balance control was assessed by using unpredictable lateral waist pulls; volitional balance control was assessed during a standing cued reaching task. Primary outcome measures: number of recovery steps and recovery step length in response to the unpredictable lateral waist pulls (reactive balance), overall excursion of the net center of pressure (volitional balance). Secondary outcome measures: the Berg Balance Scale, Falls Efficacy Scale, and 5 Item Physiological Profile Assessment. Results: Post-training, participants in the reactive balance training group increased recovery step length to a greater degree than those in the voluntary balance training group. Both groups decreased the number of recovery steps required and increased the net center of pressure excursion and postural sway component of the 5 Item Physiological Profile Assessment. There were no significant changes in Berg Balance or Falls Efficacy Scale scores. Conclusions: Our results supported our hypothesis that reactive balance training improves reactive balance control to a greater extent than volitional balance training. This suggested that the different control processes engaged by the two training protocols should be taken into account when designing effective neurorehabilitation programs to enhance balance and reduce fall risk.

Disclosures: **D.N. Savin Jr:** None. **J. Barton:** None. **M.W. Rogers:** None.

Poster

069. Posture and Gait: Injury and Disease

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 69.02/EE23

Topic: D.16. Posture and Gait

Support: NIH R01 HD069769

Title: Does visual dependency affect standing balance in adults with Cerebral Palsy?

Authors: *Y. YU, I. CHUDNOVSKAYA, S. SNELL, R. LAUER;
Temple Univ., Philadelphia, PA

Abstract: Cerebral Palsy (CP) is a disorder due to brain damage before, during, or shortly after birth. Commonly mistaken as a childhood motor disorder, CP continues into adulthood with motor and sensory deficits. Individuals with CP have been shown to be more visually dependent than those with typical development (TY), which may jeopardize the control of balance in this population. The current study aimed to understand how standing balance control is affected by visual dependency. Nine adults with CP and 14 adults with TY stood in a virtual reality environment in which visual filed motion was manipulated by projecting visual scene either in pitch-up and pitch-down direction at 15 degrees/second or motionless. Five adults with CP were defined as visually dependent for having judgment errors greater than 5° in the Rod and Frame Test. Using a force platform, the trajectory of center of pressure (COP) was recorded separately in the antero-posterior (AP) and medio-lateral (ML). Approximate entropy (ApEn) was calculated and used as an index of the regularity of standing postural sway. A repeated-measure ANOVA (Group as between-subject factor, Condition as within-subject factor) was employed. A significant effect of Condition on COP in ML direction ($p = .042$) was found; regardless of the population, standing posture sway in the ML direction was perturbed by the moving visual scene. To further understand how the standing postural sway is affected by the visual dependency in individuals with CP, a second repeated-measure ANOVA was employed with Visual Dependency being between-subject factor and Condition being within-subject factor. A borderline effect of visual dependency was observed ($p = .060$), indicating that individuals with CP who are visually dependent are more likely to move in a regular and repeated fashion. Such movement flexibility may be used as a means to actively obtain sensory information to orient oneself in the surrounding environment. Future studies are needed to develop potential intervention to reduce visual dependency in this population.

Disclosures: **Y. Yu:** None. **I. Chudnovskaya:** None. **S. Snell:** None. **R. Lauer:** None.

Poster

069. Posture and Gait: Injury and Disease

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 69.03/EE24

Topic: D.16. Posture and Gait

Title: Bracing the trunk and neck alters the spatiotemporal parameters of gait

Authors: *D. M. RUSSELL[1], K. J. KELLERAN[2], S. MORRISON[1];
[1]Physical Therapy & Athletic Training, [2]Human Movement Sci., Old Dominion Univ., Norfolk, VA

Abstract: Older individuals typically display declines in walking performance which include: slower speed, slower cadence, shorter stride length, and greater stride width. These factors have been characterized as a more "cautious" gait pattern. The trunk and neck play an important role in dissipating the transmission of forces from the ground to the head, but this more cautious gait appears to be associated with a stiffer torso. We hypothesize that an increase in body stiffness in older adults, reducing the ability to dissipate forces, may in part be responsible for the change in gait parameters observed with aging. Therefore the aim of this study was to determine if experimentally manipulating the neck and trunk mobility of young, healthy people alters the spatiotemporal parameters of gait. Twelve healthy adults performed 3 walking trials on a flat, straight 70 m walkway, under four different bracing conditions: (1) Control (no brace), (2) Neck brace, (3) Trunk brace, and (4) Neck and Trunk braces. Participants walked across a 6.1 m pressure sensitive GAITRite mat placed in the middle of the walkway. Spatiotemporal parameters of gait could then be computed based on the recording of each footfall on the mat. Participants were instructed to maintain their most comfortable walking pace during each trial. The control condition was always performed first, while the order of the other conditions was randomized. The mean walking speed decreased across the four bracing conditions, but this narrowly failed to reach the level of significance (p = .066). There was no significant difference in the cadence of walking under the different bracing conditions (p =.64). Stride length significantly decreased with bracing (p = .002), while step width increased (p = .014). Overall these results indicate that stiffening the trunk in young, healthy individuals leads to a similar gait pattern observed in older adults. Therefore, reduced mobility of the neck and torso may in part contribute to the decrements in walking observed with aging.

Disclosures: **D.M. Russell:** None. **K.J. Kelleran:** None. **S. Morrison:** None.

Poster

069. Posture and Gait: Injury and Disease

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 69.04/EE25

Topic: D.16. Posture and Gait

Support: VH Horne Fund

UW Graduate School

Title: Compulsory joint torque coordination interfaces for neuromuscular training and rehabilitation of human walking

Authors: *W. BOEHM[1], K. GRUBEN[2];
[2]Kinesiology, Biomed. & Mechanical Engin., [1]Univ. of Wisconsin Madison, Madison, WI

Abstract: Walking is an essential means of navigating the world for most humans, yet common approaches to restore this ability after neurological insult disrupts walking fall short of their perceived potential. It is theorized that optimal walking restoration therapy remains elusive due to current therapies approaching the challenge with a scope focused on externally driving the kinematic patterns of typical walking rather than addressing the underlying muscular coordination issues. Our work reveals a specific role of muscle coordination in balance control during walking. Humans coordinate hip, knee, and ankle torque during walking to produce a force of the foot on the ground (*F*) appropriate for regulating whole body angular momentum such that they do not tip over. That specific pattern of force produced through the gait cycle results from coordination of 1) ankle torque that produces heel-to-toe center of pressure shift and 2) hip and knee torques that direct *F* near the center of mass (CM). Immediately following a stroke, it has been shown that the hip and knee torque coordination component is altered in a way such that it would tip the person over if used during walking. Thus, to restore walking functionality, rehabilitation intervention must address this atypical coordination choice and require patients to practice producing the desired (typical) coordination pattern. This approach differs from many current therapies that prescribe the path of the lower limb without constraining the muscle coordination pattern used by the patient. While the limb may be forced through a typical kinematic pattern, that pattern is not necessarily voluntarily controlled by the subject and thus, the underlying muscular activation is not practicing the patterns needed to reproduce those kinematics outside of the rehabilitation setting. We have designed and constructed a rehabilitation device that requires the user to produce the desired muscular coordination (reflected in the direction of the limb endpoint force *F* produced) in order to operate. The device measures and provides feedback on the direction of *F* that the patient produces and adjusts resistance accordingly such that the device allows motion only when the appropriate coordination is learned. Our initial application aims to restore walking after a stroke, however, the system has broad application in neural and physical rehabilitation, fitness and sport-specific training, and injury prevention.

Disclosures: **W. Boehm:** None. **K. Gruben:** None.

Poster

069. Posture and Gait: Injury and Disease

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 69.05/EE26

Topic: D.16. Posture and Gait

Title: Changes in postural sway as a result of sports concussion

Authors: *K. S. THOMAS, M. MAGAL;
Mathematics and Sci., North Carolina Wesleyan Col., Rocky Mount, NC

Abstract: The incidence of sports related concussions are a growing public health concern. Assessments used to determine return-to-play criteria following a concussive incident are generally related to postural control due to the ability to indirectly measure neurophysiological decline. This study investigated the effects of sports related concussion on postural control. Twelve healthy young NCAA Division III athletes (6 males, 6 females; ages 20.58±1.00) were recruited to participate in the study. Athletes from women's soccer (WSOC), women's basketball (WBB), and football (FB) with a documented concussion in the last year (EXP) were matched with an athlete within the same sport without history of a sports concussion (CON). Participants stood on a Bertec (BP5050) force plate and performed five postural conditions (quiet stance (Eyes Open, Eyes Closed), Anterior-Posterior (A-P) sway, Medio-lateral (M-L) sway, and change of direction sway) for 3-30s trials. For the sway conditions a metronome set at 60Hz in which participants were instructed to keep up with. For the change of direction sway condition, participants began swaying in either the M-L or A-P directions to the metronome and asked to change directions using auditory cues "forward" or "backward" and "left" or "right". The presentation of the auditory cue was randomized to occur within a 10-12s period throughout each trial to control for anticipatory responses. Overall, the results of this study revealed no statistically significant differences in the amount (path length, 95% ellipse), variability (standard deviation - SD), and structure (Approximate Entropy - ApEn) of COP motion between those athletes with a history of concussion compared to those athletes that had not sustained a concussion. These findings are contrary to previous findings which indicated that postural control variables were compromised in those athletes that had sustained a concussion compared to those athletes that hadn't. In conclusion, the postural system of young athletic adults was able to rapidly compensate and adjust to the postural sway tasks regardless of concussion history.

Disclosures: **K.S. Thomas:** None. **M. Magal:** None.

Poster

069. Posture and Gait: Injury and Disease

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 69.06/EE27

Topic: D.16. Posture and Gait

Support: American Heart Association

Title: Modulation of the reactive-stepping response to forward-slip perturbations: Effect of central versus peripheral cues on compensatory step initiation with the paretic side

Authors: *P. PATEL[1,2], T. BHATT[1,2];
[2]Physical Therapy, [1]The Univ. of Illinois At Chicago, Chicago, IL

Abstract: Background: The limb preference demonstrated for reactive stepping for people with hemiparetic stroke is the uninvolved (or non-paretic limb). Such preference can however, decrease stability and increase fall-risk due to the inefficient vertical limb support being provided by the paretic limb during step execution. The purpose of this study was hence to determine if providing explicit cues could enhance step initiation from the paretic limb and further to determine the effectiveness of central versus peripheral cues for the same. Methods: Thirteen subjects with chronic hemiparetic stroke (57.7±6.60participated in the study. After being given two familiarization trials subjects were exposed to large-magnitude (velocity 0.67 m/s for 0.04s) forward-slip stance perturbations on the motorized Activestep treadmill (uncued condition, UNC). Subjects were then exposed to three trials of explicit verbal instructional cuing asking them to try their best to initiate a step with their paretic limb (central cueing, CC). The last condition was implicit peripheral cuing (PC) where the non-paretic limb was weighted down with 5% bodyweight equivalent ankle cuffs in order to induce a “constraint” effect. Subjects received three trials of each condition. Kinematic data was recorded using a passive marker system and EMG from bilateral tibialis anterior muscles recorded via wireless sensors. The reaction time (EMG onset), compensatory step characteristics (step initiation time and backward step length) and postural stability were computed and analyzed. Results: Compared to the UNC, (100% step initiation with the non-paretic limb), for the PC condition 90% successful step initiation was with the paretic-limb and even greater for the CC condition (46.66% paretic step initiation). There was no difference in step kinematics (reaction time and compensatory step length) for paretic limb steps between the CC and PC conditions. There was no significant difference in step kinematics between the paretic limb and non-paretic limbs; however, postural stability was significantly greater at instance of step touchdown when steps were initiated with the paretic limb. Conclusion: External cuing can significantly enhance reactive step ability in individuals with hemi-paretic stroke; explicit verbal cues targeting central attentional resources had a significantly greater impact than peripheral cues inducing a limb constraint. Improved step ability with the paretic limb can improve postural stability and decrease fall risk from unexpected

external perturbations. The effect of externally-cued training for improving reactive stepping needs to be further explored.

Disclosures: **P. Patel:** None. **T. Bhatt:** None.

Poster

069. Posture and Gait: Injury and Disease

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 69.07/EE28

Topic: D.16. Posture and Gait

Title: Effects of a single session of French Auriculotherapy on pain intensity and postural control in individuals with low back pain: A randomized clinical trial

Authors: *P. B. DE FREITAS, JR[1], A. USHINOHAMA[2], B. P. CUNHA[2], L. O. P. COSTA[3]; [1]Inst. de Ciencias da Atividade Fisica e Esporte, Univ. Cruzeiro Do Sul, Sao Paulo, Brazil; [2]Programa de Pós-Graduação em Ciências do Movimento Humano, Univ. Cruzeiro do Sul, São Paulo, Brazil; [3]Programa de Mestrado e Doutorado em Fisioterapia, Univ. Cidade de São Paulo, São Paulo, Brazil

Abstract: Pain is defined as an unpleasant perceptual experience related to a real or a potential tissue injury. It is subjective and depends on individuals' cultural, social, and emotional states. Low back pain (LBP) is prevalent mainly in the economically active population and causes high economic burden to healthy systems worldwide. LBP negatively affects person's ability to perform several motor tasks. For instance, individuals with LBP increase their body sway while they are asked to stand as still as possible mainly in challenging situations. The French auriculotherapy (FA) is a form of acupuncture applied to the external ear and show positive results in pain relief. Thus, the aim of the study was to examine the effects of a single session of FA on pain sensation and on body sway during postural tasks with different degrees of complexity. Eighty young adults, males and females, with chronic LBP and pain intensity equal or larger than 4 at the day of testing in a 0 to 10 pain scale were assessed. Participants were randomly allocated in two groups of 40 individuals: FA group (FAG) and placebo group (PG). Initially, the level of the participants' pain intensity (0-10) was asked. Next, they were asked to stand as still as possible on a force plate either with feet placed in parallel or in semi-tandem stance and either with eyes open or closed. Then, the participants of FAG were treated with FA

for 20 min and the participants of PG were treated with inactive ultrasound with circular movement of its head being slightly applied to the pain site. Immediately after the treatment, the level of pain intensity was asked again and the postural test was repeated. Pain intensity and center of pressure (COP) sway area were the primary and secondary outcome measured. Results of non-parametric tests revealed that pain intensity decreased in both groups after treatment ($p<.001$), but no difference between AFG and PG was observed after the treatment ($p=.063$). For postural control, results revealed effect of treatment in a single and more complex postural situation (i.e. semi-tandem stance with eyes closed - STEC) and only for AFG. Specifically, only AFG reduced COP sway area in this postural condition after treatment. Also, the only difference between AFG and PG was found in STEC condition after the treatment ($p=.049$), with COP sway area being lower in AFG than in PG. In conclusion, AF and placebo reduce pain intensity but only AF affects positively the performance of the postural control system in a more complex postural task. This effect could be caused by a decrease in nociceptive and a consequent increase in proprioceptive activity in the lumbar region, which could improve information about body center of mass position.

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Poster

069. Posture and Gait: Injury and Disease

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 69.08/FF1

Topic: D.16. Posture and Gait

Support: IRME

CNRS

Paris Descartes

ENSAM

Title: Mechanisms behind the transition from acute to chronic whiplash-associated disorders

Authors: ***P.-P. VIDAL**[1], J. LECOMPTE[2], D. WANG[1], S. BLANCHO[3], P. LINDBERG[1], C. DE WAELE[1], J. ADRIAN[4], E. CHIAROVANO[1], B. SANDOZ[2], S. LAPORTE[2];
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Nationale Supérieure d'Arts et Métiers, Paris, France; [3]Inst. pour la Recherche sur la Moelle épinière et l'Encéphale, Paris, France; [4]CEESAR, Nanterre, France

Abstract: Whiplash is usually defined as an injury of the neck, which most often occurs following car rear-end collision. In the present case they consist mostly in soft tissues injuries since we excluded patients with fractures and dislocations. The main concern with whiplash is that a large proportion of these patients experience disabling symptoms for months if not for years following the accident. The initial and most prevalent complaints are neck and upper back pain. Several other symptoms, the whiplash-associated disorders (WAD), are frequently associated: dizziness and unsteadiness, headache, concentration and memory disturbances, upper limb weakness, paresthesias, and blurred vision. At the chronic stage, sub-acute and chronic symptoms may also include fatigue, sleep disturbances, depression and anxiety. As quoted above, the main problem is that about 50% of patients experience chronic symptoms with considerable direct and indirect costs. In that context, early detection of the WAD patients at risk of developing chronic syndromes is important because it would allow designing better preventive treatment for high-risk individual. However, the great heterogeneity of the methods employed in the studies of WAD has resulted in interesting but often disparate if not contradictory results on that matter. The mechanisms behind the transition from acute to chronic WAD remain to be elucidated and in particular the evaluation of the impact of multiple risk factors in a single patient. In order to tackle with that problem, we set out to combine several methods of investigations in the same WAD patients at the acute stage of whiplash and we have repeated these observation six months later. At both stages, in the thirty WAD patients we investigated and in controls, a neurologist and a neurootologist conducted thorough clinical examinations and clinical tests. Then a project psychologist interviewed participants and the patients were asked to fill in a range of questionnaires. Two projects neurophysiologists submitted also the patients to more specialized test concerning head and postural control. Finally, the patient underwent computed tomography of the neck including a tractograhy study of the descending tract in the spinal cord. The patients had whiplash injury grades II and III. Grade II was defined as neck complaints and musculoskeletal signs; grade III required additional signs (decreased or absent deep tendon reflexes, weakness, and sensory deficits). We found that, when combined, selected neurootological tests, neuropsychological factors, some abnormalities in neck mobility and postural control could predict transition from acute to chronic WAD.

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Poster

069. Posture and Gait: Injury and Disease

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 69.09/FF2

Topic: D.16. Posture and Gait

Support: Oregon Clinical and Translational Research Institute (OCTRI), grant number (KL2TR000152) from the National Center for Advancing Translational Sciences (NCATS) at the National Institutes of Health (NIH)

CTSA grant number (UL1TR000128)

Title: Sensory augmentation for balance control in chronic post-concussive syndrome

Authors: *L. A. KING, M. MANCINI, F. B. HORAK;
Neurol., Oregon Hlth. & Sci. Univ., Portland, OR

Abstract: The control of postural sway depends on the dynamic integration of multisensory information in the central nervous system. Augmentation of sensory information, such as during auditory biofeedback (ABF), has been shown to improve postural control when natural sensory information is limited by environmental conditions or pathology. Since impaired postural control is commonly disrupted in chronic post concussive syndrome, the goal of this pilot study was to determine if using ABF to augment sensory information would improve postural control in people with chronic post-concussive syndrome. Methods: Four subjects with chronic non-resolving balance disorders post-concussion participated. The mean age was 19.5 years 4, range 17-25 and the mean time since injury was approximately 4 months (2 sports concussion, 2 falls). Each person was actively being treated for non-resolving balance disorders in our university rehabilitation center. For this pilot study, each participant underwent a balance assessment using an inertial sensor around the waist to measure postural sway under varying sensory conditions. Specifically, subjects were asked to stand quietly for 30 seconds with eyes closed on firm and foam surface, first without and then with ABF. During the trial with ABF subjects were asked to correct their sway according to the feedback, by keeping the volume as low and as balanced as possible. Here we report the percentage change in sway area ($(SA-SA_{ABF}/SA)*100$) during ABF compared to non-ABF trials for the feet together conditions with and without foam. Results: Our preliminary results showed a mean decrease of 43.1% (±35) sway area using ABF compared to non-ABF while standing with feet together in firm surface ($p=0.09$) and a significant mean decrease of 50.3% (±29) sway area using ABF compared to non-ABF while standing with feet together on foam ($p=0.04$) in 4 patients with post-concussive syndrome. Interestingly, the control subject we tested showed an increase of 40% and 24% for the firm surface and foam, respectively. Conclusion: Large sway may indicate a decreased ability of the nervous system to detect, centrally process and/or correct postural sway. People with chronic non-resolving balance

deficits after concussion found the system easy-to-use and they were able to correctly follow the audio information, when available. Such results, while preliminary, are promising and suggest that auditory biofeedback may be beneficial for rehabilitation of balance control in this population.

Disclosures: **L.A. King:** None. **F.B. Horak:** None. **M. Mancini:** None.

Poster

069. Posture and Gait: Injury and Disease

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 69.10/FF3

Topic: D.16. Posture and Gait

Title: Quantifying the relationship between step variability and dynamic instability in cerebellar ataxic gait

Authors: *W. ILG[1], Z. FLESZAR[1], C. SCHATTON[1], B. MUELLER[1], N. LUDOLPH[1], M. A. GIESE[1], M. SYNOFZIK[2];
[1]Cognitive Neurology, Section Computat. Sensomotorics, Ctr. For Integrative Neurosci., Tuebingen, Germany; [2]Dept. of Neurodegeneration, Hertie Inst. for Clin. Brain Research,, Tuebingen, Germany

Abstract: Background: The cerebellum is well-known to be crucially involved in balance and locomotion. One of the most characteristic and sensitive signs of cerebellar damage is gait ataxia. Clinically, ataxic gait is typically characterized by an increased step width, variable foot placement and a resulting instable stumbling walking path with very high movement variability and high risk of falling (1). However, the exact features of dynamic instability in ataxic gait have not yet been assessed in detail. Here, we aimed to provide a quantitative analysis of this dynamic instability for different gait phases in ataxic gait. Methods: We analysed gait patterns from 15 patients suffering from cerebellar degeneration or degeneration of afferent pathways (median age: 61, median SARA: 15.7) compared to 15 age-matched healthy subjects. Subjects were instructed to walk normally at a self-determined pace. For quantifying dynamic instability we used the approach of extrapolated centre of mass (XCoM) (2,3). Based on this, a measure of stability is established by determining the 'margin of stability' b, the minimum distance from XCoM to the boundaries of the base of support (BOS). Results: The dynamic stability measure b averaged over the whole gait cycles revealed no significant differences between patients. More meaningful measures concerning stability were obtained by the analysis of stability conditions at

the foot contact of the stepping leg. Compared to controls, patients showed a significant lower stability margin b in mediolateral ($p<0.001$), but not in anterior-posterior direction ($p=0.15$). In addition, variability of b was significantly higher in patients for both directions ($p<0.01$). The variability in b correlated with the clinical SARA score ($r=0.55$,$p=0.03$), confirming variability as a striking feature in ataxic gait. Further analyses will include the relationship between dynamic stability measures and temporal as well as spatial measures of step variability. Conclusion: Our results indicate that the reported measures allow capturing and characterizing dynamic instabilities in ataxic gait. Therefore, these measures are suitable to serve as outcome and monitoring parameter for quantifying changes in balance control in intervention studies. In addition, the analysis of instabilities in specific gait phases could help to distinguish different patterns of ataxic gait (e.g. cerebellar vs. afferent degeneration). References: (1)Ilg W, and Timmann D. Mov Disord 28: 1566-1575, 2013. (2)Pai YC, and Patton J. J Biomech 30: 347-354, 1997. (3)Hof A, et al. J Biomech 38:1-8, 2005

Disclosures: **W. Ilg:** None. **Z. Fleszar:** None. **C. Schatton:** None. **B. Mueller:** None. **N. Ludolph:** None. **M.A. Giese:** None. **M. Synofzik:** None.

Poster

069. Posture and Gait: Injury and Disease

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 69.11/FF4

Topic: D.16. Posture and Gait

Title: The influence of dual tasking on dynamic balance in patient with cerebellar ataxia

Authors: ***B. KANG**, J. PARK;
physical education, Korea Univ., Seoul, Korea, Republic of

Abstract: The purpose of this study was to investigate the effect of dual tasking on the postural control during standing in patients with cerebellar ataxia (CA). It was hypothesized that the CA patients would exhibit different sway characteristics of the center of pressure (COP) and center of mass (COM) by complexity of the secondary cognitive task compared with normal controls. Total 12 CA patients (mean age 45.8, male-7, female-5) and age-matched normal controls participated in this study. Participants were instructed to perform three balance tasks (non-dual, dual ML movement, and dual AP movement) with two different dual-task complexities. Variability, complexity, coupling and symmetric index from the left, right and overall COPs and COMs were measured. Results demonstrated that CA patients showed deficits in balance and

postural control with increased dual-task complexity during overall sway movements, and decreased coupling between left and right limb movements. However, there was no significant difference in symmetric index. With the higher difficulty in cognitive task, CA patients took longer to stabilize their COP and to regain balance, whereas normal controls showed no change between conditions. In addition, CA patients had a greater COM resultant velocity during recovery in the dual-task compared with the single-task condition. These findings suggest that CA patients had compensatory strategies in dual tasking resulting in simple and combined postural movement patterns.

Disclosures: **B. Kang:** None. **J. Park:** None.

Poster

069. Posture and Gait: Injury and Disease

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 69.12/FF5

Topic: D.16. Posture and Gait

Title: Cervical spinal cord injury model for studying locomotor dysfunction

Authors: *S. K. KARADIMAS[1], S. GOSGNACH[2], K. SATKUNEDRARAJAH[1], M. FEHLINGS[1];
[1]Toronto Western Hospital, UHN, Toronto, ON, Canada; [2]Univ. of Alberta, Edmonton, ON, Canada

Abstract: Introduction: Cervical injury is the most common form of SCI and can result from either an acute traumatic or a progressive compressive injury. The most common form of progressive compressive injury is Cervical Spondylotic Myelopathy (CSM). Cervical SCI leads to devastating locomotor deficits arising from disruption of descending input onto locomotor CPGs residing in the lumbar enlargement. In the past, majority of studies examining locomotor disruption that ensues SCI have utilized acute injury models, which provide a limited understanding of the locomotor changes. For the first time, this study examines changes in locomotor function during the progressive modifications of neural networks that make up the locomotor circuitry. Methods: Chronic compressive cervical SCI was induced in C57Bl mice by inserting a piece of aromatic polyether underneath the C5-6 lamina without the need for laminectomy. This aromatic polyether absorbs phosphate anions and gradually increases calcium phosphate sedimentation to induce new bone formation. A sham operation group was included. MRI was used to confirm progressive compression of the cord. Detail gait (CatWalk) and

hindlimbs kinematic analysis (Photron FASTCAM) were conducted to evaluate locomotor dysfunction. Lumbar motoneuron excitability was evaluated via a detail analysis of H response at various stimulation frequencies. Gait and kinematic analysis were also performed in human CSM patients. ANOVAs were used for the statistical analysis. Results: All compressed mice displayed a progressively significant decrease in hindlimb stride length and swing phase in injury mice compared to controls. In addition, progressively significant increases were observed in running time, hindlimb base of support and hindlimb stance phase duration in injury mice compared to controls. Interestingly, human patients with compressive cervical SCI exhibited a significant decrease in stride length and swing speed as well as significant increase in running time, base of support and hindlimb stance phase duration. Gait analysis also proved that CSM mice exhibit deficits in left-right alternation. Electrophysiological recordings from the lower extremities demonstrated a significant increase in the excitability of lumbar spinal circuitry following chronic compressive cervical SCI as assessed by Hmax/Mmax ratios. Conclusion: This study indicates that CSM mice exhibit locomotor phenotype that mirrors the disruption seen in human patients. Thus, this novel mouse model of progressive locomotor dysfunction provides the opportunity to study the progressive modulation of lumbar CPGs during the course of the disease.

Disclosures: **S.K. Karadimas:** None. **S. Gosgnach:** None. **K. Satkunedrarajah:** None. **M. Fehlings:** None.

Poster

069. Posture and Gait: Injury and Disease

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 69.13/FF6

Topic: D.16. Posture and Gait

Title: Sub-concussive head impact affects multisensory processing for upright standing

Authors: *J. J. JEKA, S. HWANG, L. B. MOORE, P. AGADA, R. TIERNEY; Kinesiology, Temple Univ., Philadelphia, PA

Abstract: Sub-concussion is an underrecognized phenomenon resulting from low levels of head impact that has the potential to cause significant neurological damage long-term. It has been suggested that head impact affects multimodal sensory processing for postural control, but this has never been observed at low levels of head impact. Here we used soccer heading, a safe and well-controlled human model, to study whether intermodal processing is affected by sub-

concussive impacts . Healthy young adult (ages 18-25) soccer players with at least 5 years of soccer heading experience participated. Soccer balls were projected from a JUGS machine at a speed of 25 mph as subjects performed 10 standing headers over 10-minutes at the beginning of the second test session (0-2hr post-test). Body kinematics was measured in a pre-, 0-2hr post-, 24hr post-heading repeated measures design. For multimodal sensory perturbations, subjects received an 80Hz vibratory stimulus to their bilateral Achilles tendons (stimulus turns on-off at 0.28Hz), a ±1mA bilateral monopolar galvanic stimulus (GVS) at 0.36Hz, and a visual motion stimulus at 0.2Hz at low and high amplitude during 135sec trials. Gain and phase for each modality relative to body kinematics were calculated and compared between test sessions. The results showed that intermodal gain relative to GVS was significantly decreased in 0-2hr post session compared to the pre- and 24h post-session. Gains relative to vision and vibration did not change between test sessions. These results reflect that mild mechanical insults affect intermodal vestibular system processing rather than visual and proprioceptive processing, suggesting that even sub-concussive impact may have detrimental consequences due to long-term repetitive exposure.

Disclosures: **J.J. Jeka:** None. **S. Hwang:** None. **L.B. Moore:** None. **P. Agada:** None. **R. Tierney:** None.

Poster

069. Posture and Gait: Injury and Disease

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 69.14/FF7

Topic: D.16. Posture and Gait

Title: Gait characteristics of children with spastic diplegia cerebral palsy

Authors: *C. W. CHAU, K. CARROLL, M. DONAHUE, A. KRAHMER, S. VILLAREALE; Nazareth Col., ROCHESTER, NY

Abstract: Children with Spastic Diplegia Cerebral Palsy (CP) have deficits in locomotion. The aim of this study is to examine gait characteristics in young children with CP and at specific age ranges. Four children with CP (3 male, 1 female, ages 4-13) and Gross Motor Function Measure (GMFM) score of II were studied. All subjects walked independently without assistive device or orthotics on the GAITrite© (CIR System Inc. NJ), a carpeted walkway (3ftx12ft) embedded with electronic pressure sensors that record foot prints. Two to five trials of walking at self-selected pace were videotaped. Spatiotemporal gait parameters were recorded and analyzed with the

GAITrite system which included step length, step cycle duration, velocity, cadence, stance and swing phase duration (as a percent of cycle duration), base of support width, and step length discrepancy (difference between left and right step length). Data from the 4 year old (yo) child with CP was compared to age-matched typically developing (TD) children previously recorded in the laboratory. Data from 7-13 yo children with CP were compared to age-matched normative values reported in the literature. Comparisons between age groups were also made. The preliminary results from the 4yo child with CP showed a decrease in cycle duration (-289ms), step length (-4.2cm), stance duration (-25.5%), an increase in velocity (+34.7cm/s), cadence (+52.9steps/min), swing duration (+25.5%), base of support width (+4.47cm), and step length discrepancy (+6.5cm) as compared to the 4yo TD children. Changes in gait from 4yo to 13yo child with CP included a decrease in velocity (136% to 76% of age-matched control), cadence (141% to 72%), swing duration (161% to 95%), step length discrepancy (6.9 to 3.05 cm), an increase in step length (90% to 97%) and stance duration (56% to 104%). At age 13, the step cycle duration (1389ms ± 172) was longer than that reported in TD children. Our results suggest that the locomotor pattern of the 4yo child with CP was irregular with a decrease in stance and stability. As the age of children with CP increased, the stance duration increased and a more regular gait pattern emerged, possibly due in part to delayed maturation of the locomotion pattern or increased energy efficiency in locomotion tasks. While the step length was slightly decreased at 4 yo child with CP, it was similar to age-matched controls from 7 to 13 yo despite temporal changes suggesting that the spatial parameter is robust. However, the progressive decline in velocity and cadence observed at 13 yo child with CP was consistent with existing literature suggesting that gait in children with spastic CP worsened over time.

Disclosures: **C.W. Chau:** None. **K. Carroll:** None. **M. Donahue:** None. **A. Krahmer:** None. **S. Villareale:** None.

Poster

069. Posture and Gait: Injury and Disease

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 69.15/FF8

Topic: D.16. Posture and Gait

Title: The Effects of sensory information distortion by vibrational stimulus on balance control in patients with cerebellar ataxia

Authors: *D.-H. KIM, J. PARK;
Physical Educ., Korea Univ., Seoul, South Korea, Korea, Republic of

Abstract: It has been reported that damage in the cerebellum results in deficits in maintenance of balance and posture. However, there remains a lack of empirical studies investigating the degree to which the involvement of proprioceptive information in balance tasks in patients with cerebellar disease. This study aimed to examine the influence of muscle spindle vibration on the kinematic characteristics of the static balance and postural control in cerebellar ataxic patients. Nine patients with cerebellar dysfunction and 9 age- and sex-matched normal controls participated in the study. The experimental task consisted a total of 8 conditions including with/without leg muscle vibration and with/without visual blocking per 4 situations using stable and unstable platform respectively, and we measured various kinematic variables, such as sway velocity, sway area, and the length of the sway path of the center of mass (COM). The findings revealed that cerebellar ataxia patients demonstrated an increase in COM sway velocity, sway area, and the length of the sway path in all conditions compared with normal controls. They showed the largest sway amplitudes when vibrational stimulus was applied on muscle spindles in tasks on the unstable platform with visual block. These results indicate that balance control was greatly affected by distortion of sensory information in cerebellar ataxic patients suggesting that proprioceptive input of the leg muscle spindles might be involved in sensory integration processes of the cerebellum for maintenance of balance and posture.

Disclosures: **D. Kim:** None. **J. Park:** None.

Poster

069. Posture and Gait: Injury and Disease

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 69.16/FF9

Topic: D.16. Posture and Gait

Support: Dept. of Veterans Affairs Career Development Award #I01BX007080

Medical Research Foundation of Oregon

NIH Grant R37 AG006457

Title: The Effects of Parkinson’s disease on adaptation of compensatory stepping

Authors: *D. S. PETERSON[1,2], B. W. DIJKSTRA[3], Y. P. T. KAMSMA[3], F. B. HORAK[1,2];
[1]Oregon Hlth. & Sci. Univ., Portland, OR; [2]Portland Veterans Affairs Med. Ctr., Portland, OR;
[3]Human Movement Sci., Univ. of Groningen, Groningen, Netherlands

Abstract: Falls are common in people with Parkinson's disease (PD), and compensatory steps after a postural perturbation (e.g. a slip) are critical to avoid falls. Postural motor training for PD may provide a means to improve stepping responses; however, whether compensatory stepping can be altered with repeated perturbation exposure, and whether changes are retained or generalize to other postural tasks is not well understood. We measured how stepping responses change after repeated postural perturbations in PD and HO, and whether changes are retained over 24 hrs. Seven PD (on levodopa) and 10 HO completed 25 forward and 25 backward postural perturbations, in which the support surface moved quickly under their feet resulting in a compensatory step. Participants also completed 10 perturbations in the medial-lateral (ML) direction. Twenty four hours later, participants completed 10 forward/backward perturbations (retention assessment), and 10 ML perturbations (generalization assessment). Center of mass (COM) displacement, number of steps, and step length/latency were measured. For forward perturbations (backward compensatory stepping), both HO and PD reduced COM displacement over the 50 trials (Repeated measures ANOVA; main effect for block ($f(4, 56)=18.4$, $p=<0.001$), suggesting improved compensatory responses to perturbations, and these effects are retained (HO: $p=0.02$; PD: $p=0.01$). Number of steps following a forward perturbation reduced over time in HO ($f(4,36=10.8$, $p<0.001$), and trended toward reduction in PD ($f(4,20)=1.9$, $p=0.14$). During backward perturbations (forward stepping), COM and number of steps became smaller in HO ($f(4,36=8.17$; $p=0.02$), but not in PD. Improvement in stepping responses did not generalize to lateral stepping for either group. These preliminary results suggest that while HO and PD improve stepping responses with perturbation training, improvements may not generalize to other balance tasks. This provides support for further investigation of perturbation-type training for fall prevention, however additional research is necessary to identify ways to improve generalization of learning to multiple perturbations.

Disclosures: **D.S. Peterson:** None. **B.W. Dijkstra:** None. **Y.P.T. Kamsma:** None. **F.B. Horak:** None.

Poster

069. Posture and Gait: Injury and Disease

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 69.17/FF10

Topic: D.16. Posture and Gait

Title: Effects of locomotor retraining based on the motor learning principle on rehabilitation of gait function in patients with cerebellar disease

Authors: *S.-J. IM, J.-H. PARK;
Physical Educ., Korea Univ., Seoul, Korea, Republic of

Abstract: It has been suggested that the cerebellum is critical for automatic processes of error detection and correction in maintenance of balance and posture. Accordingly, damage to this neural structure frequently results in deficits in static and dynamic balance control. These observations provide important implications for physiotherapeutic interventions for recovery from cerebellar dysfunction. The present study investigated the effects of an explicit strategy for locomotor relearning/training on rehabilitation of gait function in patients with cerebellar disease (CD). A total of 23 patients with degenerative cerebellar disease participated in a 16-week rehabilitation program that initially emphasized conscious awareness and control of the center of gravity (COG) with proper trunk and limb alignment to stabilize balance and posture during body-weight shifting ambulation. Eventually, patients were trained to reach later stages of learning characterized by more accurate and stable performance with minimal demands of attention reflecting increased automaticity in movement control. The results demonstrated that the lateral variability of COG including other gait variability such as stride length and width during gait activities were greatly reduced after intervention indicating improved walking stability in CD patients. In addition, significant improvements were observed in clinical tests that evaluate functional balance and gait capabilities. The results from this study suggest that the benefit and clinical applicability of a gait re-education program developed based on the motor learning principle are evident for the recovery of walking capacity in patients with cerebellar dysfunction.

Disclosures: **S. Im:** None. **J. Park:** None.

Poster

069. Posture and Gait: Injury and Disease

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 69.18/FF11

Topic: D.16. Posture and Gait

Title: The effect of obstacle height in stepping strategies in children with autism spectrum disorder

Authors: *S. CHOI, J. PARK;
Physical Educ., Korea Univ., Seoul, Korea, Republic of

Abstract: Obstacle crossing is a complex locomotor task requiring precise limb movements and maintenance of balance. However, the locomotor characteristics of obstacle crossing in children with autism spectrum disorder (ASD) have not been described before. This study investigated how obstructions with different heights influence the capability to plan and execute stepping movements over an obstacle in ASD children. Twelve children with ASD (age: 8~10, IQ: 50~70) and 12 age-matched normal children (age: 8~10 IQ: above 90) participated in the study. Two different obstacle heights were used; 1) 5 cm - similar height of threshold and door sill of bath room, and 2) 15 cm - such as precast pavers in everyday life. The results indicated that ASD children crossed the obstacle through high toe clearance and short take-off and landing distances with relatively large foot abduction and low walking velocity as compared with normal children. Such different stepping characteristics in ASD children are considered as an adaptive control strategies to avoid tripping over obstacles and compensate for body imbalance when negotiating obstacles. In conclusion, the finding of this study suggests that impairments of balance and coordination made children with ASD more vulnerable *in situ*ations that require gait challenges such as stepping over an obstacle, reducing their capability to plan and execute avoidance behaviors.

Disclosures: **S. Choi:** None. **J. Park:** None.

Poster

070. Visuomotor Coordination

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 70.01/FF12

Topic: D.17. Voluntary Movements

Support: BBSRC (UK) BB/I008101/1

Title: The effect of visual feedback on interlimb transfer of training during a ballistic movement

Authors: *A. R. BUICK[1], K. L. RUDDY[1,2], M. L. RANKIN[1], R. G. CARSON[1,3];
[1]Psychology, Queen's Univ. Belfast, Belfast, United Kingdom; [2]Neural Control of Movement

Lab., ETH, Zurich, Switzerland; [3]Trinity Col. Inst. of Neurosci. and Sch. of Psychology, Trinity Col. Dublin, Dublin, Ireland

Abstract: Training one limb on a specific motor task leads to strength gains and performance improvement when making the same movement with the opposite, untrained limb. This phenomenon of cross-education has the potential to be influenced through visual feedback (Carson & Ruddy, 2012). The current study sought to manipulate visual feedback of the training limb to examine its effect on performance transfer and also whether this differs depending on the side of the body that is trained. Twenty-four healthy adult volunteers (9 male, mean age 21.6 yrs, right-hand dominant) took part, twelve of whom trained with their left side whilst the remainder performed training with their right side. Each participant completed two sessions (Mirror/Non-Mirror) during which they performed 20 trials (15 movements per trial) of ballistic wrist extension movements, attempting to improve upon their peak acceleration with each subsequent movement. In the Mirror condition, participants were asked to complete the task whilst looking at the reflection of their moving limb, whereas in the Non-Mirror condition participants were required to fixate on a white cross placed on a black drape obscuring their view of the training limb. Performance of this same movement task (Non-Mirror) by the opposite (untrained) limb was assessed prior to, at the mid-point and following training. Transfer was calculated as the change in performance of the transfer limb expressed relative to the peak maximum change in performance exhibited by the training limb (i.e. regardless of the training block in which this occurred). Analysis revealed that training limb percentage improvement data was not normally distributed ($W=0.88$, $p<0.001$), thus the adjusted boxplot (Hubert & Vandervieren, 2004) was used to remove outliers and results indicated that an improvement greater than 18% was considered evidence of learning. Transfer was evident regardless of the side performing the training and the type of visual feedback (>50% across all conditions) and this effect was sustained for at least 10 minutes after the cessation of training. Mixed ANOVA revealed that although there was a trend towards larger rates of transfer when the left limb performed training compared to the right, this did not reach significance ($F(1, 16) = 3.729$, $p = .07$) and there was no effect of using mirrored visual feedback ($F(1, 16) = 2.859$, $p = .11$).

Disclosures: **A.R. Buick:** None. **M.L. Rankin:** None. **K.L. Ruddy:** None. **R.G. Carson:** None.

Poster

070. Visuomotor Coordination

Location: Halls A-C

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Program#/Poster#: 70.02/FF13

Topic: D.17. Voluntary Movements

Support: BBSRC (UK) BB/I008101/1

Title: Relationships between structural and functional cortical connectivity and cross education of motor function

Authors: *R. G. CARSON[1,2], K. L. RUDDY[3,1,2], N. WENDEROTH[3], D. G. WOOLLEY[4], A. LEEMANS[5];
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Abstract: The mechanisms through which the training of one limb leads to improved performance of the opposite (untrained) limb, were investigated using multimodal methods of quantifying structural and functional cortical connectivity. Twenty-four participants underwent a resting-state functional Magnetic Resonance Imaging (rs-fMRI) scan, along with a Diffusion Weighted Imaging (DWI) sequence, prior to an upper limb (wrist) training protocol that engenders transfer of performance to the untrained limb. An equivalent rs-fMRI scan was undertaken following training - 300 resisted discrete ballistic left wrist flexion movements, executed as fast as possible. Participants were instructed to maximise the peak acceleration of the movement. Performance of the right limb on the same task was assessed prior to, at the mid-point of, immediately after, and one week following training. On average, the improvement in performance of the untrained limb was 112% of that observed for the trained limb. At retention, performance of the untrained limb was 57% greater than on commencement. An ROI-ROI analysis revealed that functional connectivity in the resting motor network - between left and right supplementary motor area (SMA), and between left anterior cingulate cortex (ACC) and right posterior primary motor area (M1p), was elevated following training. These changes were not however correlated with or predictive of individual levels of transfer. Analysis of the DWI data using Constrained Spherical Deconvolution (CSD) based tractography indicated that the apparent fibre density (AFD) of the fibre bundles connecting left and right SMA was negatively correlated with (rpb=-0.71,p=0.001) and predictive of transfer both acutely (slope=-655.72,t=-5.01,p<0.001) and one week later (rpb=-0.48, p=0.002, slope=-491.713, t=-3.56,p=0.002). Fractional anisotropy (FA) in the same tracts displayed similar negative correlations with acute transfer, although these were expressed less reliably (rpb=-0.44,p=0.06, slope=-834.75, t=-2.03, p=0.06). A replication including only structural connectivity measures was conducted for a separate group (n=17), wherein both FA and AFD in the tracts connecting bilateral SMA were negatively correlated with and predictive of transfer (FA: rpb=-0.51,p=0.03, slope= -1054.24, t=-2.26, p=0.04; AFD: rpb=-0.70, p=0.002, slope= -338.44, t=-3.05, p=0.008). The findings suggest

that, at least for this motor task, interhemispheric interactions between bilateral SMA play an instrumental role in relation to cross education, and that the structural organisation of the intercalating white matter pathways is related to the level of transfer.

Disclosures: **K.L. Ruddy:** None. **R.G. Carson:** None. **N. Wenderoth:** None. **D.G. Woolley:** None. **A. Leemans:** None.

Poster

070. Visuomotor Coordination

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 70.03/FF14

Topic: D.05. Visual Sensory-motor Processing

Support: VR-M-03026 (to SG)

VR-M 2011-1062 (to AAK)

VR-NT-6049 (to SG)

EU- Human Brain Project

Title: Visuomotor processing in optic tectum - A microcircuit mechanism for selection of action

Authors: *A. A. KARDAMAKIS, B. ROBERTSON, S. GRILLNER;
Karolinska Inst., Stockholm, Sweden

Abstract: Movements towards and away from visual stimuli in the environment are critical for survival. The optic tectum is engaged in selecting which stimuli to attend to, and to generate the required gaze shifts, but the underlying physiological mechanisms remain unclear. To address this, we developed an isolated eye-brain preparation, allowing whole-cell recordings from identified premotor cells in response to visual stimuli, using the lamprey, belonging to the oldest vertebrate group. We first show that the tectal visuomotor pathway is conserved throughout evolution, with parallel excitatory and inhibitory inputs, arising from the superficial layer, converging onto premotor cells in the deep layer mediating orienting or evasive movements. Retinal afferents from the local visual receptive field of a given tectal output cell, provide both monosynaptic excitation and disynaptic inhibition mediated via local GABAergic neurons, while stimulation outside the receptive field yields only inhibition. The local excitatory synaptic response becomes suppressed by concurrent visual stimuli outside the receptive field. This

suppression is mediated through retinal projections to other parts of the optic tectum and further relayed through inhibitory neurons. When inhibition is blocked, the suppression induced by competing stimuli is cancelled and the excitatory responses are enhanced. These excitatory responses are driven by retinal afferents monosynaptically, exposing the bottom-up nature of target selection implemented within the optic tectum. Stimulation of the lateral pallium generates eye and head motor responses and heavily sends projections to the deep layer of the optic tectum (Ocana et al., submitted). We, thus, explore the top-down role of pallial input (homologue to the neocortex in mammals), in modulating the response patterns of tectal output cells to direct retinal input. We used a whole-brain preparation with a sagittal section to expose the tectal layers to record evoked excitatory synaptic inputs with whole-cell recordings from tectal output cells while stimulating the lateral pallium. Taken together, this tectal mechanism, which relies on local excitation and global suppression via competitive inhibition, can also be modulated by pallial/cortical inputs and is most likely conserved throughout vertebrate phylogeny.

Disclosures: **A.A. Kardamakis:** None. **B. Robertson:** None. **S. Grillner:** None.

Poster

070. Visuomotor Coordination

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Program#/Poster#: 70.04/FF15

Topic: D.17. Voluntary Movements

Support: Grant-in-Aid for Young Scientists (B) (#25750267), Ministry of Education, Culture, Sports, Science and Technology (MEXT)

Title: Size of visual error does not affect learning of cognitive strategy under mirror-reversal transformation

Authors: *S. KASUGA[1], M. KURATA[1], M. LIU[2], J. USHIBA[1];
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Abstract: Humans are capable of adapting their movements to novel environments, thanks to the sophisticated and implicit error correction system. However, such system interferers with the adaptation when the visual information is mirror reversed (MR), because normal trial-by-trial error correction could further aggravate the movement error and make the motor control system unstable as even very small errors could be amplified with trials. This error-amplification

mechanism could make performing even a simple reaching task quite hard without alterations in the error correction rule by cognitive effort. However, it is unclear what triggers such alterations; is it dependent on the size of the visual error or some other signal(s)? To examine these possibilities, we manipulated the gain of visual errors using a virtual-reality display when participants made arm-reaching movements under the MR transformation. We compared the alteration in the error correction rules between the groups where the gain is normal and reduced. Fifteen participants were asked to make 900 trials (a 100 trials of baseline block and 800 trials of perturbation blocks) of straight and fast horizontal reaching movements to a straight-ahead target on the virtual reality display with KINARM exoskeleton (BKIN Technologies, Canada) over 2 days. The participants were randomly assigned to each of the following two groups; the normal-gain group (i.e., the cursor was displayed by simply flipping the signs of x-coordinate of the fingertip at the same scale) and the reduced-gain group (i.e., the cursor was displayed by flipping the signs of x-coordinate of the fingertip while reducing the amplitude in the visual x-coordinate by 20% from the fingertip). Our results showed that in both groups angular errors were accumulated under the MR transformation even though there was only a single target, but suddenly decreased after a few trials. This process was repeated for many times over the experiment. Counterintuitively, the accumulation of errors stopped at significantly smaller size of visual errors in the reduced-gain groups ($P < 0.001$). The number of trials where errors were accumulated was not different between the groups ($P = 0.26$). The finding suggests that the alteration in the error-correction rules from implicit adaptation to cognitive strategy is not dependent on the size of visual errors, but on other error-signals such as size of proprioceptive errors or the number of trials where errors were accumulated.

Disclosures: **S. Kasuga:** None. **M. Liu:** None. **M. Kurata:** None. **J. Ushiba:** None.

Poster

070. Visuomotor Coordination

Location: Halls A-C

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Program#/Poster#: 70.05/FF16

Topic: D.17. Voluntary Movements

Title: How visuomotor adaptation in one hand acts on kinesthetic control in the other

Authors: *F. A. KAGERER;
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Abstract: Previous research suggests a (non-dominant) left arm advantage for proprioceptive feedback utilization. This experiment determined the robustness of kinesthesia-motor internal representations of either the dominant or non-dominant hand against a visuo-motor perturbation of the contralateral hand. A bimanual center-out task was used to determine cross-modal interference between the two hands when one was exposed to a 60° visual feedback (fb) rotation (either abrupt, or gradual, increasing in steps of 10°), and the other had to rely on kinesthetic fb. 54 participants (21.2 (+-1.3) yrs, right-handed, 27 female) were assigned to 4 groups: right hand abrupt visual perturbation - left hand kinesthetic mode, lh abrupt visual perturbation - rh kinesthetic mode; rh gradual visual perturbation - lh kinesthetic mode, lh gradual visual perturbation - rh kinesthetic mode. Participants simultaneously moved two joysticks under a horizontal monitor displaying two home positions (17 cm apart), two targets per home position (either 90° or 270°, 7.5cm away), and the movement trace produced by the joystick (sampled at 75Hz). Both hands moved in the same direction, either 'up' or 'down'. After 12 trials each of visual baseline (both hands visible), and kinesthetic baseline (one hand without movement trace), the contralateral (visible) hand was exposed to 120 trials of visual fb rotation, followed by 48 trials of veridical visual fb. Variables of interest were initial directional error (IDE, calculated across the first 160ms of movement), representing the feedforward component, and movement time (MT). Results showed no IDE baseline differences between groups, or hands. At the end of exposure in the abrupt condition the 'visible' hands had adapted to similar levels in both groups (rh perturbed, or lh perturbed), as shown by the respective IDE values. In both groups, the kinesthetically controlled hand deviated from an originally straight movement vector in the same direction that the visually perturbed hand took. Both hands ended up with similar degrees of deviation; the left hand responded earlier in the exposure phase to the right hand perturbation, than the other way round. Similar results were found for the gradual condition. MTs in the abrupt condition were faster for the 'invisible' left hand when the right hand was perturbed, than for the right hand when the left hand was perturbed (1076.6 vs. 1462.9 ms), although MTs of the respective 'visible' hands were not significantly different. Results indicate minimal asymmetries between, and similar robustness of kinesthetic-motor representations of either hand when a visuo-motor perturbation is present in the contralateral hand.

Disclosures: F.A. Kagerer: None.

Poster

070. Visuomotor Coordination

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 70.06/FF17

Topic: D.17. Voluntary Movements

Title: Performing, but not learning, a reaching task with one arm while learning the same task with the other leads to complete transfer of visuomotor adaptation across the arms

Authors: *Y. LEI[1], J. WANG[2];
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Abstract: The extent to which motor learning is generalized across the limbs is typically very limited. In this study, we investigated how two motor learning hypotheses could explain the phenomenon of limited interlimb transfer. According to one hypothesis, we predicted that reinforcement of successful actions by providing binary error feedback regarding task success or failure during initial training would increase the extent of interlimb transfer following visuomotor adaptation (experiment 1). According to the other hypothesis, we predicted that performing a task repeatedly with one arm without providing performance feedback (which prevented learning the task with this arm), as the same task was concurrently learned with the other arm, would increase the extent of transfer (experiment 2). Thus, in experiment 1, our subjects adapted to a 30-degree CCW visuomotor rotation with the left arm first (training session), then again with the right arm (transfer session). During the training session, some subjects received vector error feedback that provided movement direction and amplitude information, while others received binary error feedback. In experiment 2, our subjects adapted to the same visuomotor rotation with the left arm, then again with the right arm. During the training session, however, some subjects performed reaching movements with the right arm toward a target location that they would reach to either prior to or following complete adaptation to the visuomotor condition (i.e., 0-deg. or 30-deg. CW target location, respectively). Results indicated that providing binary error feedback, as compared with vector error feedback, had no influence on the extent of transfer. In contrast, repeatedly performing (but not learning) a specific task with the right arm while the same task was learned with the left arm led to (nearly) complete transfer. This suggests that the absence of motor instances associated with specific effectors and task conditions is the major reason for limited interlimb transfer, and that reinforcement of successful actions during initial training is not beneficial for interlimb transfer. These findings indicate crucial contributions of effector- and task-specific motor instances to optimal motor learning and advance our understanding of the neural processes involved in motor learning, especially those involved in model-free learning.

Disclosures: **Y. Lei:** None. **J. Wang:** None.

Poster

070. Visuomotor Coordination

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Topic: D.17. Voluntary Movements

Title: Performing a reaching task with one arm passively while learning the same task with the other leads to substantial transfer of visuomotor adaptation across the arms

Authors: S. BAO[1], Y. LEI[1], *J. WANG[2];
[1]Univ. of Wisconsin - Milwaukee, Milwaukee, WI; [2]Dept Kinesiology, Univ. of Wisconsin, MILWAUKEE, WI

Abstract: The extent of interlimb transfer of motor learning is typically limited. We have demonstrated that performing, but not learning, a reaching task with one arm repeatedly while learning the same task with the other can lead to complete transfer of visuomotor adaptation across the arms. Based on the finding, we argued that limited interlimb transfer is primarily due to the absence of motor instances that are effector and task specific. In the present study, we examined whether performing a reaching task passively with one arm while learning the task with the other would also lead to complete transfer of visuomotor adaptation across the arms. We reasoned that if effector- and task-specific instances played a substantial role in increasing the extent of interlimb transfer, they should have similar effects whether the instances were accrued actively or passively during reaching movements. In this study, our subjects adapted to a rotated visual display during reaching movements with the left arm first (training session), then with the right arm (transfer session). During the training session, the subjects also performed reaching movements passively with the right arm for 10 trials following every 20 trials with the left arm. The passive movements were induced by a robot arm that moved the right arm in such a way that the instantaneous velocity of the moving arm either changed as that of an active movement would (i.e., bell-shaped velocity profile) or remained constant throughout the movement. Visual feedback was provided only for the left arm during the training, and for the right arm during the transfer session. Our results indicate that the extent of interlimb transfer was greater in both passive conditions, as compared with a condition in which the right arm did not perform reaching movements during the training session. This suggests that providing appropriate effector-specific instances during initial training can facilitate interlimb transfer of motor learning substantially regardless of whether the instances were produced during active or passive movements. We also observed that the extent of transfer was greater when the velocity of the passive movements changed, than when it did not, throughout the movement. This suggests that the similarity in movement velocity between the passive movements performed during the training session and the active movements performed during the transfer session also plays a role in shaping the effector-specific instances that are beneficial for generalization of motor learning.

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Poster

070. Visuomotor Coordination

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Topic: D.17. Voluntary Movements

Support: Neotia Foundation

Title: Visuomotor adaptation influences perceptual decision-making

Authors: N. KUMAR, J. A. MANJALY, *P. K. MUTHA;
Indian Inst. of Technol. Gandhinagar, Ahmedabad, India

Abstract: Accurately predicting the sensory consequences of movement commands using a "forward model" and combining them with actual feedback relayed via sensory systems is thought to yield better perceptual estimates of limb and environmental state than those possible with sensory feedback alone. However, sensory predictions are accurate only if the forward model accurately reflects the properties of the body, the environment and the relationship between the two. This accuracy is maintained via adaptation, in which the forward model is updated based on differences between the predicted and actual sensory consequences of action. Here we investigate how updating sensory predictions through motor adaptation influences perceptual processing and subsequent decisions based on the outcome of those perceptual processes. Twelve young, healthy subjects adapted to a 10 degree visuomotor rotation while using a stylus to trace a trajectory displayed on a computer screen in front of them. Subjects showed clear within- and between-trace adaptation such that the applied rotation was effectively canceled with training. Subsequently, subjects were asked to report the color of a target that moved on the screen among several distractors while they also moved their hand, which was not directly visible. Target motion was either random, matched to that of the hand, or along a path that was rotated by 10 degrees relative to hand motion. Surprisingly, subjects were much more accurate in reporting target color if it moved along the rotated path compared to veridical or random motion. When between-trace adaptation was prevented by means of a secondary cognitive task, subjects showed greater accuracy in reporting the color of a target moving along the rotated path for a few initial trials, reflecting learning that occurred within the last trace of the adaptation block. However, with time subjects shifted towards more accurately reporting the color of the target moving along the actual hand path. These results demonstrate the strong

influence of adaptation and updating of predictive mechanisms that adaptation entails on perception and decisions based on perceptual processing. Perceptual decisions can be clearly modified by manipulating the relationship between movement commands and their sensory consequences, and the degree of modification may depend on how strongly the adapted state is reinforced.

Disclosures: **N. Kumar:** None. **P.K. Mutha:** None. **J.A. Manjaly:** None.

Poster

070. Visuomotor Coordination

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Topic: D.17. Voluntary Movements

Title: Specificity of visuomotor adaption to movement dynamics

Authors: *M. J. CROSSLEY[1], J. L. FAN[2], R. B. IVRY[2];
[1]UCB, Psychology, [2]UC Berkeley, Berkeley, CA

Abstract: Recent work suggests that the encoding of motor memories is context-dependent, incorporating information about recently past sensorimotor states (e.g., hand position and velocity). Participants can learn to compensate for opposing clockwise (CW) and counterclockwise (CCW) force fields when reaching to a fixed target if the reaches start at unique positions (Howard et al., J Neurophysiology 2012), even though the perturbation was limited to a part of the trajectory that was common to both movements. However, participants failed to compensate when the context was cued statically (e.g., with visual landmarks), or when there was a delay prior to the perturbed phase of the movement. We extend these results in two key ways. First, we show that a similar pattern holds for visuomotor adaptation. Participants made two-stage reaching movements, moving from a start position to a central location and then on to a target. The direction (CW or CCW) of a 20 deg rotation was defined by the particular start-target pair, with the rotation only applied to the second, center-out component. Adaptation was strong when the two components were executed as an integrated smooth movement. In contrast, adaptation was weak when participants paused at the center for 1 s. Second, learning with dynamic cues exhibited marked retention across test blocks. Here we used a design composed of four phases: baseline (80 movements with no rotation), acquisition (80 movements with a CW rotation), washout (160 movements with no rotation), and reacquisition (80 movements with a CW rotation). A set of start-target pairs with one direction of rotation was

used in the acquisition and reacquisition blocks. A different set of start locations, paired with the same targets was used in the washout with no rotation. Changing the dynamic cues in the washout block prevent the expression of an aftereffect. However, when the initial context was reintroduced in the reacquisition block, adaptation returned immediately to a level approximately equal to that observed at the end of initial acquisition, and continued at similar rate. These findings underscore the influence of dynamic cues in both the encoding and retention of sensorimotor memories.

Disclosures: **M.J. Crossley:** None. **J.L. Fan:** None. **R.B. Ivry:** None.

Poster

070. Visuomotor Coordination

Location: Halls A-C

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Program#/Poster#: 70.10/FF21

Topic: D.17. Voluntary Movements

Title: The effects of spacing practice and perturbation session on the motor memory consolidation during the acquisition of visuomotor adaptation task

Authors: *Y.-G. SONG[1], J.-H. PARK[2];
[1]Physical Education, Korea Univ., Korea Univ., Seoul, Korea, Republic of; [2]Korea UNIV., Seoul, Korea, Republic of

Abstract: The memory consolidation hypothesis maintains that the processing of a memory continues long after the completion of a practice repetition. The present study was designed to identify the factors that influence the effectiveness of the consolidation processes, leading to a relatively stable and long-term motor learning in the production of a visuomotor adaptation that required 30° rotation (i.e., clockwise). Four groups of participants (n=36) practiced the task conditions on each group as follows: (i) 1 day group (AAA), (ii) 3 day group (AAA), (iii) 1 day group (ABA) and (iv) 3 day group (ABA). Specifically, 1 day group (AAA) and 3 day group (AAA) performed in 3 sessions of 6 blocks each, and the practice sessions were separated by 10 minutes or 24 hours (1 day). 1 day (ABA) and 3 day (ABA) also practiced the same practice sessions as the above groups, then additional perturbation condition (-30° rotation; counter-clockwise) in 1 sessions of 1 block each, and the practice sessions were separated by 10 minutes or 24 hours (1 day). The distance error (movement accuracy) and velocity (movement time) were calculated using the Motion Tracking System. The results demonstrated that as the amount of the practice increased, distance error and velocity were improved. However, no significant

difference was found among these groups, but no 1 day (AAA) and 3 day (AAA) perturbation groups were improved compared with 1 day (ABA) and 3 day (ABA) perturbation groups. The 3 day group (AAA) also showed accuracy and velocity during the retention test than remaining 3 groups, significant differences were indicated among these groups. These findings suggest that practicing additional tasks after the regular training sessions could perturb memory consolidation processes for visuomotor adaptation learning. In addition, longer space between practice sessions may facilitate production of fast pointing movements during visuomotor rotation tasks. Taken together, consistent with the notion of memory consolidation, these findings suggest that distribution of practice over a relatively long period of time (day), rather than perturbation (i.e., interference), is a more important factor for the enhancement of learning in the production of a visuomotor task.

Disclosures: **Y. Song:** None. **J. Park:** None.

Poster

070. Visuomotor Coordination

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Topic: D.17. Voluntary Movements

Support: KO1HD059983

RO1NS085122

RO1HD58301

Title: Motor behavior and associated primary motor cortex excitability differ during relearning of visuomotor gain when unlearning occurs either via washout or a period of inactivity

Authors: ***M. YAROSSI**[1,3,2], S. ADAMOVICH[3,2], J. W. KRAKAUER[4], E. TUNIK[2,1]; [1]Grad. Sch. of Biomed. Sci., [2]Rehabil. and Movement Sci., Rutgers Biomed. Hlth. Sci., Newark, NJ; [3]Biomed. Engin., New Jersey Inst. of Technol., Newark, NJ; [4]Dept. of Neurol., The Johns Hopkins Hosp., Baltimore, MD

Abstract: Recently, savings has been posited to relate to memory of actions rather than of cerebellar-dependent internal models [Huang 2011]. In addition, retention and savings of adapted reaching behavior to a visuomotor rotation has been shown to depend on the form of the unlearning phase interposed between learning and re-learning [Kitago 2013]. We investigated the

possible role of the primary motor cortex (M1) in the learning, unlearning, and relearning of a visuomotor gain by tracking its excitability throughout these phases of adaptation. Healthy right-handed subjects (N=23) participated after providing informed consent. Seated with hands under a display, data glove equipped subjects were given visual feedback of a virtual hand and instructed to make ballistic target directed right index finger flexion movements. Subjects completed 2 baseline blocks (40 trials), 4 initial learning blocks (30 trials), 5 unlearning blocks (30 trials), and 4 re-learning blocks (30 trials). Discordant visual feedback was provided by applying a 0.5 (low gain) scaling factor to the glove data during learning and relearning blocks. Subject groups experienced one of two types of unlearning: either reversion to veridical gain (Washout), or a period of inactivity (Time). In a third condition (Control), extent was manipulated through changes in target angle, i.e., a new mapping was not required. M1 excitability was assessed using TMS-evoked resting motor evoked potentials (MEPs) recorded from the first dorsal interosseous in a 1-minute period following each block. In the Washout group, we observed canonical adaptation, deadaptation (with aftereffects) and re-adaptation (with savings) behavior. The Time group displayed similar initial learning but little unlearning such that relearning started at a high residual gain level. Patterns of M1 excitability were similar for Washout and Time during baseline, learning and unlearning. During relearning, however, despite equal asymptotic performance, MEPs were significantly larger in the Washout group (Students t-test, $p<.05$), suggesting increased excitability was related to savings rather than residual adaptation. Control subjects increased peak velocity to meet the demand of larger target angles in the learning and re-learning blocks with nominal change in excitability. This dissociation between excitability and performance implies that M1 excitability assays learning rather than motor performance. We suggest that for visuomotor gain adaptation M1 excitability relates to both active selection and retrieval of actions when target errors are present and that these processes are distinct from forward-model updating [Jordan 2014].

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Poster

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Topic: D.17. Voluntary Movements

Support: 863 Program, 2012AA011602

Title: Savings as an indicator of motor generalization for visuomotor learning

Authors: *C. YIN, K. WEI;
Peking Univ., Beijing, China

Abstract: For studies of motor adaptation, people typically adapt to a certain novel perturbation and their learning and generalization are then assessed by unexpectedly removing that perturbation. The performance during these immediate catch trials was termed after-effect; it is universally used for quantifying directional generalization for visuomotor learning such as visuomotor rotation or gain. However, after-effect decays rapidly thus it has to be tested immediately after learning. Researchers even resort to use refresher trials (original training trials) to maintain the "learning" during the measurement of generalization. Ironically, despite of the importance of refresh trials, its frequency varies across those generalization studies, leaving it hard to compare generalization results across studies. As compared to fragile aftereffect, saving effect, defined as enhanced learning rate at the time of recall, is a robust indicator of learning with much slower decay. Using savings to quantify directional generalization has never been tested. In this study, we trained subjects with visuomotor rotation and evaluated their directional generalization with both savings and after-effects, either immediately after training or 24 hours later. When tested immediately after learning, the generalization indexed by savings was unimodally shaped, similar to but still broader than the aftereffect-based generalization. Interestingly, this unimodal generalization was largely unchanged 24 hours later when the aftereffects disappeared almost completely. Generalization curve has been related to neuronal representations of motor learning and generalization for long. Its exact shape should be reproducible to enable us to make any concrete inference about the neural substrate of motor learning. The aftereffect, given its sensitivity to decay as suggested by our findings, might not be a good candidate for assessing generalization. On the other hand, saving is relatively stable against decay and it does not require refresh trials to maintain the fragile visuomotor learning. Furthermore, savings has also been related to retention, a hallmark of successful motor learning. We thus propose to use saving, as an alternative to aftereffect, for studying motor generalization specifically and for investigating motor learning in general.

Disclosures: **C. Yin:** None. **K. Wei:** None.

Poster

070. Visuomotor Coordination

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Program#/Poster#: 70.13/FF24

Topic: D.05. Visual Sensory-motor Processing

Title: Motor and visual directional tuning after visuomotor adaptation

Authors: ***S. HAAR**[1], O. DONCHIN[2], I. DINSTEIN[1,3];
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Abstract: What happens to directional tuning when reaching movements are dissociated from their visual feedback by rotating the visual field? Do neural populations in motor system areas remain tuned to the reaching direction or shift their tuning according to the visual rotation? We recorded simultaneous movement kinematics and fMRI activity while subjects performed 'out and back' reaching movements to four targets spaced 45° apart. The experiment included three conditions: 1) Baseline - visual and motor mapping were matched. 2) Rotated - the cursor movement was rotated by 45° with respect to the hand movement. 3) Washout - the visual and motor mappings were matched again. A multivariate classification algorithm was trained to identify movement direction according to voxel-by-voxel fMRI patterns in each of several brain areas. The classifier was trained using a subset of trials and then tested by decoding excluded trials. The direction of movements was successfully decoded with above-chance accuracy rates in multiple motor and visual areas when training and testing the classifier on trials within each condition (i.e. within-condition decoding). However, when training the classifier on baseline trials and decoding rotated trials, motor brain areas including primary motor cortex (M1), dorsal premotor cortex (PMd), and supplementary motor area (SMA) exhibited above chance decoding for the original movement direction (as in the baseline condition) while visual brain areas including early visual cortex and superior parieto-occipital cortex (SPOC) exhibited above chance decoding for the rotated visual target location rather than the actual movement direction. Most interestingly, decoding levels in the anterior intraparietal sulcus (aIPS), an intermediate visuomotor area, were at chance level for both visual and motor aspects, suggesting that directional tuning in this area was altered by the visuomotor adaptation. Additional analysis revealed mixed tuning to both original motor targets and rotated visual targets. We interpret these results to suggest that in humans, like monkeys, directional tuning of low level motor areas is not affected by visuomotor adaptation, but directional tuning in higher parietal areas such as aIPS is altered.

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Poster

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Topic: D.17. Voluntary Movements

Support: NATIONAL AGENCY FOR SCIENCE AND TECHNOLOGY PROMOTION

Title: Time course of motor memory consolidation in visuomotor adaptation

Authors: *P. CAFFARO[1], J. VILLALTA[2], V. DELLA-MAGGIORE[2];
[1]Dept. of Physiology, Sch. of Medicine, Univ., Argentina; [2]Dept. of Physiology, Sch. of Medicine, Univ., CABA, Buenos Aires, Argentina

Abstract: Visuomotor adaptation is the process by which our motor system adjusts movements to a novel environment. Although abundant evidence points to the formation of long-term motor memories for this type of learning, several previous studies have failed at characterizing the time course of memory consolidation using behavioral protocols of retrograde interference (e.g. Caithness et. al., 2004). This may in part be due to the fact that anterograde effects are very strong, often masking retrograde effects. Here, we took advantage of this phenomenon to explore the time course of motor memory consolidation. Subjects adapted to two opposite visual rotations of 30 degrees following a protocol of anterograde interference. They performed 6 blocks of pointing movements to 8 visual targets using a joystick (1 cycle = 8 targets; 11 cycles per block). Participants were divided in 6 groups, which experienced a clockwise rotation (A), followed, after a variable interval of 1min, 15min, 1h, 3h, 5,5h and 24 h, by a counterclockwise rotation (B). The rate of learning for A and B was computed by fitting individual visuomotor error for all cycles using a single exponential function with a constant (Zarahn et. al., 2008). We assumed that a reduction in the rate of learning of B would reflect the consolidation of the memory for A. In contrast to our own predictions and to previous studies on sensorimotor adaptation (Shadmehr et. al., 1997; Krakauer et. al., 2005), our preliminary results point to a non-monotonic pattern of anterograde interference. Reference: - Caithness et. al., 2004 J Neurosci. 2004 Oct 6;24(40):8662-71. - Zarahn et. al., 2008 J Neurophysiol. 2008 Nov;100(5):2537-48. - Shadmehr et. al., 1997 J Neurosci. 1997 Jan 1;17(1):409-19. - Krakauer et. al., 2005 J Neurosci. 2005 Jan 12;25(2):473-8.

Disclosures: **P. Caffaro:** None. **J. Villalta:** None. **V. Della-Maggiore:** None.

Poster

070. Visuomotor Coordination

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 70.15/FF26

Topic: D.17. Voluntary Movements

Support: NATIONAL AGENCY FOR SCIENCE AND TECHNOLOGY PROMOTION

Title: Training in native coordinates interferes anterogradely with adaptation to rotated visual feedback

Authors: *J. VILLALTA, A. STOLKINER, E. VIGNETTA, V. DELLA-MAGGIORE; Dept. of Physiology, Buenos Aires Univ., Ciudad Autonoma De Buenos Aires, Argentina

Abstract: Motor adaptation is a type of motor learning that allows maintaining accurate movements in the presence of environmental or internal perturbations by creating new sensorimotor maps. A typical experiment involves a session of familiarization during which subjects move to a visual target in native -unperturbed- sensorimotor coordinates (null trials), followed by an adaptation session to a visual perturbation. Often, null trials are used in savings protocols to equate the initial level of error across adaptation and re-adaptation sessions (to "wash out" aftereffects)[1]. Recently we have shown that a relatively small number of null trials (2 blocks of 88 trials) presented just before the test session can interfere with memory retrieval, thereby reducing the amount of savings [2]. Our results suggest that practice in unperturbed conditions is not innocuous but may lead to the formation of a new visuomotor memory. Here, we addressed this hypothesis by investigating if, like perturbations, null trials can directly interfere with new learning. To test this hypothesis we trained two groups of naïve subjects on a visuomotor adapation task to a -40º optical rotation (6 blocks of 88 trials). The experimental and control groups were preceded by 7 and 1 blocks of null-trials, respectively. We found a decrease in the amount of memory retention measured 1 min after adaptation in the Experimental relative to the Control group ($p=0.04$) . Our results support the idea that null trials form a memory that competes with those formed during adaptation to rotated visual feedback. [1] Krakauer JW et al. J Nerosci. 2005 Jan 12;25(2):473-8 [2] Villalta JI et al., Cereb Cortex. 2013 Dec 19. [Epub ahead of print]

Disclosures: **J. Villalta:** None. **A. Stolkiner:** None. **E. Vignetta:** None. **V. Della-Maggiore:** None.

Poster

070. Visuomotor Coordination

Location: Halls A-C

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Topic: D.17. Voluntary Movements

Title: In experts, contribution of explicit and implicit processes to visuomotor adaptation is different than in novices

Authors: *C. LEUKEL[1,2], W. TAUBE[2], A. GOLLHOFER[1];
[1]Univ. of Freiburg, Freiburg, Germany; [2]Univ. of Fribourg, Fribourg, Switzerland

Abstract: Motor learning may be based on explicit and implicit processes. Explicit processes are changes in motor performance caused by conscious decisions that require declarative knowledge about the task. In contrast, implicit processes are behavioral changes that are subconsciously driven. Recent studies argue that there exist at least two forms of implicit processes that differ with respect to the rate of learning and the retention of the acquired movement(s) (Smith et al., 2006). In the present study, we investigated contribution of explicit and implicit processes to visuomotor adaptation in subjects with different levels of expertise. Experienced handball players (N = 30) and novices (N = 30) were compared when performing standardized free throws. We used prismatic glasses to induce a visuomotor adaptation and performed three different experiments (subjects were equally assigned to the protocols): in experiment 1, we recorded the aiming strategy of the subjects that informs about contribution of explicit processes. In experiment 2, explicit processes to adaptation were blocked by verbal instructions about the throwing procedure, thus we assessed how adaptations were performed with solely implicit processes. In experiment 3, retention of the adapted throwing movement was tested while contribution of explicit processes was blocked like in experiment 2. The results of experiment 1 indicate that contribution of explicit processes was significantly larger in experts than in novices. Results of experiment 2 showed that experts adapted significantly slower than novices when contribution of explicated processes was blocked. Finally, results of experiment 3 indicate that experts retained the adapted movement longer (i.e. slower forgetting) than novices. These results have important implications. First, they suggest that the standard models of skill acquisition (e.g. Fitts and Posner, 1967), that propose a reduction of explicit and enhancement of implicit processes with increased levels of expertise, may require further critical considerations. Second, the results of experiment 2 and 3 suggest that experts use different implicit processes than novices. Adaptation took longer and retention was better in experts. Thus, one may speculate that learning in experts is optimized in a sense that acquired movements can be retained longer than in novices. A slower rate of learning by these implicit processes would be counteracted by a higher contribution of explicit processes in experts. Fitts PM, Posner MI (1967) Belmont: Brooks/Cole. Smith MA, Ghazizadeh A, Shadmehr R (2006) PLoS Biol 4:e179.

Disclosures: **C. Leukel:** None. **W. Taube:** None. **A. Gollhofer:** None.

Poster

070. Visuomotor Coordination

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Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 70.17/FF28

Topic: D.17. Voluntary Movements

Support: James S. McDonnell (USA)

National Agency for the Promotion of Science and Technology (Argentina)

Title: Time course of changes in functional connectivity induced by visuomotor adaptation: A 24 h resting-state fMRI study

Authors: *V. M. DELLA MAGGIORE[1], J. I. VILLALTA[1], N. KOVACEVIC[2], A. R. MCINTOSH[2];
[1]Dept. of Physiol., Univ. of Buenos Aires, Buenos Aires, Argentina; [2]Univ. of Toronto, Rotman Res. Inst., Toronto, ON, Canada

Abstract: Motor adaptation is a type of motor learning that allows maintaining accurate movements in the presence of environmental or internal sensory perturbations by adjusting motor output. The neural substrates of motor adaptation have been extensively explored in human and non-human primates during acquisition. Yet, when interested in identifying plastic changes associated with learning, the "online" approach is limited by kinematics and dynamics confounds that are very difficult to control for. In this study, we used resting-state fMRI, an offline experimental approach, to characterize the time course of changes in functional connectivity triggered by visuomotor adaptation, throughout a 24 h period. Twenty two normal subjects performed a visuomotor task that required hitting 8 visual targets using a joystick. Experimental subjects (n = 11) did so under a 40 degree clockwise rotation, whereas no perturbation was applied to controls (n = 11). A total of six resting-state runs were acquired following a standard resting-state protocol. One of them was acquired before adaptation (baseline), and the remaining five runs 15min, 1h, 3h, 5.5h and 24 h after adaptation. After standard fMRI preprocessing, data was denoised using ICA and bandpass filtered between 0.08 and 0.009Hz. Twenty-two regions of interest (ROIs) of 5mm ratio were chosen based on previous functional studies carried out during visuomotor learning. Time series were extracted for these ROIs and correlated with each other for each run and each subject. A multivariate statistical approach (PLS, Mcintosh and Bookstein, 1996) was then used to identify a brain pattern that distinguished experimental and control groups based on the time course of functional connectivity. The analysis revealed that the connectivity of the right posterior cerebellum (lobule VIII), left putamen, right inferior frontal

gyrus and left ventral premotor cortex increased as a function of time for the control group. In contrast, the connectivity between the posterior parietal cortex and i) the left primary motor cortex, ii) the left dorsal premotor cortex and iii) the left somatosensory cortex increased as a function of time for the experimental group. The level of connectivity between these regions peaked 5.5 h after adaptation and decreased thereafter. We hypothesize that the connections strengthened during visuomotor adaptation underlie motor memory consolidation associated with this type of learning. References: Mcintosh and Bookstein (1996). Neuroimage;3 (3 Pt 1):143-57

Disclosures: **V.M. Della Maggiore:** None. **J.I. Villalta:** None. **N. Kovacevic:** None. **A.R. McIntosh:** None.

Poster

070. Visuomotor Coordination

Location: Halls A-C

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Program#/Poster#: 70.18/FF29

Topic: D.05. Visual Sensory-motor Processing

Support: Fondazione del Monte di Bologna e Ravenna

MIUR

EYESHOTS

Monash Vision Group

ARC-Special Research Initiative "Bionic Eye"

Title: From visuomotor to motor activity in the caudal part of the macaque superior parietal lobule: A comparison study between areas PEc and V6A

Authors: *G. DAL BO[1], K. HADJIDIMITRAKIS[1,2], R. BREVEGLIERI[1], C. GALLETTI[1], P. FATTORI[1];
[1]Dip. Farmacia e Biotecnologie, Univ. of Bologna, Bologna, Italy; [2]Dept. Physiol., Monash Univ., Clayton, Victoria 3800, Australia

Abstract: In the posterior parietal cortex, there is a constellation of areas collectively involved in the visuomotor transformations necessary for controlling goal-directed actions. Two neighboring areas of the caudal part of the superior parietal lobe, V6A and PEc, show different cytoarchitecture and connectivity profiles, but have neurons with similar functional properties that are involved in the control of reaches. While we have recently demonstrated that during reaches in 3D space both depth and direction information are represented in V6A (Hadjidimitrakis et al., 2013), the encoding of reach depth has never been investigated in PEc. The aim of this work was to investigate the effect of both target direction and depth on the activity of PEc neurons, and to compare the direction and depth tuning between PEc and V6A in several phases of the reaching task performed in the 3D space. Single unit activity was recorded from three Macaca fascicularis monkeys performing foveal arm reaching towards visual targets placed at eye level at different depths and directions in the peripersonal space. Reaching actions were perfomed in a dark environment. We found that PEc neurons were affected by direction and depth, in some cases by both of them, but these modulations were not equally distributed over

the several phases of the reaching task. In fact, the effect of direction was more common than depth when the monkey was fixating the target without performing any arm movement (28% vs. 14%). The opposite happened after the onset of arm movement, when depth (30%) and jointly depth and direction (27.5%) signals became predominant. In V6A, in contrast, direction and depth information were jointly encoded in the majority of cells during both target fixation and movement execution. It is worthwhile to note that, while both PEc and V6A cells processed depth information during the arm movement, in V6A, but much less in PEc, this type of information started to be encoded at fixation onset, that is well before the onset of arm movement. Altogether, these findings support the idea that while both posterior parietal areas are involved in the visuomotor transformations for reaches in 3D space, there is a posterior-to-anterior gradient from a more visuomotor representation in V6A, to a predominantly motor encoding of the reaching action in PEc.

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Poster

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Program#/Poster#: 70.19/FF30

Topic: D.17. Voluntary Movements

Support: NIH R01 AG031769

Title: Low-gain visual feedback improves reaction time in older adults

Authors: ***M. KWON**[1], Y.-T. CHEN[1], A. GARNER[1], D. W. SOLIS[1], F. RACKARD[1], V. J. PEDIGO[1], B. DANCOSE-GIAMBATTISTO[1], C. R. SUE-WAH-SING[2], E. A. CHRISTOU[1,3]; [1]Applied Physiol. and Kinesiology, [2]Hlth. Sci., [3]Physical Therapy, Univ. of Florida, Gainesville, FL

Abstract: Reaction time slows significantly with aging likely due to deficiencies in visual information processing. The purpose of this study was to determine whether the age-related slowing in reaction time was associated with deficiencies in visual information processing. Nine young (20.4 ± 1.1 years) and five older adults (71.8 ± 5.6 years) participated. Participants were asked to match a constant force equal to 15% maximum with ankle dorsiflexion for 20 s and dorsiflex their ankle as fast as possible in response to a visual stimulus. We manipulated visual

information processing by changing the visual gain of force feedback prior to the visual stimulus. Specifically, in a counterbalanced order, subjects performed the constant force task either with low-gain visual feedback (LG, 0.05°; lower visual information processing required) or with high-gain visual feedback (HG, 1.2°; higher visual information processing required). Reaction time was quantified as the interval between the onset of the stimulus and the onset of ankle dorsiflexion force. Reaction time was slower in older adults (607.5 ± 58 ms) than young adults (477.6 ± 16.8 ms). Interestingly, high gain visual feedback exacerbated the age-associated differences in reaction time. Young adults exhibited similar reaction time with low- and high-gain visual feedback (LG: 476.1 ± 17.9 ms; HG: 479.2 ± 16.7 ms). In contrast, the reaction time of older adults was slower with the high-gain visual feedback condition (LG: 590.3 ± 63.0 ms; HG: 624.8 ± 55.4 ms). In summary, these results suggest that reaction time in older adults can be influenced by the gain of visual feedback. Consequently, training paradigms to reduce reaction time in older adults may benefit from lowering the gain of visual feedback.

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Poster

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Topic: D.17. Voluntary Movements

Support: NIH Grant P20 GM103645

Veterans Administration N9228C

Title: Brain activity during gradual visuomotor adaptation

Authors: P. BÉDARD, *J. N. SANES;
Neurosci., Brown Univ., PROVIDENCE, RI

Abstract: Interactions in a constantly changing environment require that the brain forms new sensory-motor relationships. While prior work has extensively assessed behavioral and brain concomitants of sudden adaptations, much less is known about gradual adaptation. Prior work suggested that cerebellar processing is more important for sudden than for gradual adaptation

(Criscimagna-Hemming et al. 2010). We used functional MRI to measure whole brain activity as healthy adults reached quickly to visual targets using a cursor to guide movements. In a null condition, the cursor had "normal' hand-cursor mapping, while for a rotation condition, we artificially rotated the cursor by 0.25º counterclockwise each trial, to reach 30° rotation at the end of the learning condition. Participants performed 6 sequential conditions: null (80 trials), resting state (8 min), learning (160 rotation trials), washout (80 null trials), resting state (8 min), and recall (80 rotation trials, 30° rotation). We used a random-effect model to assess brain activation during learning and recall, not yet analyzing the resting state data. Reaching error remained low during application of the gradual rotation, but progressively increased across learning to ~5º when the final 30° rotation occurred. Participants exhibited after-effects, suggesting they had formed a new memory. No participant expressed awareness of the gradual perturbation. At recall, the amount of savings had similarity to a separate group of participants who experienced a sudden visual perturbation. When participants gradually adapted to the visual rotation, we found more activation in various cerebellar areas bilaterally, thalamus, left putamen, anterior cingulate cortex, left primary motor cortex, left somatosensory cortex, and left superior parietal cortex. At recall, we found a wide activation pattern that had similarity to that observed during learning, including activation in cerebellum, left primary motor cortex, left somatosensory cortex, and left parietal cortex. The results of Criscimagna-Hemminger et al. (2010) may predict reduced cerebellar activation during gradual adaptation. However, we found robust cerebellar activation during gradual adaptation and recall, more than we would have expected from earlier results with sudden adaptations (e.g., Bédard and Sanes 2014). The strong cerebellar activation during the learning, and also during recall may relate to the size of error which was relatively high compared to the null phase. References: Bédard P, Sanes JN (2014) Neuroimage in press; Criscimagna-Hemminger SE, Bastian AJ, Shadmehr R (2010) J Neurophysiol 103:2275-84.

Disclosures: **P. Bédard:** None. **J.N. Sanes:** None.

Poster

070. Visuomotor Coordination

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

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Topic: D.05. Visual Sensory-motor Processing

Support: Mitsui Sumitomo Insurance Welfare Foundation

Title: Age-related changes in the contribution of reflexive and voluntary corrections of spatial errors in target reaching

Authors: *D. KIMURA, K. KADOTA, Y. HIRAMATSU, H. KINOSHITA;
Grad. Sch. of Med., Osaka Univ., Toyonaka, Japan

Abstract: When reaching a visual target, a sudden shift of the target position elicits a fast reflexive motor-tracking response, so called a "target jump response (TJR)", providing automatic correction of motor errors towards new target position. The effects of aging on the spatial component of this response remain unknown. In the present study, aging-related changes in the spatial accuracy of TJR, and relationship between the TJR directional error and reaching variability were investigated using aged and young individuals. The participants were 15 elderly [mean ± standard deviation (SD) age, 70 ± 5 years] and 16 young (25 ± 5 years) individuals. All were healthy without any neurological or orthopedic problems. The right hand was used to reach and touch a visual target presented in the center of a screen located 0.5 m in front of the participant. After the initiation of reach motion, the target presented in the center either shifted (5° right, left, above, or below the screen center,36 trials for each location) or remained at the same spot (48 trials). In the target-shift trial, the participants were required to correct their reaching trajectory towards a new target location as quickly as possible. Hand kinematics during reaching were monitored using a three-dimensional motion capture system (sampling frequency = 500 Hz), and its velocity was computed subsequently. The directional error of TJR was obtained by the angle between the direction of target-shift and the vector of hand velocity computed at 80 ms before the onset of voluntary correction movement. Repeated measures ANOVA (group × target location) revealed that none of the main and interaction effects on the median values of the directional error was significant, indicating that aging did not affect the reflexive visuomotor transformations for TJR. On the other hand, ANOVA on directional error's SD, a measure of inter-trial variability of the arm trajectory just before touching the target, revealed a significant group effect. Aging thus reduced trial-to-trial consistency in voluntary control of the visuomotor transformations. Furthermore, the directional error's SD for TJR was correlated significantly with the arm trajectory' SD for the young participants, but not for the elderly participants. The findings suggest that TJR plays a role in decreasing variability of limb trajectory for reaching. Aging seems to affect this function.

Disclosures: **D. Kimura:** None. **K. Kadota:** None. **Y. Hiramatsu:** None. **H. Kinoshita:** None.

Poster

070. Visuomotor Coordination

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 70.22/GG1

Topic: D.05. Visual Sensory-motor Processing

Support: HHMI

Title: Linking vision and action in *Drosophila*

Authors: *J. D. SEELIG, R. FRANCONVILLE, V. JAYARAMAN;
Janelia Farm Res. Campus, HHMI, ASHBURN, VA

Abstract: Studies from many labs and in different insects have implicated a central brain region called the central complex in a broad range of visuomotor functions including sun-compass navigation [1], short-term memory for orientation [2], long-term memory for visual patterns [3], visual place learning [4], and locomotion [5]. In *Drosophila*, behavioral genetics experiments have suggested the involvement of specific central complex cell types in such functions [2-4], but little is known about their physiology [6]. Over the past few years, we have developed techniques to monitor neural activity using two-photon calcium imaging in head-fixed behaving flies in a virtual reality arena. We record from genetically identified neural populations during tethered flight and tethered walking on an air-supported ball. Recently, we identified a major source of visual feature input to a substructure of the central complex, in the form of orientation-tuned responses [6]. We will discuss our results from experiments in which we continue to probe the central complex's role in visuomotor integration in flies. [1] Heinze, S., Homberg, U. (2007). Maplike representation of celestial E-vector orientations in the brain of an insect. Science, 315(5814). [2] Neuser, K., Triphan, T., Mronz, M., Poeck, B., & Strauss, R. (2008). Analysis of a spatial orientation memory in *Drosophila*. Nature, 453(7199). [3] Liu G., Seiler H., Wen A., Zars T., Ito K., Wolf R., Heisenberg M., & Liu L. (2006). Distinct memory traces for two visual features in the *Drosophila* brain. Nature, 439(7076). [4] Ofstad T.A., Zuker, C.S., & Reiser M.B. (2011). Visual place learning in *Drosophila* melanogaster. Nature, 474(7350). [5] Guo, P. and Ritzmann, R. E. (2013). Neural activity in the central complex of the cockroach brain is linked to turning behaviors. J. Exp. Biol., 216(Pt 6). [6] Seelig, J.D., Jayaraman, V. (2013). Feature detection and orientation tuning in the *Drosophila* central complex. Nature, 503(7475).

Disclosures: **J.D. Seelig:** None. **R. Franconville:** None. **V. Jayaraman:** None.

Poster

070. Visuomotor Coordination

Location: Halls A-C

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Program#/Poster#: 70.23/GG2

Topic: D.05. Visual Sensory-motor Processing

Support: HHMI

Title: Direct observation of ON and OFF pathways in the *Drosophila* visual system

Authors: *J. STROTHER, A. NERN, M. REISER;
Janelia Farm Res. Campus, Ashburn, VA

Abstract: Visual motion perception is critical to many animal behaviors, and flies have emerged as a powerful model system for exploring this fundamental neural computation. Although numerous behavioral studies have suggested that fly motion vision is governed by a relatively simple neural circuit, the implementation of this circuit has remained mysterious for decades. Several recent studies have shown that key neurons associated with this circuit are selective for light increments (ON) or decrements (OFF), but the origin and role of this selectivity in motion vision remains unclear. We examined activity in the neuropil responsible for visual motion detection, the medulla, of *Drosophila* melanogaster in response to a range of visual stimuli using two-photon calcium imaging. We confirmed that the input neurons of the medulla, the LMCs, are not responsible for light-on and light-off selectivity. We then examined the pan-neural response of medulla neurons and found prominent selectivity for light-on and light-off in layers of the medulla associated with two anatomically defined pathways (L1/L2 associated). We next examined the activity of prominent interneurons within each pathway (Mi1 and Tm1) and found that these neurons have corresponding selectivity for light-on or light-off. These results provide direct evidence that motion is computed in parallel light-on and light-off pathways, demonstrate that this selectivity emerges in neurons immediately downstream of the LMCs, and specify where crucial elements of motion computation occur.

Disclosures: **J. Strother:** None. **A. Nern:** None. **M. Reiser:** None.

Poster

070. Visuomotor Coordination

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 70.24/GG3

Topic: D.05. Visual Sensory-motor Processing

Title: Parallel descending pathways for visually-evoked escape in *Drosophila*

Authors: *M. PEEK, S. NAMIKI, G. M. CARD;
HHMI Janelia Farm, Ashburn, VA

Abstract: To understand how nervous systems accomplish fast visual-motor control, we investigated the neural basis of visually-evoked escape behavior in the fruit fly, *Drosophila* melanogaster. Previously we demonstrated that identical looming stimuli evoke two different modes of escape. The fast, unsteady mode requires a pair of descending interneurons, the giant fibers, whereas the neural substrates for the slower, steady mode remain unknown. To determine how slower escapes are controlled, we performed an anatomical search of the Janelia GAL4 expression pattern database for descending neurons that connect the same visual and motor centers linked by the giant fibers. These neurons could form information pathways parallel to the giant fibers to control slow escapes. We found six uniquely identifiable pairs of descending neurons that putatively receive the same visual input as the giant fibers. To assess their visual responses, we are performing somatic whole-cell patch clamp experiments while displaying sets of visual primitives. For one pair, loom-evoked excitatory responses largely overlap with the giant fibers. This pair could provide a parallel pathway for visual information to be conveyed to takeoff motor centers. In future work, we aim to study the behavioral consequences of the activity of these descending neurons.

Disclosures: **M. Peek:** None. **S. Namiki:** None. **G.M. Card:** None.

Poster

070. Visuomotor Coordination

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Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 70.25/GG4

Topic: D.05. Visual Sensory-motor Processing

Title: Functional investigation of a visual projection neuron in the *Drosophila* visual system

Authors: *M. MORIMOTO, M. WU, A. NERN, G. M. RUBIN, M. B. REISER;
HHMI Janelia Farm Res. Inst., Ashburn, VA

Abstract: Fly visual systems have been used extensively for nearly a century to study diverse aspects of vision. While the investigation of local motion detection (mediated by columnar circuits of the medulla) has received much attention, the functions of other pathways within the

optic lobes of flies have been much less explored. To functionally characterize prominent anatomically described pathways we are currently investigating the encoding properties of neurons that project from the lobula to the central brain (so-called visual projection neurons) in *Drosophila*. In recent work, thermogenetic activation of a one such cell-type induced flies to take off, implicating this cell-type as a likely conduit for visual features that elicit escape. Using split-GAL4 lines for *in vivo* two-photon GCaMP imaging, we are imaging from several cellular compartments of this cell-type, while presenting various visual stimuli. Preliminary data from axonal imaging reveal a receptive field size which is consistent with the small spatial extent of this cell type's dendrites. Questions such as how retinotopic information is being conveyed, and whether a predator's loom may be encoded by this cell-type, are currently under investigation.

Disclosures: **M. Morimoto:** None. **M. Wu:** None. **A. Nern:** None. **G.M. Rubin:** None. **M.B. Reiser:** None.

Poster

070. Visuomotor Coordination

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 70.26/GG5

Topic: D.05. Visual Sensory-motor Processing

Title: Neural substrates for looming detection in *Drosophila* melanogaster

Authors: *C. R. VON REYN, W. R. WILLIAMSON, P. BREADS, A. NERN, G. M. CARD; Janelia Farm Res. Campus, HHMI, Ashburn, VA

Abstract: To navigate through complex, dynamic environments, an animal's nervous system must be able to quickly recognize looming threats, approaching on a direct collision course, and engage appropriate evasive maneuvers. How neural circuits extract looming information and drive a behavioral response is still under investigation. "Looming detector" neurons have been identified in the visual system of many vertebrate and invertebrate species. These neurons encode information about the size, velocity, and time to collision of an approaching object - essential information for coordinating evasive maneuvers. In *Drosophila melanogaster*, genetic tools can be applied to further elucidate circuits and mechanisms underling looming-evoked behavioral responses. Using these tools, we have previously shown how giant descending interneurons (the GFs) drive a rapid escape takeoff in response to a looming threat. The visual neurons supplying looming information to the GF circuit remain unknown. Likely candidates are

the lobular columnar neurons type 4 (LC4s) whose axon terminals project to the same optic glomerulus as the GF dendrites. However, the LC4 population of approximately 60 retinotopically distributed neurons differ morphologically from canonical "looming detectors" that consist of a pair of tangential neurons spanning the entire visual field. In addition, the LC4s are thought to be glutamatergic, while data suggests that cholinergic transmission provides the main excitatory drive to the GFs. Here, using selective genetic labeling and targeting, we investigate anatomical and functional connectivity between the GFs and LC4s. Through pharmacological manipulations, we determine the neurotransmitter involved in LC4 to GF communication. Finally, through selective genetic activation and silencing, we investigate whether the LC4s provide the main source of looming information to the GF pathway.

Disclosures: C.R. von Reyn: None. **W.R. Williamson:** None. **P. Breads:** None. **A. Nern:** None. **G.M. Card:** None.

Poster

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Topic: D.05. Visual Sensory-motor Processing

Support: MAX PLANCK SOCIETY

DFG BO3746/1-1

Title: Size discrimination in the retinotectal system

Authors: C. A. TRIVEDI[1], S. J. PREUSS[1], C. VOM BERG-MAURER[1], S. RYU[1], *J. H. BOLLMANN[2];
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Abstract: Visual space is mapped onto the retina and retinal ganglion cells (RGCs) transmit visual information via their axons to several brain areas for further information processing. Extraction of distinctive features from the visual environment such as direction of motion can already occur at the level of the retina. Therefore, different subtypes of RGCs could carry a rich repertoire of information about visual features to their downstream targets in the central nervous system (CNS). Recent advances in how the 'feature space' at the level of RGCs is mapped onto

their post-synaptic partners has revealed a laminar organization in terms of wiring specificity that may influence local processing and extraction of visual information in RGC target areas. In particular, direction selectivity has been well studied; both at the level of the retina and at the level of its targets such as the optic tectum (superior colliculus), LGN and the visual cortex. However, little is known about how RGC inputs are processed and what kind of local computations occur in retino-recipient brain areas. How these visual processing channels may influence perception and as a consequence, visually guided behavior is also not very well understood. We and others had recently shown that appetitive and aversive behavior in larval zebrafish can be elicited depending on the size of the visual stimulus. This stimulus-response mapping provides a direct link between the processing of stimulus properties and its influence on behavior. Therefore, we performed experiments to determine how objects of different sizes are represented in the population of RGC axons and their downstream targets in the optic tectum in larval zebrafish. Using a combination of transgenic fish lines expressing genetically encoded calcium indicators, functional two photon imaging and statistical analyses, we show that there may be a size selective categorization of visual input to the tectum. Furthermore, our data suggests that the optic tectum has parallel processing channels for visual objects of different sizes. Experiments to determine the morphology of these functional cell classes in the optic tectum may help elucidate the structure-function relationship and wiring specificity of RGC axons to these post synaptic partners with respect to stimulus size processing.

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Poster

070. Visuomotor Coordination

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Program#/Poster#: 70.28/GG7

Topic: D.05. Visual Sensory-motor Processing

Support: Max Planck Society

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Title: Size-filtering circuits in the retino-tectal pathway

Authors: *S. J. PREUSS, C. A. TRIVEDI, C. M. VOM BERG-MAURER, S. RYU, J. H. BOLLMANN;
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Abstract: Processing sensory information is one of the most important functions of the nervous system and the basis of selecting appropriate behavioral output. The optic tectum (homologous to the mammalian superior colliculus), the major retino-recipient area in teleosts, is known to play a vital role in information processing during visually guided behaviors. Visual stimuli detected and preprocessed by the retina get directly mapped to different input layers of tectal neuropil by retinal ganglion cell (RGC) axons. Tectal neurons pick up these signals and further process stimulus features, like motion direction and the size of an object. This processing may be crucial for extracting salient cues in a dynamic environment and for eliciting suitable motor patterns; for example attractive or aversive behavior to prey- or predator-like cues, respectively. Superficial interneurons (SINs) are a subpopulation of tectal neurons thought to play an important role in size-filtering of visual cues during prey capture behavior. Here, we perform a detailed analysis of SINs as putative size-selective elements in the retino-tectal pathway. Using realistic prey- and non-prey-like stimuli previously shown to elicit attractive and aversive behavior, we record size-selective neural responses in this morphologically distinct cell type. Two-photon targeted whole-cell recordings were used to measure the input tuning curves of individual SINs at high temporal resolution. We observed that excitatory inputs of SINs are tuned to the size of moving objects. Several types of input size tuning were observed, suggesting that SINs fall into different functional subclasses. Simultaneously, we labeled SINs through the patch-pipette with a fluorescent dye for subsequent morphological analysis. Arborization profiles of individual SINs were compared with the laminar organization of the tectal neuropil in a transgenic zebrafish line, expressing GFP in a subset of RGCs. We observed that the size tuning of synaptic inputs correlated with the arborization depth of individual SINs in a systematic way. Furthermore, in response to whole-field flash stimuli, SINs arborizing in different layers exhibited markedly different ON or OFF input currents, suggesting that the laminar target zone determines, at least partly, the synaptic input patterns in this cell type. In addition, Ca2+-imaging and current clamp recordings in SINs showed that the spike output pattern correlated with their synaptic input tuning properties, which suggests that SINs may serve different layer-specific roles in the processing and categorization of behaviorally relevant visual stimuli.

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Poster

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Topic: D.05. Visual Sensory-motor Processing

Title: Distinct classes of orientation/direction-tuned 5HT3A receptor-positive interneurons in the mouse visual cortex

Authors: *Y. CHEN[1], V. D. J. BONFARDIN[1], R. A. MEASE[1], N. L. ROCHEFORT[1,2], H. MONYER[3], A. KONNERTH[1];
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Abstract: Information processing in the sensory cortex relies on interactions between excitatory and inhibitory neuronal networks. Inhibitory GABAergic interneurons can be classified into three groups: those expressing the Ca2+-binding protein parvalbumin (PV), those expressing somatostatin (SST) and those expressing the 5-hydroxytryptamine-3A receptor (5HT3AR). The 5-HT3AR-positive interneurons represent the major population of inhibitory neurons in the supragranular layers of the neocortex. However, the properties of these interneurons *in vivo* are largely unknown. In this study, we used transgenic mice expressing enhanced green fluorescent protein (GFP) under the control of the 5HT3A promoter to characterize the spontaneous and visual stimulation-evoked activity of 5-HT3AR-positive interneurons in layers 1 and 2/3 of mouse primary visual cortex. 5-HT3AR-positive interneurons represent a diverse population, including the vasoactive intestinal peptide (VIP)-expressing neurons with a bipolar/bitufted morphology and multipolar neuropeptide Y (NPY) expressing cells. Here, we used two-photon imaging guided whole-cell and cell-attached recordings to characterize their activity *in vivo*. In layer 2/3, bipolar/bitufted 5-HT3AR-positive interneurons exhibited a low spontaneous firing rate (1.03 ± 0.27 Hz, n = 10) and were highly tuned to the orientation of drifting grating stimuli. By contrast, presumed neuroglialform interneurons in layer 2/3 had high spontaneous firing frequencies (3.89 ± 0.69 Hz, n = 13) and increased firing rates for 4/8 up to 7/8 directions. Thus, these interneurons are more broadly tuned than bipolar cells. A separate class of layer 2/3 interneurons had high spontaneous firing frequencies (2.55 ± 0.88 Hz, n = 4) and responded with a decrease in firing for 4/8 up to 7/8 directions. In layer 1, we identified a class of interneurons with high spontaneous firing rates (5.33 ± 1.05 Hz, n = 10) and multipolar dendritic arborization, which increased their firing level for 4/8 up to 7/8 directions. In addition, we found another class of interneurons with low spontaneous firing rates (0.47 ± 0.18 Hz, n = 6) that decreased their firing level for 4/8 up to 7/8 directions. Finally, we identified a third class of layer 1 interneurons with low spontaneous rates (0.45 ± 0.12 Hz, n = 7) and multipolar dendritic arborization, which were highly direction-tuned. In conclusion, our results identify distinct classes of 5-HT3AR-

positive interneurons with specific activity patterns both spontaneously and in response to visual stimulation.

Disclosures: **Y. Chen:** None. **V.D.J. Bonfardin:** None. **R.A. Mease:** None. **N.L. Rochefort:** None. **H. Monyer:** None. **A. Konnerth:** None.

Poster

070. Visuomotor Coordination

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Topic: D.05. Visual Sensory-motor Processing

Title: Activity-control of layer 2/3 pyramidal cells by 5HT3A receptor-positive interneurons in the mouse visual cortex *in vivo*

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Abstract: Information processing in the sensory cortex relies on interactions between excitatory and inhibitory neuronal networks. In the supragranular layers of the neocortex, an abundantly present population of 5-HT3AR-positive inhibitory interneurons interacts with layer 2/3 pyramidal cells. However, the nature and the extent of these interactions *in vivo* and their specific roles in controlling spontaneous activity remained unexplored. Here, we selectively expressed the light-sensitive proton pump ArcheorhodopsinT (ArchT-GFP) in 5-HT3AR-positive interneurons in layers 1 and 2/3 in order to study the impact of their silencing on the ongoing spontaneous activity of layer 2/3 pyramidal cells in the primary visual cortex of isoflurane-anesthetized mice. In developing this approach, we first characterized ArchT-GFP-expressing interneurons in brain slices. Infected interneurons were illuminated with an optic fiber by a 100 msec light pulse (561nm) at different light intensities (6.9, 11.7, 23.3, 32.2, 47.7 and 54.8 mW/mm2). Infected interneurons showed a linear relationship between light intensity and the amplitude of the resulting photocurrent (67.26 ± 9.14 pA up to 146.22 ± 19.05 pA; n = 8 - 11

interneurons for each point). Then, we confirmed, by using *in vivo* two-photon imaging guided cell-attached recordings, that infected 5-HT3AR-positive interneurons can be effectively silenced. A complete block of activity was achieved by light intensities of about 23 mW/mm2 (n = 22 cells). By combining optogenetic stimulation of 5-HT3AR-positive interneurons with cell-attached and whole-cell recordings of pyramidal cells in layer 2/3, we identified two opposite effects. In 11/33 pyramidal cells, we observed a strong decrease in spontaneous activity (-55.09 ± 7.98% n = 5 cells and 100% in n = 6 cells), reflecting an indirect connection with 5-HT3AR-positive interneurons via some other interneurons. In the majority of pyramidal cells (22/33), we observed a dramatic increase of activity (up to 10-fold), demonstrating a direct and particularly powerful connection with 5-HT3AR-positive interneurons. Overall, our results demonstrate that 5-HT3AR-positive interneurons are, without exception, important determinants of the spontaneous activity of layer 2/3 pyramidal cells *in vivo*.

Disclosures: **V.D. Bonfardin:** None. **Y. Chen:** None. **A. Stroh:** None. **I. Nelken:** None. **H. Monyer:** None. **A. Konnerth:** None.

Poster

071. Interlimb Coordination

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Topic: D.17. Voluntary Movements

Support: NIH Grant R01NS076589

VA Grant 3397626

Title: Coupling of bimanual forces during independent and cooperative hand movements

Authors: ***T. BOYANOSKI**, M. A. PEREZ;
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Abstract: Many bilateral motor tasks engage simultaneous activation of distal hand muscles. However, the mechanisms involved in the control of force coupling between hands remain poorly understood. In the present study, we examined human force and electromyographic (EMG) activity in the first dorsal interosseous (FDI) muscle during symmetric and asymmetric increasing levels of bilateral isometric voluntary contractions (5%, 10% and 30% of maximal

voluntary contraction, MVC) into index abduction when subjects controlled, on a computer screen, two independent cursors (dual-task) or a single 2D cursor moved by both hands (single-task) as fast and accurately as possible. During unilateral contractions subjects were able to accurately discriminate increasing levels of force with both hands. We demonstrate a linear increase in all forces in the dual-task, regardless of the level of force and hand tested. When the right hand exerted more force the left hand also generated more force, suggesting a strong bias to produce identical bilateral forces regardless of the force level tested. In contrast, during the single-task subjects were able to discriminate asymmetric and symmetric forces exerted by each hand, regardless of the level of force and hand tested. Trial-by-trial peak forces revealed a strong correlation between peak forces and EMG activity during the bimanual dual-task compared with the single-task. Our results demonstrate that the ability to perform independent movements with both hands can be maximized by coordinated control of a single object, which is influenced by the visual feedback.

Disclosures: **T. Boyanoski:** None. **M.A. Perez:** None.

Poster

071. Interlimb Coordination

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Topic: D.17. Voluntary Movements

Support: NIH Grant R01NS076589

VA Grant 3397626

Title: Object properties influence symmetry of bilateral reach to grasp movements after tetraplegia

Authors: *F. J. CALABRO, M. A. PEREZ;
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Abstract: Most spinal cord injuries in humans result in bilateral anatomical damage to the spinal cord. Recent evidence has indicated that physiological interactions between bilateral hand and arm muscles are impaired after injury. However, the extent to which motion of one arm affects the movement of the other arm is unknown. Using kinematics and electromyographic (EMG)

recordings we studied unilateral and bilateral reach to grasp movements to a small and large cylinder in uninjured controls and in patients with chronic incomplete cervical spinal cord injury (SCI). We demonstrate in controls that movement time during hand opening and closing was delayed with bilateral compared to unilateral movements, regardless of the object or arm tested. In contrast, movement of the weaker arm in patients delayed the stronger arm during hand opening and closing when reaching for a large and small object, respectively. No object specific influences were observed from the stronger to the weaker arm. Patients showed impaired modulation of EMG activity in an intrinsic finger muscle during grasping at times when bilateral delays were observed for each object compared to controls. Thus, our results demonstrate that the symmetry of bilateral reach to grasp movements in humans with SCI can be selectively influenced by the more affected arm according to object properties, likely related to impairments in the bilateral control of EMG activity in hand muscles. We argue that the flexibility of the stronger arm to inputs during bilateral movements may open a critical window to target bilateral control of movement after human SCI.

Disclosures: **F.J. Calabro:** None. **M.A. Perez:** None.

Poster

071. Interlimb Coordination

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Topic: D.17. Voluntary Movements

Title: Increase of interhemispheric coherence during acquisition of asymmetric bimanual movements

Authors: ***S.-W. PARK**, J. COWENHOVEN, D. STERNAD;
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Abstract: Bimanual asymmetric movements are common in everyday life. Previous studies have shown that the corpus callosum plays a vital role not only to enable, but also to constrain bimanual coordination. While symmetric movements are easy, many skilled movements, such as playing the piano, require individuated control of the two hands. How does interhemispheric communication change over asymmetric motor learning? Healthy humans practiced an asymmetric bimanual task, which required independent performance of the two arms. EEG was measured during practice and in a follow-up session two months later. We hypothesized that interhemispheric coherence increases as the asymmetric bimanual performance improved. Six

healthy right-handed subjects were instructed to rotate their forearms in the horizontal plane and to move their right arm to a target cue as fast as possible without disturbing the continuous oscillations in the left arm. During each 45s trial, subjects performed 10 discrete movements triggered at random phases of the ongoing rhythmic arm. Subjects practiced for 10 daily sessions, followed by a 2-month retention session. Each session comprised 16 bimanual trials (45s) and 2 control trials (right-only and left-only). After each trial, subjects received feedback about right-arm peak velocity and their left-arm perturbation, measured by deviations from smooth oscillations. The task was to simultaneously achieve high peak velocity, while minimizing perturbations. Cortical activity during the task was measured using 64-channel EEG electrodes in the beginning, the middle, and the end of practice, as well as the beginning of follow-up sessions. For control, subjects also performed bimanual symmetric reaching for 16 trials. The electric potential was aligned with the visual cue onset. Coherences between EEG electrodes in the motor area (FC1-6, C1-6, CP1-6) were analyzed in alpha and beta frequency regions (8 and 20 Hz, respectively). Behavioral results showed that all subjects increased their right-arm peak velocity over practice. Five out of six subjects reduced the left arm perturbation, although not to zero. All subjects significantly increased their peak velocity in the symmetric condition as well. For the EEG, interhemispheric coherence at 20 Hz increased after 5 sessions for all subjects only in the asymmetric condition. The increased coherence was not maintained until the end of practice. In sum, increased interhemispheric communication was associated specifically with acquisition of the asymmetric bimanual skill. These results suggest that enhanced beta-band coherence plays a crucial role to acquire a novel asymmetric bimanual coordination.

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Poster

071. Interlimb Coordination

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Topic: D.17. Voluntary Movements

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Title: A theoretical framework for simultaneously explaining motor learning effects in unimanual and bimanual movements

Authors: *K. TAKIYAMA;
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Abstract: Motor learning in unimanual and bimanual movements have been intensively investigated, but distinct theoretical frameworks were proposed for each of them. This separate modeling failed to explain relationships or transfer of learning effects (generalization) between those movements. Here, we propose a novel model to simultaneously explain motor learning in unimanual and bimanual reaching movements by extending a framework of motor primitives. We analytically proved that, only when preferred reaching directions (PDs) of each primitives are modulated between unimanual and bimanual movements, a framework of motor primitive can reproduce the following generalization between unimanual and bimanual movements which cannot be explained by any conventional models: generalization between a parallel type of bimanual and unimanual movements is partial when the number of target is one (Nozaki et al., 2006, Nat Neurosci), but the generalization is perfect when the number of target is eight (Wang et al., 2013, PLoS One). Our results are independent of shape of tuning curve (activity function of motor primitives), distribution of PDs in unimanual movements, and how different the PDs between unimanual and bimanual movements, which suggests our analytical and numerical results are robust. Although we focused on parallel types of bimanual movements in the analytical and numerical calculations, additional analytical calculation enabled to clarify relationships between unimanual and other kinds of bimanual movements. Furthermore, the PD modulation revealed a novel relation between unimanual and parallel types of bimanual movements: compatibility of specialization and generalization, i.e., after trained with bimanual movements, generalization to other bimanual movements is large but restricted to bimanual movements similar to trained movements (specialization) and generalization to unimanual movements is slight but extended to wide-range unimanual movements (generalization).

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Poster

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Title: Age-related changes in interhemispheric communications during the preparation of bimanual movements

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Abstract: Introduction. For successful bimanual performance, it is necessary to regulate complex interhemispheric communication (excitation and inhibition). Studies using functional neuroimaging revealed that older adults increase activity of prefrontal brain areas during task performance, suggesting that interactions between prefrontal cortex and primary motor cortex (M1) are likely to be involved in the regulation of motor performance in older adults. However, the exact role of these prefrontal regions in bimanual coordination in older adults is yet to be determined. Here, using a dual-site transcranial magnetic stimulation protocol (dsTMS), we investigated the nature of interactions between prefrontal (non-primary motor regions) and the contralateral M1 during the preparation of a bimanual coordination task in older adults. Methods. A group of healthy young and older volunteers participated in three experimental sessions while performing a bimanual coordination task with their index fingers rotating dials. Participants were instructed to perform isofrequency (1:1) and non-isofrequency movements (3:1, 1:3). In separate sessions, we investigated the interaction between the dorsolateral prefrontal cortex (DLPFC), dorsal premotor (PMd), or M1 and the contralateral M1 assessing interhemispheric inhibition (IHI) in both directions (i.e., from the right to the left hemisphere, and vice versa) at two time points (onset of preparatory cue (PC0ms) and 50ms prior to the imperative stimulation (IS-50ms)) during the preparatory period. Results. For DLPFC-M1 IHI, young adults showed facilitation at IS-50ms relative to PC0ms irrespective of movement ratio and hemisphere (left and right). Interactions between left PMd and right M1 and M1-M1 in both directions became facilitatory from IS-50ms to PC0ms in young adults. Importantly, these facilitations were absent in older adults showing no IHI modulations during the preparatory period. Conclusion. The present findings demonstrate an absence of modulation in IHI in older as compared to young adults, indicating that the declined ability to functionally modulate interhemispheric interactions with advancing age may underlie degraded bimanual coordination performance in older adults.

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Poster

071. Interlimb Coordination

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Topic: D.17. Voluntary Movements

Title: Effect of single pulse transcranial magnetic stimulation of unilateral motor cortex on bimanual grip coordination: A pilot study

Authors: *R. PATEL, S. AMANO, K. OKI, B. C. CLARK, S. L. HONG;
Ohio Musculoskeletal and Neurolog. Inst., OUHCOM, Athens, OH

Abstract: The purpose of this pilot study was to test the effect of a single pulse transcranial magnetic stimulation (TMS) of unilateral motor cortex during a bimanual grip force task. We tested the hypothesis that a single TMS pulse would disrupt bimanual grip force coordination. Four right-handed healthy young individuals (21± 2years, 174.2±8.8 cm, and 63.9±10.9kg) were asked to generate bimanual grip forces simultaneously with both hands at a target (5% of maximum bimanual grip force). Subjects were only provided with visual feedback of the sum of the forces generated across both hands. The task was performed under two conditions: 1) single pulse TMS stimulus to M1 at an unpredictable time (STIM), and 2) a sham condition (SHAM). Both conditions were conducted for the left and right hemispheres, with a total of five trials per condition (20 trials in total). For each subject, stimulus intensity was set at 100% of active motor evoke potential, and the conditions were presented in random order. Bimanual coordination dynamics were evaluated using 1-second windows from 3 seconds pre- and post-stimulus. The circular standard deviation (CirSD) of the Hilbert relative phase of the dominant and non-dominant grip forces was calculated. The windows were averaged to the three 2-second epochs before (PRE), after (POST) and at the time of stimulus (ST). A 3-way (2 Side x 2 Condition x 3 Time) ANOVA was conducted on the results and post-hoc LSD tests were employed when needed. We observed a significant Condition x Time interaction (p=.03), where the TMS pulse led to an increased CirSD exclusively during the ST window [PRE: .026±.004, ST: .0.48±.011, POST .027±.001 in RH; PRE: .026±.006 ST: .061±.020, POST: .026±.007 in LH], but only for the STIM condition (PRE to ST: p=.03, ST to POST: p=.02). Our results revealed that unilateral motor cortex stimulation could lead to a disruption in bimanual coordination during the 2-second window surrounding the stimulus. The lack of an effect of or interaction with Side indicated that the pattern or degree to which bimanual coordination was disrupted was not affected by hand dominance. These results indicate that the single pulse TMS is able to generate a perturbation to bimanual coordination that persists for over one second, an effect that is present regardless of which hand is stimulated. Further investigation with larger sample size is in process.

Disclosures: **R. Patel:** None. **S. Amano:** None. **K. Oki:** None. **B.C. Clark:** None. **S.L. Hong:** None.

Poster

071. Interlimb Coordination

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Topic: D.17. Voluntary Movements

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F32 NS076206

Title: Representation of bimanual reach plans in the posterior parietal cortex

Authors: ***E. F. MOOSHAGIAN**, C. WANG, L. H. SNYDER;
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Abstract: The parietal reach region (PRR) in the posterior parietal cortex represents reach plans. The representation is biased for contralateral reaches, though individual neurons in PRR represent reach plans for the contralateral, ipsilateral, or either limb. Since lesions in PRR affect only contralateral limb movements, the utility of the ipsilateral reach representation is unclear. The representation of both contralateral and ipsilateral reach plans makes PRR a candidate region for bimanual coordination. Bimanual coordination shows a range of patterns. The two arms might move together as if to catch a football or they might move independently as in playing the piano. Here, we asked if reach planning activity in PRR is affected by movements of both arms and whether it depends on the pattern of bimanual coordination. We considered four possible profiles for bimanual reach planning activity in the PRR: First, PRR neurons might code just the contralateral movement and be uninvolved in bimanual reach planning. Second, planning activity might be the average of activity preceding planned contralateral and ipsilateral limb movements. Third, activity might be a linear combination of activity preceding planned contralateral and ipsilateral limb movements. Fourth, planning activity might be a non-linear combination of activity preceding planned contralateral and ipsilateral limb movements. To test for representation of bimanual reach plans in the PRR, we trained two male rhesus macaques (Macaca mulatta) to make three types of reaches to visual targets: unimanual (left or right arm) reaches to a single target accompanied by an eye movement to that target, bimanual reaches and an eye movement to a single target, and bimanual movements to different targets with the animal

free to choose which target to saccade to. Most PRR neurons showed higher firing during planning of reaches with the contralateral compared to the ipsilateral arm. For these neurons, planning activity for bimanual reaches did not differ from the contralateral reach condition. Approximately 20% of neurons showed a different pattern of firing. In these neurons, planning activity for bimanual reaches was differentially modulated depending on whether the planned bimanual reach was to be made to the same or separate targets. Thus, bimanual reach planning activity in PRR is a non-linear combination of the unilateral planning responses, suggesting that PRR is involved in bimanual coordination.

Disclosures: **E.F. Mooshagian:** None. **C. Wang:** None. **L.H. Snyder:** None.

Poster

071. Interlimb Coordination

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Topic: D.17. Voluntary Movements

Title: Reduced intracortical inhibition is associated with bimanual common and dual goal tasks

Authors: W.-W. LIAO[1], S. KANTAK[2], J. BARTON[3], J. WHITALL[1], *S. MCCOMBE WALLER[1];
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Abstract: Objective: Stroke is the leading cause of long-term disability worldwide with approximately 75% of stroke survivors reporting functional upper extremity dysfunction. Many activities of daily living (ADL's) comprise upper extremity (UE) tasks involving varying degrees of collaboration between the two arms/hands. Some tasks involve a common goal in which the two hands share a single focus, such as picking up a box. Others involve separate goals, such as picking up two different items at the same time. We hypothesize that fundamentally different control mechanisms are involved in each of the two types of tasks, and that this will have important implications for the rehabilitation therapies administered to populations whose UE movement has been impaired. In this pilot we present preliminary results of testing to determine the intracortical inhibition and facilitation to upper extremity muscles during unimanual, bimanual common goal and bimanual separate goal isometric tasks in healthy subjects. Subjects: Six healthy adults. Methods: Intracortical inhibition and facilitation (SICI, ICF) of the dominant

hemisphere were assessed bilaterally for the biceps during three conditions; 1) unimanual isometric task, 2) bimanual common goal isometric task and, 3) bimanual dual goal isometric task. Results: In this preliminary data set, a significant reduction in SICI ($p < .05$) was found for both bimanual common goal tasks and bimanual dual goal tasks compared to the unimanual task condition. While SICI was reduced for bimanual common goals tasks compared to bimanual dual goal tasks it did not reach significance. Discussion: Reduced intracortical inhibition seen during the two bimanual tasks conditions is indicative of a disinhibitory mechanism, at least within the dominant hemisphere, underlying bimanual arm activation compared to unilateral arm activation. In this preliminary data set the two bimanual conditions did not differ in terms of intracortical inhibition. Further work will include increasing the sample size to see if this finding remains consistent and will include an exploration into interhemispheric inhibition to further determine the effects of these different task conditions on interhemispheric interactions. Conclusion: A reduction in SICI or disinhibition was seen during bimanual dual goal and common goal tasks when compared to unimanual task performance. This may have implications for consideration of training approaches for individuals following stroke.

Disclosures: **W. Liao:** None. **S. Kantak:** None. **J. Barton:** None. **J. Whitall:** None. **S. McCombe Waller:** None.

Poster

071. Interlimb Coordination

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 71.09/GG18

Topic: F.01. Human Cognition and Behavior

Support: Marie Curie International Incoming Fellowships

JSPS Postdoctoral fellowship for research abroad

UCL-NTT Communication Science Laboratories Grant, European Research Council, ERC, 260424

Title: Learning history of unimanual action affects bimanual coordination

Authors: ***A. SARAIVA**[1], N. HAGURA[2], T. KIMURA[3], H. GOMI[3], S. BESTMANN[1]; [1]Sobell Dept of Motor Neurosci. and Movement Disorders, Inst. of Neurology, Univ. Col.

London, London, United Kingdom; [2]Inst. of Cognitive Neuroscience, Univ. Col. London, London, United Kingdom; [3]NTT Communication Sci., Human & Information Sci., Atsugi, Japan

Abstract: Bimanual object manipulation requires distributing the output force between the two hands. This distribution pattern depends both on the variability and the maximum voluntary contraction (MVC) of each hand (O'Sullivan et al 2009). In daily life, however, such bimanual activities are often preceded by unimanual activities, and differ between the left and right hand. Here, we asked whether the preceding schedule of visuomotor gain adaptation for each hand affects the force distribution pattern during bimanual coordination. Participants (n=24) pressed on a left or right force sensor with their left or right index finger to reach a desired force output level (unimanual task, UM). Force, normalised to MVC, was presented online as a vertical moving bar that had to reach a visual target. Each UM task was followed by a bimanual task (BM), where the summed normalised output of both hands was presented, and the target was reached by pressing both force sensors concurrently. Critically, there were three types of force gain change, one for each UM, in which the effect of each schedule was subsequently assessed in BM. Following a baseline UM and BM schedule in which no gain changed occurred, the gain of the force increased (gain-up) differently for both hands: one increased abruptly, and the other increased gradually. In the final UM, the gain decreased back to the baseline (gain-down), with the decrease pattern now reversed between the two hands. While the schedule of gain change differed between the two hands initially, both hands reached the same gain at the end of each UM. The force sharing pattern during BM was significantly affected by the gain change schedule during UM. After gain-up, the hand which was exposed to gradual gain change increased its force contribution during BM. Surprisingly, this effect persisted after gain-down. Further analysis showed that the different gain schedule in the UM lead to differences in within-trial variance of force output between the two hands during BM, and that asymmetry of the variance significantly explained the force distribution pattern. Our data suggest that the bimanual coordination pattern during force sharing is not static; the preceding unimanual activity can dynamically change the way the two hands coordinate and combine forces.

Disclosures: **A. Saraiva:** None. **N. Hagura:** None. **T. Kimura:** None. **H. Gomi:** None. **S. Bestmann:** None.

Poster

071. Interlimb Coordination

Location: Halls A-C

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Topic: D.17. Voluntary Movements

Support: KAKENHI 26242062

NEXT Program #LS034

Grant-in-Aid for JSPS Fellows

Title: Effect of the number of training contexts on motor learning transfer from bimanual to unimanual movement

Authors: *T. HAYASHI[1,2], D. NOZAKI[1];
[1]The Univ. of Tokyo, Grad Sch. Educ, Tokyo, Japan; [2]JSPS research fellow, Tokyo, Japan

Abstract: It is still controversial how motor memories of a limb are shared between unimanual and bimanual movements. Our previous study has showed partial transfer of learning of a force field from bimanual to unimanual movement (Nozaki et al., Nat Neurosci 2006). In contrast, a recent study has demonstrated almost complete transfer (Wang et al., PLoS One 2013) when bimanual training was performed for reaching toward 8 targets instead of just one target in our previous study. However, in their study, the movement directions were changed not only for the opposite arm but also the trained arm, making the mechanisms of full transfer ambiguous. To examine the problem more directly, we investigated how the number of movement directions of the opposite arm for the bimanual training influenced the amount of learning transfer to unimanual movement, while the movement direction of the trained arm kept constant. Eight right-handed participants performed bimanual reaching movements training under the presence of a velocity dependent force field to the left arm (KINARM End-Point Lab, Bkin Technologies, Canada). There were 4 training sets in which the number of targets for the opposite right arm was gradually increased from 1 to 8 targets [1 (0 deg), 2 (0, 180 deg), 4 (0, 90, 180, 270 deg) and 8 targets (0, 45, 90, ⋯, 315 deg)]. At the end of each training set, error clamp trials were randomly interleaved to quantify the aftereffect of the left arm for unimanual and bimanual (both hands were moved forward) movements. In the first training set, the aftereffect of the left arm was significantly greater for bimanual movement than for unimanual movement. Thus, consistent with previous findings, the motor learning acquired bimanually was only partially transferred to unimanual movement. However, even when the number of targets for the opposite right arm was increased (2nd to 4th training set), the aftereffect of unimanual movement did not increase. Thus, the number of the training contexts was unlikely to be related the amount of motor learning transfer. Future experimental and/or theoretical studies would be necessary to reconcile the present results with those of Wang's study and to clarify how the motor memories are shared between unimanual and bimanual movements.

Disclosures: **T. Hayashi:** None. **D. Nozaki:** None.

Poster

071. Interlimb Coordination

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 71.11/GG20

Topic: D.17. Voluntary Movements

Title: Dynamic dominance revealed through transcranial magnetic stimulation during bimanual isometric grip force production: a pilot study

Authors: *S. AMANO, R. PATEL, K. OKI, B. C. CLARK, S. HONG;
Ohio Univ., Athens, OH

Abstract: Dynamic-dominance hypothesis suggests that handedness could result from differential specialization of limbs in a motor performance. The purpose of this pilot study was to test this hypothesis using unilateral transcranial magnetic stimulation (TMS). Four right-handed healthy young individuals (21± 2years, 174.2±8.8 cm, and 63.9±10.9kg) were asked to generate bimanual grip forces simultaneously with both hands at a target (5% of maximum bimanual grip force). Subjects were only provided with visual feedback of the total force generated across both hands. The task was performed under two conditions: 1) single pulse TMS condition where a pulse was delivered to M1 at an unpredictable time (STIM), and 2) a sham condition (SHAM). Both conditions were conducted for the left and right hemispheres, with a total of five trials per condition (20 trials in total). For each subject, stimulus intensity was set at 100% of active motor threshold, and the conditions were presented in random order. Detrended Fluctuation Analysis was performed to obtain α-values for 1-second windows between three seconds pre- and post-stimulus, as an index of the spread of fluctuations in the grip force dynamics of the individual hands. The average ratio of α-values of the left (LH) to the right (RH) hand during the three 2-second epochs before (PRE), after (POST) and at the time of stimulus (ST) were used for statistical comparisons. A 3-way (2 Side x 2 Condition x 3 Time) ANOVA was conducted on the results and Sidak-corrected post-hoc tests were employed when needed. A significant main effect of Side was observed where LH α-values consistently exceeded the RH across the three TIME epochs ($p=.01$). A significant 3-way interaction ($p<.01$) revealed that a Side x Time interaction existed only for the STIM condition ($p<.01$), but not for the SHAM. We observed significant changes in the α-value ratio from PRE to ST for both sides (RH: 1.10±.14 to .78±.07, LH: 1.06±.14 to x1.48±.08; both p's=.02). Furthermore, the α-value ratio was significantly higher during PRE when the RH was about to be stimulated (1.10±.14) in comparison to the LH (1.06±.14; $p=.02$). Our results reveal that: 1) LH consistently exhibited slower grip force fluctuations, 2) TMS led to slower grip force fluctuations in the stimulated hand, and 3) unlike

RH, the LH appeared to exhibit anticipatory behavior (faster force fluctuations) prior to the delivery of stimulus to the contralateral side. While further investigation with larger sample size is needed, these observations support the dynamic-dominance hypothesis, where the dominant limb primarily controls the dynamics of a task, and the non-dominant limb is specialized for position stabilization.

Disclosures: **S. Amano:** None. **R. Patel:** None. **K. Oki:** None. **B.C. Clark:** None. **S. Hong:** None.

Poster

071. Interlimb Coordination

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 71.12/GG21

Topic: D.17. Voluntary Movements

Support: European Commission through MOVE-AGE, an Erasmus Mundus Joint Doctorate programme (2011-0015)

Research Fund of Flanders (G.0708.14)

Title: Neural activity as a correlate of task difficulty in bimanual coordination

Authors: *L. M. RUEDA DELGADO[1], E. SOLESIO JOFRE[1], A. DAFFERTSHOFER[2], S. P. SWINNEN[1];
[1]KU Leuven- Fac. of Kinesiology and Rehabil. Sci. (faber), Leuven, Belgium; [2]Res. Inst. MOVE, Vrije Univ. Amsterdam, Amsterdam, Netherlands

Abstract: *Background.* Studies using fMRI have shown that increased task difficulty is associated with increased brain activation in different areas [1, 2]. An analogue neural correlate of task difficulty regarding bimanual motor performance using electrophysiology has yet to be examined. Coordination of the upper limbs to achieve a common goal is seminal for challenging motor capabilities. *Methods:* Using a 128-channel EEG system, we recorded the brain electrical activity of 26 participants during a bimanual coordination task consisting on the rotation of two dials while online augmented visual feedback of performance was provided via the PC screen. Three conditions were assessed: the 'simple' 1:1 coordination mode, a more challenging 1:3 polyrhythm (where the right hand moved faster than the left hand), and its more difficult symmetry partner (i.e., the 3:1 task, where the left hand moved faster than the right hand). Motor

performance was quantified as deviation from the target. After artifact-removal, we extracted modulations of the theta band via the Hilbert amplitude of the 4-8Hz bandpass filtered EEG. Principal component analysis (PCA) was applied to determine spatial modes dominating these changes in theta activity. *Results*. Motor performance dropped significantly with increases in task difficulty. EEG power was primarily signified by theta activity. PCA revealed a significant increase of theta power above frontal regions as task difficulty increased. This frontal theta activity is therefore likely to be a proper biomarker for task difficulty modulation during motor performance. *Conclusion*. In line with fMRI studies [1, 2] and EEG studies [3], we found altered fronto-central theta activity with modulation of bimanual task difficulty. This might also be linked to error detection [4]. The better performance during 1:1 coordination was accompanied by a lower theta power indicating low task difficulty or a higher level of 'expertise' on the task. Conversely, performing the more difficult 3:1 mode was associated with higher theta power. Monitoring frontal theta activity may thus serve as a predictor for experienced differences in motor task difficulty. References 1. Debaere et al. (2003). Neuroimage 19: 764–776. 2. Ullen et al. (2003). J Neurophysiol 89: 1126–1135. 3. Meyer et al. (2014). J NeuroEng and Rehabil 11, 24. 4. Van Driel et al. (2012). J Neuroscience 32(47):16795-16806

Disclosures: **L.M. Rueda Delgado:** A. Employment/Salary (full or part-time):; PhD student in KU Leuven. **E. Solesio Jofre:** A. Employment/Salary (full or part-time):; Post-doc in KU Leuven. **A. Daffertshofer:** A. Employment/Salary (full or part-time):; Professor at VU University Amsterdam. **S.P. Swinnen:** A. Employment/Salary (full or part-time):; Professor at KU Leuven.

Poster

071. Interlimb Coordination

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 71.13/GG22

Topic: D.17. Voluntary Movements

Title: Cortical EEG dynamics related to a delay in selective response execution/inhibition during bimanual Go/Stop tasks

Authors: *K. YAMANAKA;
Showa Women's Univ., Tokyo, Japan

Abstract: Previous studies demonstrated a difficulty in selective inhibition of one motor response with concurrent execution of another response. However, it remains unclear the

underlying cortical mechanism of the selective response execution/inhibition. The aim of this study is to investigate the cortical mechanism of the difficulty to selective response execution/inhibition. Eight right-handed female volunteers conducted bimanual go/stop tasks, in which they were instructed to click two computer mice bimanually when two indicators moving with a constant velocity reached targets simultaneously (both-go), to avoid the bimanual clicks when both indicators simultaneously stopped just before they reached the targets (both-stop), to avoid a left-hand click with executing of a right-hand click when a left indicator stopped unilaterally just before the target (selective right-go/left-stop), and to avoid a right-hand click with executing of a left-hand click when a right indicator stopped unilaterally just before the target (selective left-go/right-stop). For each participant, stop-signal reaction times (SSRTs) in a non-selective (both-stop) and two selective-stop conditions (right-go/left-stop and left-go/right-stop) were calculated from the %correct in stop-response and mean go-response time. Surface electroencephalography (EEG) was recorded during the task and analyzed by a traditional event-related potential (ERP) and a single-trial-based EEG power and phase dynamics. SSRTs in a non-selective condition was significantly smaller than those in two selective-stop conditions, suggesting a difficulty in selective response execution/inhibition. Next, ERPs in the right-go/left-stop and left-go/right-stop conditions about 150-200 ms after the stop-signal onset were slightly lateralized in the opposite hemispheres. These lateralized ERP responses indicate presence of additional EEG responses for delayed go-responses. Finally, power increase and inter-trial phase-locking in the theta-band EEG occurs broadly over the motor-related areas about 150-200 ms after the stop-signal onset. And the phase angles of the EEG oscillation at the ipsilateral motor-related areas consistently precede those at the contralateral ones, suggesting that the lateralized cortical activation patterns are dependent on the responding hand. These results suggest that the difficulty of the selective response execution/inhibition is related to the presence of the additional processes for the delayed go-responses.

Disclosures: K. Yamanaka: None.

Poster

071. Interlimb Coordination

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Topic: D.17. Voluntary Movements

Support: Child Health Research Appeal Trust

Epilepsy Research Foundation

MRC Grant G1002276

Title: Can a single hemisphere control the synchronised execution of asymmetric bimanual actions?

Authors: D. NOBBS[1], L. HAMMETT[1], L. BERTHOUZE[1,2], *F. VARGHA-KHADEM[1,3]; [1]UCL Inst. Child Hlth., London, United Kingdom; [2]Univ. of Sussex, Brighton, United Kingdom; [3]Great Ormond Street Hosp., London, United Kingdom

Abstract: Healthy participants use both cerebral hemispheres to perform asymmetric, bimanual actions. For the hemispherectomised patient, this is not possible: one hemisphere must provide cerebral control of both limbs. Here, we asked if hemispherectomised patients synchronise asymmetric, bimanual movements as healthy participants do. We obtained motion capture recordings from a 33 year old with Sturge-Weber syndrome who underwent left-sided hemispherectomy at 8.5 years and three healthy, age and sex matched controls. As previously reported (Vargha-Khadem et al., 1997) the patient retained contralesional hand function post-operation. The task required the participants to press an empty soap dispenser with the left or right hand (pressing hand) whilst placing the other beneath the dispenser (receiving hand). We computed the average percent of each trial for which the tangential velocity of both arms was simultaneously above 20% of their respective maximum velocities ('simultaneous movement'). For the controls, this was 80.45% (SD = 4.51) when pressing the dispenser with the left hand and 81.42% (SD = 5.85) when pressing with the right. For the patient, this was 37.14% (SD = 10.19) when pressing with the left hand and 21.64% (SD = 11.35) when pressing with the right. This revealed a strong tendency for the patient to move each arm separately. We then computed the average difference between reach onset of the receiving hand and reach onset of the pressing hand and calculated this as a percentage of total reach duration of the pressing hand ('onset latency'). For the controls, onset latency was 0.48% (SD = 6.41) when pressing with the left hand and -1.60% (SD = 6.33) when pressing with the right, indicating left and right reaches began almost simultaneously. For the patient, when pressing with the left hand, the right arm began moving later and onset latencies were highly variable: average onset latency was 52.13% (SD = 31.83). When the patient pressed with the right (paretic) hand, left and right reaches were on, average, entirely sequential: onset latency was 99.52% (SD = 59.94). The cause of this previously unreported deficit is unknown but evidence suggests that asymmetric bimanual movements require independent planning and monitoring for each side, functions performed bilaterally by structures such as the superior parietal cortex (Diedrichsen et al., 2006). After hemispherectomy, cortical preparation of both actions must be completed by one hemisphere, perhaps by a shared neural substrate. We speculate that asymmetric, bimanual actions cannot be prepared simultaneously by one hemisphere, hence such movements are planned and performed sequentially.

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Poster

071. Interlimb Coordination

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 71.15/GG24

Topic: D.17. Voluntary Movements

Title: What promotes interhemspheric interaction during bilateral tasks? Evidence from intermuscular coherence

Authors: *S. LEE[1,3], H. B. NGUYEN[2,3], M. L. HARRIS-LOVE[4], P. S. LUM[5,3];
[1]Biomed. Engin., [2]Catholic Univ. of America, Biomed. Eng., Washington, DC; [3]Ctr. for Applied Biomechanics and Rehabil. Res., MedStar Natl. Rehabil. Hosp., Washington, DC;
[4]Interdisciplinary Program in Neurosci. and Rehabil. Med., Georgetown Univ., Washington, DC;
[5]Biomed. Engin., Catholic Univ. of America, Washington, DC

Abstract: Bilateral movement training has been shown to improve upper limb functionality of those affected by neurological diseases such as stroke. Interhemispheric interaction, such as motor cortex disinhibition, is postulated as one of the underlying mechanisms facilitating neural plasticity during bilateral tasks, but how such interaction between the hemispheres can be modulated by task conditions has not been quantified in detail. In this study, therefore, we aimed to examine how different types of feedback provided to subjects can affect neural interactions underlying their bimanual coordination, by examining EMG-EMG coherence between task-relevant muscles of the limbs participating in the task. Twelve healthy subjects participated in an experiment in which they generated bilateral isometric hand forces using extension or flexion moments at their elbow joints. During task performance, we provided subjects with three different types of feedback; 1) visual feedback (i.e., tilting bar representing force ratio between hands); 2) visual feedback imposing a task constraint (i.e., balancing of a ball placed on the bar); and 3) haptic feedback provided by a physical apparatus that coupled the two hands together and required equal forces in the two hands to perform the task. EMG-EMG coherence of three muscle pairs, 1) agonist muscle pairs (left and right biceps during elbow flexion; left and right triceps brachii during elbow extension), 2) antagonist muscle pairs (left and right triceps during flexion; left and right biceps during extension), and 3) agonist-antagonist muscle pairs (left triceps-right biceps and left biceps-right triceps), were calculated. The coherence values in the alpha (8-15Hz) and beta (16-35Hz) bands were found to be the major contributors to the total coherence, and the intermuscular coherence was modulated by the different types of feedback during bilateral elbow extension, but not during bilateral elbow flexion. Specifically, the

coherence between the agonist muscle pair (elbow extensors) in the condition 3 (haptic feedback) was significantly higher than those in the other conditions ($p = 0.006$). The coherence values of the other muscle pairs, i.e., antagonist muscle pair (elbow flexors) and agonist-antagonist pair (flexor-extensor), were also the highest in the condition 3, but the difference between the conditions was relatively smaller. Results of this study suggest that sensory modality of the feedback provided to subjects should be properly chosen to promote interhemispheric interaction during bimanual task, and that tasks that mechanically couple the two hands may be the most effective bilateral method of rehabilitation.

Disclosures: **S. Lee:** None. **H.B. Nguyen:** None. **M.L. Harris-Love:** None. **P.S. Lum:** None.

Poster

071. Interlimb Coordination

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Topic: D.17. Voluntary Movements

Support: NIH Grant R01 NS037844

Title: Bilateral force transients in the upper limbs following single-pulse microstimulation in the ponto-medullary reticular formation

Authors: *T. J. HIRSCHAUER[1], J. A. BUFORD[2];
[2]Div. of Physical Therapy, Sch. of Hlth. and Rehabil. Sci., [1]Ohio State Univ., Columbus, OH

Abstract: The ponto-medullary reticular formation (PMRF) is a group of brainstem nuclei that gives rise to the reticulospinal tract, which primarily contributes to postural stability and motor control of proximal muscles. The PMRF receives input from both the ipsilateral and contralateral motor cortex and projects to the spinal cord where it synapses on motor neurons and commissural interneurons. These bilateral projections are a potential source of motor recovery following a unilateral motor cortex injury, such as a stroke. While previous studies have assessed EMG responses to microstimulation, they have not determined the resultant movement and force output patterns generated by stimulation of reticulospinal neurons in primates. In this study, muscle activity was recorded using intramuscular EMG electrodes implanted bilaterally in 24 muscles (12 on each side) of the trunk and arms in two M. fascicularis monkeys. Force responses were measured by two force-sensitive joysticks, which the monkeys were trained to grasp while performing bilateral force control tasks. A tungsten microelectrode was placed in the

pontomedullary reticular formation and 2000 30 μA pulses were applied at 5 Hz to 163 reticular formation locations. Force responses to stimulation were collected with the force transducers and analyzed using Spike2 data acquisition and analysis software and MATLAB. Preliminary data analysis identified significant EMG or force responses from 92 sites (56.4%) using stimulus-triggered averaging. Most stimulation sites produced bilateral muscle activation consistent with facilitation of the ipsilateral flexor muslces and contralateral extensors and suppression of the ipsilateral extensors and contralateral flexors in the upper limbs. Force responses revealed a corresponding pattern of motion, toward the body in the ipsilateral arm (directed medially and posteriorly) and away from the body in contralateral arm (directed laterally and anteriorly). These force transients were characterized by amplitudes of 0.05 N with onset latencies of about 15 ms. This work demonstrates that force transients in response to single-pulse microstimulation of reticulospinal neurons can be detected with sensitive force transducers using stimulus-triggered averaging and that these force outputs agree with the general pattern of ipsilateral arm flexion and contralateral arm extension observed in EMG recordings.

Disclosures: **T.J. Hirschauer:** None. **J.A. Buford:** None.

Poster

071. Interlimb Coordination

Location: Halls A-C

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Program#/Poster#: 71.17/GG26

Topic: D.17. Voluntary Movements

Title: Muscle relaxation of the foot induces decrease in muscle activity of hand sustained-contraction

Authors: *K. KATO[1,2], J. WATANABE[3], K. KANOSUE[4];
[1]Grad. school of Sport Sci., Fuculty of Sport Sciences, Waseda Univ., Saitama, Japan; [2]Japan Society for Promotion of Sci. Res. Fellow, Tokyo, Japan; [3]Grad. school of Sport Sci., Tokorozawa, Japan; [4]Fac. of Sport Sci., Saitama, Japan

Abstract: Introduction Muscle relaxation is an important aspect to make a fine control of the body in daily life, but the mechanism has not been well understood. It has been suggested that muscle relaxation is accomplished by an active process in the brain, and not just the end of contraction (Toma et al., 1999). We have recently observed that EMG level of contraction in one limb decreased during the relaxation of the ipsilateral remote limb (Kato et al., 2014). Furthermore, we utilized transcranial magnetic stimulation (TMS) and suggested that muscle

relaxation of foot increased intracortical inhibition for the hand (Kato et al., in preparation). However, in the period of voluntary muscle relaxation of foot muscle, the time-dependent change in EMG activity of hand muscle during sustained contraction has not been well understood. The objective of this study was to clarify how the relaxation of foot muscle influences on EMG activity of hand muscles by utilizing simple reaction task. Methods Ten participants sustained isometric contraction in their right pronated hand with 2, 5 and 10% MVC, then relaxed/contracted their right foot from dorsiflexed/resting position in response to an auditory signal. Force level and EMG activity of hand were recorded based on the relaxation/contraction onset of foot. Mean force level and EMG activity of hand were calculated in each 200 ms period from 0 to 1000 ms from foot relaxation/contraction onset, and were compared with those before the signal. Results After the contraction onset of foot, mean EMG activity and force level of the hand significantly increased for 5 and 10 % sustained contraction. After the relaxation onset of foot, on the other hand, mean EMG activity and force level of the hand significantly decreased for 5 and 10 % sustained contraction. Discussion We demonstrated that muscle relaxation of foot induced temporally decrease in EMG activity and force level of hand muscle, whereas muscle contraction of foot induced temporally increase in EMG activity and force level. Remote effect has been previously observed during muscle contraction of a limb. Present study suggested that muscle relaxation also influences on muscle activity of a remote limb. References Toma K et al., J Neurosci. 1999 Kato K et al., Exp Brain Res. 2014

Disclosures: **K. Kato:** None. **J. Watanabe:** None. **K. Kanosue:** None.

Poster

071. Interlimb Coordination

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Topic: D.17. Voluntary Movements

Support: 1R56NS070879

R01 NS060830

R21 HD067906

Title: Task dependent modulation of interhemispheric inhibition

Authors: M. WISCHNEWSKI[1], G. KOWALSKI[2], S. R. BELAGAJE[1], *C. M. BUETEFISCH[1];
[1]Neurol., [2]Emory Univ., Atlanta, GA

Abstract: The role of primary motor cortex (M1) in the control of voluntary hand movements is still unclear. fMRI studies of unilateral hand motor performance reported a relationship between level of precision of a motor task and additional ipsilateral M1 activation. In the present study we determined whether the demand on accuracy of a movement influences the magnitude of inhibitory effect between primary motor cortices. We used transcranial magnetic stimulation (TMS) to measure active interhemispheric inhibition (aIHI) in the pre- movement period of a pointing task where precision varied quantitatively. Six healthy participants used a joystick to point to targets of 2 different sizes in a block design. Following the presentation of a fixation square, participants were asked to move a cursor to a target square immediately upon its appearance. Target squares were of small (high precision) or a large (low precision) sizes. Electromyographic (EMG) signal was recorded from both extensor carpi ulnaris muscles (ECU), a muscle supporting the execution of the pointing task. Active IHI was measured by applying a conditioning pulse to the left M1 and a second test pulse to the homotopic right M1 at an interstimulus interval of 10 ms. Paired stimuli were interspersed with single test stimuli applied to right M1. Measurements were obtained at target appearance (0 ms), and 300, 350, 400, and 450 ms after target appearance. We also measured IHI during rest (rIHI) using the same TMS settings. The amount of inhibitory effect of the conditioning pulse on the motor evoked potential (MEP) of the subsequent test pulse was expressed as percentage of the mean MEP amplitude evoked by the single test pulses. Across the 5 time points of aIHI measurements, there was a significant effect for target size (repeated measures ANOVA: $F(1,5) = 13.62$, $p = 0.014$) with aIHI being weaker with high precision movements when compared to low precision movements ($t(5) = 3.60$, $p = 0.014$). This difference was most pronounced in the early pre- movement period (0 - 300 ms; % mean test MEP, high precision: 77.79 ± 14.16 (0 ms), 79.09 ± 11.10 (300 ms); low precision: 64.46 ± 13.17 (0 ms), 68.58 ± 11.91 (300 ms). During this time period, aIHI in the early pre- movement period for low precision movements was similar to rIHI (66.11 ± 13.97 % mean test MEP), whereas aIHI in the high precision movements was below rIHI. The present findings suggest that during the pre- movement period the aIHI between motor cortices is modulated by the demand on accuracy of motor task. This is consistent with previous findings where bilateral M1 activation was seen during high precision movements, whereas unilateral M1 activity was seen with low precision movements.

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Poster

071. Interlimb Coordination

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Topic: D.17. Voluntary Movements

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RJ Ibey was supported through The Ireland Canada University Foundation (Dobbin Scholarship)

Title: Interhemispheric inhibition of corticospinal projections to wrist muscles

Authors: *D. A. BOLTON[1], R. J. IBEY[2,3], A. R. BUICK[1], R. G. CARSON[1,3];
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Abstract: Interhemispheric inhibition (IHI) is an electrophysiological phenomenon obtained when an initial magnetic (conditioning) stimulus (CS) is applied to one primary motor cortex (M1) shortly (6-15 ms) before a second (test) stimulus (TS) is directed to the other M1. Here, the magnitude of the motor evoked potential (MEP) generated by the TS is smaller than that elicited in isolation. The expression of IHI is typically examined via changes in the excitability of corticospinal projections to intrinsic hand muscles. This is an important consideration as the cortical representations of proximal and distal muscles in the upper limb may be distinguished in terms of their inter-hemispheric projections. Given the practical utility of examining movements generated by muscles proximal to the wrist, particularly in the context of rehabilitation, it is necessary to determine whether optimal IHI parameters established for the hand apply more generally. To address this issue, eleven healthy, right-handed adults were studied. They were seated comfortably with arms supported and elbows semi-flexed. Surface muscle EMG was recorded from the flexor carpi radialis (FCR) and extensor carpi radialis (ECR) of both arms. The TS coil was held over M1L at the optimal position to evoke a motor evoked potential (MEP) in the contralateral right FCR. The CS coil was positioned over M1R to stimulate the left FCR. For both stimulation sites, the resting motor threshold (rMT) was determined. The time course of IHI was probed using seven different ISIs (6, 7, 8, 9, 10, 12 and 15ms). Each ISI was delivered in random order within a given test block for a total of ten trials per ISI. In addition to CS-TS pairings, ten trials of TS alone were included in each test block. TS intensity was fixed at 120% rMT. Conversely, CS intensity was adjusted in five separate test blocks ranging from 110, 120,

130, 140 and 150% rMT (5 minutes rest between blocks). The order of test block presentation (i.e. different CS intensities) was randomly allocated. For FCR, IHI scaled with CS intensity and a progressive increase in IHI was observed as the ISI was extended (up to 10ms). The most consistent and pronounced IHI (per CS intensity) tended to occur at an ISI of 10ms, whereas negligible IHI was present at the 6ms ISI. A similar pattern of outcomes was observed for ECR. While the results are broadly in accordance with those obtained for hand muscles the utility of the present approach lies in the delineation of stimulus parameters that permit changes in IHI in either direction to be resolved. This is an important consideration in the study of neural adaptations associated with motor learning.

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Poster

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Topic: D.17. Voluntary Movements

Support: CIHR MOP 111175

HSFCSR catalyst

Title: Interhemispheric cortical interactions of the caudal forelimb (CFA) area with its contralateral homologue in the rat

Authors: *E. SERRANO, I. MOREAU-DEBORD, L. JEAN-CHARLES, B. TOUVYKINE, S. QUESSY, N. DANCAUSE;
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Abstract: Numerous studies have demonstrated that an interhemispheric dialogue exists between sensory and motor cortical areas of the two hemispheres. In the frontal agranular cortex of the rat, there is a large forelimb area, the caudal forelimb areas (CFA), forming a mosaic of shoulder, forearm, wrist and digits movements. The CFA of the two hemispheres are interconnected, which suggests that they can interact for the production of the corticospinal outputs. However, these interhemispheric interactions are only partially understood. In the present study, we used paired pulse stimulation protocols to investigate how one CFA can facilitate or inhibit its contralateral homologue. Following craniotomy and durectomy, anesthesia was changed from

isoflurane to ketamine (IP). A stimulating electrode placed in the CFA of one hemisphere was used to deliver a sub-threshold conditioning stimulus (C). A second stimulating electrode was placed in the opposite CFA to deliver a supra-threshold test stimulus (T). The C and T stimuli were separated by seven different randomly-presented inter-stimulus intervals (ISIs). Effects of the cortical stimulations were quantified with the recording of motor evoked potentials (MEP) from 3 forelimb muscles and one dorsal muscle in each arm. Overall, we recorded 29 protocols in 6 rats, each testing the effect of one CFA cortical site on the outputs of the opposite CFA. Our results demonstrated a strong relationship between the ISIs and the modulatory effect of the contralateral CFA. The contralateral CFA facilitated the MEP response at short ISIs (0, 2.5 ms) in 64% of our protocols and at long ISIs (20, 35 ms) in 64% of our protocols. However, at intermediate ISIs (5, 10 and 15 ms), the contralateral CFA exerted an inhibitory influence in 54% of our protocols. The data indicate that subthreshold CFA activation can provide both facilitatory and inhibitory effects on the motor outputs of the opposite CFA, depending on when it is activated in relation to the other. These results suggest that the outputs from these homologous areas are not merely summated to provide the MEP response but are instead subject to more complex temporal processing.

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Poster

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Topic: D.17. Voluntary Movements

Support: NSERC RGPIN 402663

Title: Subthreshold activation of ventral premotor cortex produces interhemispheric modulation of contralateral primary motor cortex outputs in Capuchin monkeys

Authors: *S. QUESSY, A. HAMADJIDA, M. DEA, J. DEFFEYES, N. DANCAUSE;
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Abstract: The primary motor cortex (M1) plays a pivotal role in the control of movements in primates. The numerous inputs from several premotor areas can influence its corticospinal outputs. While a lot of efforts have been devoted to the study of the input form ipsilateral ventral

premotor cortex (PMv) on M1 outputs, little is known about the influence of cortical areas of the opposite hemisphere. In the present study we document the modulation of M1outputs by interhemispheric inputs from the contralateral PMv (cPMv). We used paired-pulse stimulation protocols while recording electromyographic (EMG) activity from 8 forelimb muscles in each arm. Following a bilateral craniotomy and durectomy, anesthesia was switched from isoflurane to ketamine. Using standard intracortical microstimulation techniques, we first identified the location of the hand representation area in M1 in one hemisphere, as well as in cPMv. Then, we selected a site in the hand area of M1 where a single pulse stimulation of moderate intensity (30-300 μA) would elicit a clear motor evoked potential (MEP) in at least one of the muscles contralateral to the stimulation. This stimulating electrode would be used as the test electrode (T). A second stimulating electrode was placed in the hand representation of the cPMv to be the conditioning electrode (C). The intensity of the C stimulus was kept subthreshold (typically 75% of threshold). In different conditions, we used 6 relative timings between C and T (range = 0-20ms). Data was collected in 4 adult monkeys from which we tested a total of 11 cPMv-M1 site pairs. At each site pair, we obtained MEP from between 1 and 7 contralateral muscles, allowing us to record a total of 44 interhemispheric interactions. The MEP evoked using paired stimulations were compared to the ones obtained using T only. In general, inhibitory interactions were more common, but the strength of this inhibition depended on the latency between the C and the T. When longer delays between C and T were used (15 and 20ms), the MEPs observed often showed a strong inhibitory modulation of M1 outputs by the cPMv, while shorter delays (0-10 ms) were more likely to produce a more moderate inhibition. Less often, interhemispheric facilitatory effects were also observed but mostly at shorter ISI (0-10 ms). These results suggest that while interhemispheric inhibitory interactions between premotor and motor areas are more common, these interactions are complex and strongly temporally modulated.

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Poster

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Topic: D.17. Voluntary Movements

Support: CIHR MOP 111175

HSF CSR catalyst

Title: Interhemispheric cortical interactions of forelimb motor areas in the rat: Modulation of the caudal forelimb area (CFA) by the contralateral rostral forelimb area (RFA)

Authors: *I. MOREAU-DEBORD, E. SERRANO, B. TOUVYKINE, L. JEAN-CHARLES, S. QUESSY, N. DANCAUSE;
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Abstract: The interhemispheric interactions between motor areas are not currently well understood. In rats, movements of the forelimb can be evoked from two distinct sensorimotor areas, the caudal forelimb area (CFA) and the rostral forelimb area (RFA). Interhemispheric connections exist between RFA and CFA of the two hemispheres. However, no study has investigated the extent to which RFA can modulate the output of the contralateral CFA. The goal of the current study was to characterize these interactions with paired-pulse stimulation protocols in the rat. After a bilateral craniotomy and durectomy, anesthesia was changed from gas to ketamine. An electrode was placed in the RFA of one hemisphere to provide a sub-threshold conditioning stimulus (C) while a second electrode was placed in the contralateral CFA in order to deliver a supra-threshold test stimulus (T). Seven randomly-presented inter-stimulus intervals (ISIs; 0, 2.5, 5, 10, 15, 20, and 35 ms) separated C from T stimulation to study the effects of different delays on CS output. This output was assessed by recording motor evoked potentials (MEP) from 3 forelimb muscles (palmaris longus, extensor digitorum, biceps brachii) and one shoulder muscle (spinodeltoid) in each arm. In total, 31 paired pulse protocols were recorded in 4 rats. The results demonstrated that the modulation of CS output depends on ISI. With short (0, 2.5, 5ms) and long ISIs (15, 20, 35ms), there was clear facilitation of MEPs in 92% and 81% of protocols, respectively. RFA had an inhibitory effect on the contralateral CFA in 48% of protocols with an ISI of 10ms. Thus, the interhemispheric interactions between RFA-CFA were mostly facilitatory across ISIs. These results contrast from those obtained from paired-pulse stimulation of CFA with its contralateral homologue, for which a greater number of inhibitory responses were observed. The ISI-dependent modulation of CFA outputs by the contralateral RFA reveals that complex interhemispheric interactions between these areas can take place. The contrast between the interhemispheric influence of the contralateral RFA and CFA suggest that these two areas play a different role for the generation of coordinated movements of the two forelimbs.

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Poster

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Topic: D.05. Visual Sensory-motor Processing

Support: Sasakawa Scientific Research Grant from The Japan Science Society 26-630

Title: Observation of unilateral hand movement modulates interhemispheric inhibition on primary motor cortex; a transcranial magnetic stimulation study

Authors: *S. YAMAMOTO, Y. OKAMOTO, N. TAKESHITA, Y. UMEHARA, M. OHSHIMA, M. MONMA, Y. KOHNO, K. NUMATA;
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Abstract: Introduction: It is presumed that interhemispheric inhibition (IHI) between motor cortical areas is thought to play a critical role in motor control. However, IHI can influence adversely. Murase et al confirmed that stroke patients with abnormal IHI pattern present poor motor performance. Repetitive transcranial magnetic stimulation can modulate IHI, but it's expensive and not diffused widely. To explore an expedient mean to modulate IHI, is crucial. In this study, we investigate the effect of action observation of unilateral hand movement on interhemispheric inhibition. Method: The excitability of the left and right primary motor cortex (M1) (the first dorsal interosseous muscle controlling area) in a resting state during observation of adduction and abduction movements of a unilateral index finger displayed on a monitor were investigated in 11 healthy subjects using two transcranial magnetic stimulators (interstimulus interval, 10 ms). Results: The result indicated an increased excitability of the contralateral M1 and an increased suppression of the ipsilateral M1 in the acion observation condition compared with the condition without action observation. Discussion & Conclusions: Numata et al confirmed activation of the anterior intraparietal sulcus (aIP) during action observation by an fMRI study. The increased contralateral M1 excitability may be attributed to information transmitted from aIP via the premotor cortex, while the increased suppression to the ipsilateral M1 may be attributed to an increased interhemispheric inhibition (IHI) associated with the increased contralateral M1 excitability. Action observation is suggested to be a possible effective means to correct the imbalance of IHI occurring after brain injury and facilitate recovery from paralysis.

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Poster

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Topic: D.17. Voluntary Movements

Support: NIH Grant RO1 NS058667

NIH Grant: T32 EB009406

Title: Assessment of associated reactions in pediatric and adult onset hemiplegia

Authors: *R. L. HAWE, J. P. A. DEWALD;
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Abstract: In both children and adults with hemiplegia, high effort motor tasks in the lower extremities can result in involuntary upper extremity movements, typically in flexion or extension movement patterns called associated reactions. These associated reactions refer to involuntary movement at one body part in response to a voluntary effort in a different body part. Associated reactions are thought to be the result of an increased reliance on brainstem pathways, specifically reticulospinal or vestibulospinal systems. An example is that when children with hemiplegia run, their upper extremity moves involuntarily into a posture consistent with a flexion synergy. In this study, we aimed to quantify associated reactions in both children and adults with hemiplegia to gain insight into the use of brainstem pathways following unilateral brain injuries to the developing brain as well as mature brain. Individuals performed maximal and submaximal isometric knee flexion and extension tasks while receiving visual feedback on their lower extremity torque production. Individuals were instructed to relax their upper extremities while performing the lower extremity task, though no explicit feedback was given. EMGs were placed on shoulder, elbow, and wrist muscles bilaterally as well as quadriceps and hamstrings to measure muscle activity. All EMG activity was normalized to the maximal voluntary EMG for each muscle. Motion analysis was captured using a Microsoft Kinect system. Our findings show that associated reactions are stronger for knee extension than knee flexion, possibly due to higher neural drive to the knee extensors. Associated reactions were greater when the paretic lower limb was tested compared to non-paretic. Muscle activity and involuntary motion was greater for

maximal efforts compared to submaximal, suggesting a threshold level of neural drive prior to recruitment of brainstem pathways. The results of this study support the hypothesis that associated reactions are due to the recruitment of brainstem pathways during high effort motor tasks when available corticospinal resources are exhausted. The pattern of muscle activity and kinematics is consistent with vestibulospinal or reticulospinal innervation patterns.

Disclosures: **R.L. Hawe:** None. **J.P.A. Dewald:** None.

Poster

071. Interlimb Coordination

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Topic: D.17. Voluntary Movements

Support: IDeA CTR support – NIH/NIGMS Award Number U54GM104942

Title: Asymmetric Walkway: Novel behavioral assay for studying asymmetric locomotion

Authors: *K. TUNTEVSKI, R. ELLISON, J. M. SHAFFER, S. YAKOVENKO;
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Abstract: Behavioral assays are commonly used for the assessment of sensorimotor impairment in the central nervous system. The most sophisticated methods for quantifying locomotor deficits in rodents measure the minute disturbances of overground gait (e.g. manual BBB score or automated CatWalk measures, Noldus). However, the cortical inputs are not essential for the locomotor pattern generation produced by spinal network (Yakovenko, 2011). Thus, unconstrained walking tasks only indirectly test locomotor deficits due to the cortical impairment. In this study, we propose the novel task of measuring the locomotor phase characteristics during precise limb placement that requires cortical control (Drew et al., 2009). The peg walkway was used to set symmetrical and asymmetrical locomotor tasks mimicking lateralized movement deficits. The task imposed consistent shifts (20% of stride length) from the default symmetric foot placement corresponding to the preferred stride length. The forced asymmetric foot placement induced significant changes in the phase relationship characteristics during locomotion measured with the asymmetry and diagonality indices. The stance phase of forelimbs showed significant modulation by the task in both uninjured animals and those with impairment due to focal stroke induced by the occlusion of medial cerebellar artery. The changes in phase modulation were characterized with the model of rhythm generation based on half-

center leaky integrators. In general, the locomotor assay based on the asymmetric walkway is a sensitive measure of asymmetric adaptations.

Disclosures: **K. Tuntevski:** None. **R. Ellison:** A. Employment/Salary (full or part-time):; Neural Engineering Laboratory, Biomedical Research Center - West Virginia University. **J.M. Shaffer:** A. Employment/Salary (full or part-time):; Neural Engineering Laboratory, Biomedical Research Center - West Virginia University. **S. Yakovenko:** None.

Poster

071. Interlimb Coordination

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Topic: D.17. Voluntary Movements

Support: NSERC PGS D2-426833

MOP 84403

Title: Primary motor cortical neurons reflect perturbation and torque-related activity from ipsilateral limb

Authors: *E. A. HEMING, S. H. SCOTT;
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Abstract: It is commonly accepted that the primary motor cortex (M1) is involved in controlling the contralateral side of the body. However recent work has shown that many M1 cells are active during ipsilateral limb movements. As feedback plays an important role in voluntary motor actions, we were interested in exploring the relative prevalence of limb feedback in M1 from the ipsilateral and contralateral forelimbs. For our tasks, a non-human primate (NHP) stabilized a cursor representing the location of its right or left hand at a central virtual target. After 1000-1500ms, flexor or extensor step torques were applied to the shoulder, the elbow, or both (9 load conditions). The NHP had 1000ms to return to the target, followed by another 1500-2000ms hold period. The paradigm was then repeated but with a cursor for each hand, and simultaneous perturbations on both arms. Perturbations were selected to activate similar muscle groups or opposing muscle groups in each arm. All trials from all conditions were interleaved. During each task, we recorded the activity of M1 cells using a micro-electrode array. As expected, almost all M1 cells isolated with the array showed responses to perturbations of the contralateral limb.

However we also found about 60% of cells modulated their activity for perturbations on the ipsilateral limb, with about half of the cells showing responses to perturbations of either limb. Although overall responses tended to be larger for contralateral perturbations, about a third of bilaterally responsive neurons had earlier responses to ipsilateral perturbations than their responses to contralateral perturbations. A third of the bilaterally responsive cells also showed larger response magnitudes to ipsilateral perturbations. While individual cells modified their baseline activities between tasks, there was no population rate change between right and left tasks. Additionally, we found that cells often displayed preferred torque directions (PTD) that were different for each limb. Our results surprisingly indicate that M1 shows fast and substantial ipsilateral response to perturbation-related activity from both limbs.

Disclosures: **E.A. Heming:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); BKIN Technologies. **S.H. Scott:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); BKIN Technologies.

Poster

071. Interlimb Coordination

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Topic: D.17. Voluntary Movements

Support: Office of Naval Research (ONR), United States of America.

Title: A preliminary multi-level neurocomputational model for overt/covert self-intended and imitated arm reaching movements

Authors: *H. OH[1,2], D.-W. HUANG[3], G. E. KATZ[3], R. H. MILLER[1,2], J. A. REGGIA[3,4], R. J. GENTILI[1,2,5];
[1]Dept. of Kinesiology, Univ. of Mayland-College Park, College Park, MD; [2]Grad. Program in Neurosci. and Cognitive Sci., [3]Dept. of Computer Sci., [4]Univ. of Maryland Inst. for Advanced Computer Studies, [5]Maryland Robotics Ctr., Univ. of Maryland-College Park, College Park, MD

Abstract: Humans have the ability to actually and mentally perform self-intended complex actions such as bimanual arm reaching movements. Further, it has been proposed that the human brain incorporates a mirror neuron system that is engaged during observation and imitation of

other's actions. From a neurophysiological point of view, mental simulation theory suggests that such motor performance capabilities are enabled by a simulation network in which the fronto-parietal circuitry is critical, and that allows for performing actual, mental, observed, and imitated actions. Based on mental simulation theory, we present a neurocomputational architecture that includes high (cognitive) and low (sensorimotor) levels of processing that coherently integrate self-intended actual/mental and observed/imitated bimanual arm reaching movements. Here, as a first step, only the sensorimotor level was developed by modeling three fronto-parietal loops that learn to compute the i) inverse kinematics, ii) sensorimotor predictions and iii) visuo-spatial transformations needed to remap the frame of reference from allo- to ego-centric coordinates during the execution of bimanual arm movements. Our results show that this neural model can execute accurate and robust 3D actual/mental reaching movements while generating human-like kinematics. Also, the visuo-spatial remapping network allows for imitating arm reaching independently of the viewpoint from which an action is observed. This is consistent with previous neurophysiological studies showing that the frontal mirror system in monkeys includes independent viewpoint neurons that are activated regardless of the observation viewpoint. The long term goal of this work is to further understand the underlying neural and behavioral principles of actual and mentally-simulated motor performance, especially in the context of human imitation learning. This research was supported by the Office of Naval Research (ONR; N000141310597), United States of America.

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Poster

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Topic: D.17. Voluntary Movements

Support: NIH R00EY021252

GMU URSP

Title: The influence of visual feedback on the intralimb and interlimb generalization of motor adaptation

Authors: *K. MURTHY, J. FITZGERALD, W. M. JOINER;
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Abstract: The type of visual feedback during training in a visuomotor rotation (VMR) task has been shown to influence the magnitude of intralimb generalization. Here, we were interested in the effect of this feedback on the interlimb transfer of adaptation. First, we attempted to replicate previous studies that reported an influence of visual feedback on the extent of intralimb generalization to movement directions not experienced during training (Taylor et al. 2013). We tested 14 subjects as they made point-to-point right handed movements to a peripheral target located 9 cm at 90^o. A 30^o clockwise or counterclockwise rotation was applied (the rotation direction was counterbalanced) to the visual feedback of their unseen reaching movements. This visual feedback during training was either for the full movement trajectory (full feedback, FF) or terminal feedback once the radial target distance (9 cm) was surpassed (end point feedback, EPF). In both cases the terminal cursor position was displayed for 1.5 seconds once the movement surpassed 9 cm. Generalization was tested by requesting subjects to make movements to untrained target locations (19 target locations spaced 15^o apart, from 315^o to 225^o) without any performance feedback. We estimated the local (the width at 50% transfer) and global components (the offset from 0% transfer) of the generalization function based on fitting a Gaussian function to the data ($R^2 > 0.91$ in both feedback conditions). Surprisingly, unlike previous reports, we found a substantially broader generalization for the EPF condition—the local and global components were 41% and 17% greater for EPF than for FF. Based on this finding we tested 5 additional subjects on interlimb transfer with either FF or EPF during training. Subjects were trained in the same method as experiment 1, but the untrained left hand was used to make movements only to the trained target without feedback. Based on the results from the first experiment, we hypothesized that EPF would again result in a greater transfer of the adaptation to the untrained left limb. Preliminary results show that the feedback in this case affected the magnitude of learning transferred to the untrained limb; transfer with EPF at the trained location was substantially greater than with FF. In summary, we report that the extent of performance feedback strongly influences the amount of VMR generalization within the trained limb and transfer to the untrained limb. We hypothesize that this variation may be due to a difference in how the resulting movement error is interpreted based on the feedback, and subsequently credited (self-caused vs. externally-caused) during training.

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Poster

072. Motor Deficits

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Title: Harnessing tongue movement to decrease RMSE in wrist tracking exercise using exoskeletal assistance

Authors: S. OSTADABBAS[1], J. KIM[1], D. WU[2], K. RICHARDS[2], *A. J. BUTLER[2], M. GHOVANLOO[1];
[1]Electrical & Computer Engin., Georgia Inst. of Technol., Atlanta, GA; [2]Physical Therapy, Georgia State Univ., ATLANTA, GA

Abstract: Robot assisted therapy has been shown to produce positive clinical outcomes. Current robot-assisted technology for the upper extremity (UE) requires patients who are able to actively initiate hand motion before the robot can help to complete the movement task. We investigated the use of an innovative control system that utilizes the tongue to indirectly drive movement of the hand through an existing robotic device, thereby supplementing active movement. Subjects played an interactive target-tracking task that requires up and down movements on the screen, driven by a robotic arm (Hand Mentor: HM). The robot was interfaced with a wireless headset that detects the movements of a small magnetic tracer temporarily adhered to the tongue (Tongue Drive System: TDS). In TDS-HM system, users try to move their paretic hands actively while using their tongue to drive motion. This enables an individual with little or no active hand movement to participate in therapy and has the potential to remodel the brain's neural pathways. The theoretical construct that underpins this notion is that by linking the extensive motor cortical representation of the tongue to that of the hand, an individual with limited UE functionality following a stroke will be able to progress towards active functional movement. We have developed a TDS-HM prototype and tested it with able-bodied individuals. For the pilot study, two subjects were asked to complete a series of computer games in three consecutive days, in which they tracked three target waveforms (rectangular, sinusoidal, and triangular), while receiving visual feedback on the screen. The games are based on the concept that stroke patients have very limited range of motion (ROM). Within the active ROM, they complete the movement with their hand; outside their active ROM, they use the tongue to move their hand with robotic assistance. Limited ROM was simulated in such a way that beyond a certain wrist angle the robot prevented movements not initiated by tongue control. The TDS-HM was able to quantify wrist angles, tongue positions/commands, and tracking performance as the root mean square error (RMSE). Performance improvement between day 1 and 2 was observed as a 30% decreased in RMSE, while RMSE remained constant between days 2 and 3. The results demonstrate TDS-HM interaction can be learned effectively in 3 days. Future studies will include stroke survivors within 3 to 12 months post-injury, who have less than 10 deg. active ROM. Ultimately, TDS-HM

will allow us to understand the potential effects of controlling a therapeutic robotic exoskeleton with the tongue by gradually reducing the role of tongue motion to improve UE ROM and function in stroke survivors.

Disclosures: **S. Ostadabbas:** None. **J. Kim:** None. **D. Wu:** None. **K. Richards:** None. **A.J. Butler:** None. **M. Ghovanloo:** None.

Poster

072. Motor Deficits

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 72.02/HH3

Topic: D.17. Voluntary Movements

Title: Training-related improvement in hand force perception in older adults

Authors: ***K. KERN**[1], S. BROWN[2];
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Abstract: Deficits in manual dexterity in older adults are well documented (Olafsdottir et al., 2008; Parikh and Cole, 2012;) due, in part, to reduced force control (Cole et al., 1999; Diermayer et al., 2011; Galganski et al., 1993). Impaired perception of force may also contribute to difficulties with hand function as has been shown in force matching tasks in elderly individuals (DeSerres and Fang, 2004). While improvement in hand force control following task-based training in older adults has been demonstrated (Ranganathan et al., 2001), to what extent similar benefits occur in force perception is unclear. Thus, the purpose of this study was to determine if a sensorimotor training program could improve the ability to perceive pinch force in older adults. Eleven right hand dominant older adults (mean age 77.3 y) performed a variety of clinician-designed sensorimotor tasks in a home setting for 30-40 min/day for 6 weeks. Tasks were performed by both the dominant and non-dominant hands. Sensory tasks involved identification of object characteristics in the absence of vision. Motor tasks focused on visually-guided object manipulation and pinch force activities. Force perception was assessed before and after the intervention using a tripod pinch matching paradigm. In the ipsilateral remembered (IR) condition, participants generated a reference force equivalent to 25% of maximum voluntary force with either their dominant or non-dominant hand and then matched the reference force with the same hand. During the contralateral concurrent (CC) task, participants maintained the reference force while matching with the opposite hand. Functional, somatosensory, and cognitive ability was also assessed. Prior to training, matching errors were significantly greater in the CC

compared to the IR task. Following training, matching error decreased but only in the contralateral concurrent task ($p<0.02$). Differences were statistically significant only when matching was performed by the dominant hand ($p<0.02$). Directional error also decreased in the CC but not the IR condition ($p<.05$). Training was associated with improved tactile sensibility and manual dexterity but only for the dominant hand. Lastly, a decrease in cognitive processing was observed ($p<.05$). The results of this study demonstrate that simple object manipulation activities can improve hand force perception in older adults, particularly in tasks where force monitoring of both hands is required. Improvements in tactile function, manual dexterity, and cognition underscore the broad benefits of such training.

Disclosures: **K. Kern:** None. **S. Brown:** None.

Poster

072. Motor Deficits

Location: Halls A-C

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Program#/Poster#: 72.03/HH4

Topic: D.17. Voluntary Movements

Support: CIHR MOP-125915

Donald Sanderson Memorial Trust Fund

Title: Cognitive-motor integration in young elite athletes with a history of concussion

Authors: Y. HAMANDI[1], J. HURTUBISE[1], C. HUGHES[1,2], A. MACPHERSON[1,2], *L. E. SERGIO[1,2,3];
[1]Sch. Kinesiol & Hlth. Sci., York Univ., Toronto ON, ON, Canada; [2]York Univ. Sport Med. Team, Toronto, ON, Canada; [3]Ctr. for Vision Res., Toronto, ON, Canada

Abstract: Our research examines cognitive-motor integration during eye-hand coordination. Such integration is often required when performing non-standard visuomotor tasks, where a rule is used to align the required motor output to the guiding visual information. We propose that measuring visuomotor integration under conditions that place demands on visual-spatial and cognitive-motor processing may provide an effective behavioural means for the early detection of brain alterations associated with concussion. Previous research from our laboratory1 has shown cognitive-motor integration declines in university-level varsity athletes who have a history of concussion (but were deemed recovered at the time of evaluation). To extend our

research, the current study examines cognitive-motor integration in young asymptomatic elite athletes with a history of concussion. The participants were 187 National Hockey League draft prospects, 28 of whom had a history of concussion but were recovered at the time of evaluation during the annual NHL combine fitness testing. Participants were tested on two visuomotor transformation tasks using an Acer Iconia dual-touchscreen tablet. They made movements from a central target to one of four peripheral targets (up, down, left, right) viewed on the vertical laptop screen by sliding their finger 1) across the touch-sensitive surface displaying the targets (direct interaction) or 2) across a horizontal plane with the cursor feedback 180o reversed, so that the motion plane and cursor alignment were decoupled from guiding visual information(requiring cognitive-motor integration). Unlike our previous observations with varsity level athletes, we observed no differences in reaction time, movement time, path length, or trajectory endpoint accuracy/precision between concussion-history and no-concussion-history groups in this elite population ($p>0.05$). There are many possible reasons for our observed resiliency of skilled performance following concussion in this group, including a stronger group selection bias (combine invitation vs varsity inclusion), superior concussion management, and/or a superior movement control brain network resiliency. Future work will examine whether enhanced brain network activity is underlying the maintained motor behavioural performance, something observed for purely cognitive skills in older retired NHL athletes2. Ref: 1.Brown JA, Hughes C, Sergio LE (2011) Soc. Neurosci. annual meeting, Session #698.192. Esopenko C et al.(2013). Cog. Neurosci. annual meeting, Session #B60.

Disclosures: **Y. Hamandi:** None. **J. Hurtubise:** None. **C. Hughes:** None. **A. Macpherson:** None. **L.E. Sergio:** None.

Poster

072. Motor Deficits

Location: Halls A-C

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Program#/Poster#: 72.04/HH5

Topic: D.17. Voluntary Movements

Title: Quick, low-cost, and highly sensitive motor assessment using markerless motion capture

Authors: *P. K. JOHNSON[1], M. MCCAIN[2], S. K. CHARLES[3];
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Abstract: There is a nationally recognized need for more sensitive motor assessments to evaluate movement and diagnose motor impairments following traumatic brain injury (TBI). Properly identifying impairments and the underlying neural injuries is critical in properly diagnosing movement disorders, estimating prognosis, and prescribing an effective rehabilitation program. Unfortunately, many conventional motor assessments rely on subjective observations, fail to detect subtle impairments, and only identify movement impairments without providing insights into underlying mechanisms. The purpose of this research is to 1) develop a motor assessment method that is quick, low-cost, and highly sensitive, and 2) establish normative data for use in future comparisons. By adapting traditional motor assessment tests and automating them using custom software and the Leap Motion sensor, we have developed an integrated system that is quick, low-cost, and highly sensitive. The Leap Motion sensor, which only costs $80, markerlessly measures the position of all ten finger tips in three dimensions with a resolution up to 0.01mm and a sampling frequency up to 200 samples per second. Our custom software programs interface with this sensor and prompt the user via a graphical user interface to perform certain movements and record the user's movements as he or she progresses through a battery of motor tests. The tests include postural tremor, finger tapping, point-to-point movements, reaction time, and postural sway. Because this motor assessment method uses a sensitive sensor, it provides a large amount of movement data with high resolution in both space and time. For data from individual patients to be clinically useful, we are establishing a normative database of unimpaired motor behavior with fifty young, healthy research participants (25 male and 25 female subjects between the ages of 18 and 30). To provide a basis of comparison between our new assessment method and conventional motor assessment methods, we are including in this normative database the traditional finger tap test (using a clicker counter) and the Beery-Buktenica Developmental Test of Visual-Motor Integration. The outcomes of the motion capture assessment will be evaluated against those of the conventional exams. Our novel integrated system increases the sensitivity and objectivity of motor assessments, and the low cost, portability, and ease of use make this system easily accessible to clinicians as well as researchers.

Disclosures: **P.K. Johnson:** None. **M. McCain:** None. **S.K. Charles:** None.

Poster

072. Motor Deficits

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Program#/Poster#: 72.05/HH6

Topic: D.17. Voluntary Movements

Support: ESRC Grant RES-062-23-2183

Title: The relation between attention and tic generation in Tourette syndrome

Authors: *E. MISIRLISOY[1], V. BRANDT[2], C. GANOS[3], J. TUBING[2], A. MUNCHAU[2], P. HAGGARD[1];
[1]Inst. of Cognitive Neuroscience, UCL, London, United Kingdom; [2]Inst. of Neurogenetics, Univ. of Lübeck, Lübeck, Germany; [3]Inst. of Neurology, UCL, London, United Kingdom

Abstract: Many neuropsychiatric disorders involve abnormal attentional processing. Tourette syndrome (TS) is a disorder characterised by multiple motor and phonic tics. Systematic investigations of how attention may affect tic frequency in TS are lacking. Here, we test experimentally whether shifting attention away from tics reduces tic frequency. We developed a novel paradigm in which patients performed a self-paced finger movement task. Throughout each 1 minute trial, patients opposed a finger of their choice against the thumb of their dominant hand, approximately once every 2 seconds. Movement of each digit triggered a unique visual colour stimulus. We manipulated attentional focus by asking patients to monitor and remember their finger actions, the external colours caused by their actions, or their tics during action. Recall was tested after each trial. A group of 16 adult TS patients participated in a single testing session. They performed each task twice: once while voluntarily inhibiting tics, and once without inhibiting tics. During the 'freely tic' condition, patients had significantly fewer tics when attending to finger movements, or to the ensuing colours, compared to when attending to their tics. Attention to fingers produced the fewest tics overall. During tic suppression, there was no such pattern. Instead, tic frequency was reduced to the same level in all conditions. This suggests that effects of attentional focus on tic generation are markedly different from the effects of voluntary tic inhibition. In conclusion, focussing attention away from tics significantly reduces tic frequency. This attentional process may operate by regulating motor noise. Attentional regulation and intentional tic inhibition are distinct cognitive processes.

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Poster

072. Motor Deficits

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Program#/Poster#: 72.06/HH7

Topic: D.17. Voluntary Movements

Support: AHA 13PRE14690048

Title: The effect of paretic ankle plantarflexion in spontaneous and voluntary joint torque coupling patterns

Authors: *N. SANCHEZ, J. P. A. DEWALD, R. LOPEZ-ROSADO;
Northwestern Univ., Chicago, IL

Abstract: Introduction: Clinical observation of the loss of independent joint control after stroke has defined a dominant lower extremity synergy: the extensor synergy. This coupling pattern is characterized by spontaneous coupling of hip extension, hip adduction, knee extension and ankle plantarflexion. Clinical observation however, has not determined the joint torque patterns that drive this synergy. Research in our lab has found abnormal extension/adduction coupling, as described in the clinic, during the generation of maximal and submaximal hip extension torques in the paretic lower extremity. We have also shown that the paretic lower extremity is constrained to this abnormal extension/adduction coupling when executing a dual degree of freedom task. In the present study we explored the effect of the ankle plantar flexion in eliciting the extensor synergy. Spontaneous joint torque coupling generated during maximal and submaximal ankle plantarflexion was quantified. Furthermore, the ability of individuals post-stroke to combine ankle plantarflexion with volitional hip abduction torques or whether they are constrained to extension/adduction coupling during ankle plantarflexion was determined using a dual degree of freedom tack. Methods: Subjects were fitted into an isometric device capable of quantifying torques generated simultaneously at the hip, knee and ankle. Subjects generated maximum voluntary torques for ankle plantarflexion and hip abduction/adduction with their paretic and non-paretic or control lower extremities. Subjects then generated 25, 50 and 75% of their plantarflexion torque and attempted to combine them with maximum hip abduction torques. EMG activity of 10 lower extremity muscles was measured. Results: We quantified spontaneous extensor/adductor coupling during maximum ankle plantarflexion torques in the paretic extremity of post-stroke individual but not on the non-paretic extremity or in controls. EMGs supported this finding: greater coactivation was observed on the paretic extremity. Significantly greater EMGs were measured in the paretic Adductor Longus ($p=0.000$), Rector Femoris ($p=0.000$), Vastus Medialis ($p=0.002$) and Biceps Femoris ($p=0.003$). During submaximal ankle

plantarflexion, associated spontaneous hip adduction was quantified on the paretic lower extremity. In contrast, when instructed, individuals were able to neutralize the hip adduction torque and generate a net hip abduction torque. This indicates that neural resources are available to decouple ankle plantarflexion/hip adduction contrary to the hip ext/add which could not be decoupled in individuals with chronic hemiparesis due to a stroke.

Disclosures: **N. Sanchez:** None. **J.P.A. Dewald:** None. **R. Lopez-Rosado:** None.

Poster

072. Motor Deficits

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Program#/Poster#: 72.07/HH8

Topic: D.17. Voluntary Movements

Support: Department of Defense, Telemedicine & Advanced Technology Research Center W81XWH-09-2-0131 (Healton, PI)

Title: Transcallosal effects of chronic below-elbow amputation: Behavior and physiology

Authors: *E. CHAN[1,2], E. BRECEDA[1,3], F. SANDBRINK[3], A. DROMERICK[1,3,4], P. LUM[3,5], S. MOHAPATRA[1,2,4], R. SILVA[1], M. HARRIS-LOVE[1,4];
[1]Res. Div., Natl. Rehabil. Hosp., Washington, DC; [2]MedStar Hlth. Res. Inst., Washington, DC; [3]Washington DC Veterans Affairs Med. Ctr., Washington, DC; [4]Georgetown Univ., Washington, DC; [5]Dept. of Biomed. Engin., The Catholic Univ. of America, Washington, DC

Abstract: Background: Large-scale cortical reorganization is known to occur after unilateral below-elbow amputation. The motor cortex representation for muscles proximal to the amputation extends into territory previously representing the distal arm muscles. The physiological effects of this "affected" hemisphere reorganization on homologous muscle representations in the "unaffected" hemisphere, and on motor performance of the intact arm, are not known. Methods: A convenience sample of 3 chronic (>1 yr) below-elbow amputees (age 33 ± 21 yrs) were enrolled. Participants performed a planar center-out reaching task using the IM2 Manus robot in which they were asked to use a manipulandum to reach out toward 1 of 3 visual targets displayed on a computer screen. Repetitive reaching tasks were performed with each arm (intact or prosthetic) under two task conditions: null field and force field. In the null field condition, no perturbation was applied and no visual feedback of the manipulandum position was provided until the end of the trial. In the force field condition, a velocity-dependent force field

was applied to the manipulandum during early trials of the task and "catch" trials (force field unexpectedly removed) were randomly introduced afterwards. In a separate visit, transcranial magnetic stimulation was used to test interhemispheric inhibition of the biceps and triceps brachii representations of each hemisphere. Results: All participants performed the movements successfully with both the intact and the prosthetic arms. In the null field, 2 of the 3 participants showed greater endpoint error in the intact arm than the prosthetic arm: one participant had greater endpoint error for all 3 reaching targets (4.4 ± 0.7 cm vs. 3.5 ± 0.7 cm), and one participant had greater endpoint error for the target opposite to the reaching arm (5.1 cm vs. 3.9 cm). The third participant showed no such difference. The 2 participants with greater endpoint error in the intact than the prosthetic arm also had greater inhibition from the affected to the intact hemisphere than vice versa (55.2% vs. 35.5% and 41.7% vs. 21.6%, respectively). The participant who did not show this between-limb difference in endpoint error also did not show this imbalance in interhemispheric inhibition. In the force field conditions, between-limb differences were also observed, but were more variable among participants and reaching targets. Discussion: The preliminary analysis suggests that an imbalance in the strength of interhemispheric inhibition between proximal muscle representations after unilateral below-elbow amputation can contribute to the intact arm motor deficits that have been previously reported.

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Poster

072. Motor Deficits

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 72.08/HH9

Topic: D.17. Voluntary Movements

Support: Wellcome Trust

MRC

Title: Convergence of ipsilateral pyramidal and bilateral medial brainstem pathways onto cervical spinal interneurons after unilateral pyramidal tract lesion in monkeys

Authors: *B. ZAAIMI, S. N. BAKER;
Newcastle Univ., Newcastle Upon Tyne, United Kingdom

Abstract: We recently demonstrated that there are minimal direct connections from the ipsilateral corticospinal tract to cervical motoneurons in primates (Soteropoulos et al, 2011); this situation is unchanged after recovery from a unilateral pyramidal tract (PT) lesion (Zaaimi et al, 2012). On the other hand, the reticulospinal tract can activate primate hand muscles in healthy animals (Riddle et al., 2009), and these connections undergo changes after corticospinal lesions (Zaaimi et al, 2012), forming part of the substrate for functional recovery. Here we investigated the extent of ipsilateral cortical projections onto spinal interneurons and their convergence with pathways emerging from ipsilateral and contralateral brainstem involved in hand movement after the lesion of the contralateral pyramidal tract. Two female adult Macaca mulatta monkeys underwent a unilateral pyramidal tract lesion (left PT). After a six moths of recovery, an ipsilateral PT electode (iPT), bilateral medial longitudinal fasciculus (MLF) electrodes, and bilateral EMGs over 8 muscles were implanted. A stainless steel recording chamber was fixed above a laminectomy covering spinal segments C5-T1. Single neuron extracellular recordings were made in the intermediate zone from the right (recovered) side. During PT/MLF stimulation the animal was seated quietly. A total of 130 spinal interneurons in two monkeys were tested for responses to stimuli delivered through electrodes implanted in the iPT and right and left MLFs. Across recorded neurons which responded to at least one stimulus, convergent input from the iPT and both MLFs was the most common result (39/102 cells, 38%). Fewer neurons were excited solely by both MLFs without receiving an excitatory input from the iPT (16 cells, 16%). Cells that received an input from the iPT and only from one of the MLFs were rare (7% iPT and RMLF, and 5% iPT and LMFL). A small fraction of cells received an exclusive input from iPT (12 cells, 12%). These results show a high convergence of iPT with brainstem output onto interneurons in the spinal cord. This convergence may be enhanced by the plasticity occurring during recovery from the lesion, and could serve as a structural path for the ipsilateral cortex to control and modulate brainstem influence on hand movement.

Disclosures: **B. Zaaimi:** None. **S.N. Baker:** None.

Poster

072. Motor Deficits

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Program#/Poster#: 72.09/HH10

Topic: D.17. Voluntary Movements

Support: CIHR grant MOP111175

Spinal cord reseach chair CIHR attributed to S. Rossignol

Title: Effects of specific unilateral lesion of the primary hindlimb motor cortex on treadmill locomotion in adult rats

Authors: *M. DELCOUR, H. DELIVET-MONGRAIN, N. DANCAUSE, S. ROSSIGNOL; Univ. De Montréal / Dept. of Neuroscienc, Montreal, QC, Canada

Abstract: Motor rehabilitation strategies after spinal cord injury in humans and animals highlight that anatomical and functional plasticity occur at various levels of the Central Nervous System to support the motor recovery. It becomes therefore important to try and extricate the specific contributions of both supraspinal and spinal structures to the recovery movement such as locomotion. The present study aimed to determine how the primary motor cortex (M1) contributes to locomotion of the hindlimbs in rats. In preliminary experiments, we used intracortical microstimulation (ICMS) techniques to map the hindlimb (HL) representation in M1 of Long Evans rats and obtain stereotaxic coordinates for our lesions. In experimental rats (n = 7), we produced unilateral ischemic lesions (9 microinjections of 330 nl of Endothelin-1 diluted at 0.3 µg/µl) of the HL area. We investigated the impact of this lesion during 28 days on 1) locomotion on a treadmill, with video recordings taken at 120 Hz and speeds of 20 and 26 m.min-1 from which various kinematic parameters were extracted to evaluate the step cycle characteristics and interlimb coordination; 2) voluntary motor control of HL on a grid walk by measurements of foot faults. Preliminary results showed that the cortical lesions induced clear motor deficits of the contralateral HL during locomotion on treadmill in half of experimental rats (n = 3) while all animals exhibited deficits of locomotion on grid walk. Rats with deficits on treadmill displayed hyperextension of the ankle, drag or dorsal placement of the foot and hyperflexion of the knee. The cortical lesions also induced specific alteration of the left forelimb/HL coordination as well as of the hindlimbs coordination. On the grid walk, there was an increase of foot faults of the contralesional HL, supporting the idea that the lesions induced deficits of the voluntary control of this HL. So far, our results indicate that the locomotor impairments on treadmill decrease after the first postlesional week while deficits on grid walk persist. At the 35th postlesional day, we found, using ICMS techniques, that the HL representation in the ipsilesional M1 was completely destroyed by the lesions. In the contralesional M1, the total HL map area was larger than in control rats. Several questions are raised by these observations and will be pursued in the coming months. Is the fast recovery of the hindlimb dependent on compensatory mechanisms of preserved descending pathways or through changes occurring rapidly within the spinal cord? Are anatomical changes occurring fast enough to account for such recovery or are these mainly dependent on physiological changes in spinal and supraspinal pathways?

Disclosures: **M. Delcour:** None. **H. Delivet-Mongrain:** None. **N. Dancause:** None. **S. Rossignol:** None.

Poster

072. Motor Deficits

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Topic: D.17. Voluntary Movements

Support: March of Dimes Grant MOD 3877

Title: A novel, automated test of forelimb supination in the rat provides a sensitive measure of corticospinal tract function

Authors: ***A. SINDHURAKAR**[1], B. FLYNN[1], E. MEYERS[2], D. SLOAN[2], R. RENNEKAR, II[2], J. B. CARMEL[1,3];
[1]Burke Med. Res. Inst., White Plains, NY; [2]Biomed. Engin., Univ. of Texas at Dallas, Richardson, TX; [3]Brain and Mind Res. Inst. and Dept. of Neurol. and Pediatrics, Weill Med. Col. of Cornell Univ., New York, NY

Abstract: The corticospinal tract (CST) is the primary motor pathway for voluntary movement, and CST injury is largely responsible for motor impairment after injury. We study CST function in the rat to understand motor control, its loss with injury, and strategies for repair. Current tests of motor function in the rat are relatively insensitive to corticospinal injury. One movement that is highly impaired with CST injury in both rats and humans is forearm supination, turning the hand palm up. Supination brings the hand into position for manipulation, and loss of supination strongly impairs hand function. We developed a task to test supination in the rat. In this task, rats are placed into a reaching box and trained to reach through an aperture to a spherical manipulandum. Rats must grasp this "knob" and turn it in supination in order to receive a food reward. The knob is mounted to a rotary encoder that measures angles within a quarter of a degree of accuracy, and an attached computer samples angle position at 100 Hz. When the turn angle exceeds a user defined threshold, the computer signals a pellet feeder to give a reward and plays a reward tone. Rats associate the turning of the knob with a food reward over a few days. We then increased the angle the rats need to supinate in order to receive the reward using a continuous performance measure. This allows us to create learning curves. Training to a criterion turn angle of 60 degrees took an average of 4 weeks after which, we cut the CST at the pyramid. This lesion severely compromised the rats' ability to perform the supination task. We then

trained the rats intensively and measured their performance for 6 weeks. Rats regained only 40% of their supination ability on average compared to their baseline. Thus, we have created a novel test of rat forelimb function that measures key components of CST function: motor learning, loss with injury, and partial recovery with rehabilitation. The task is automated to allow testing and training of several rats at a time. We acquire quantitative data not just about turn angle, but about task kinetics: how quickly, smoothly, and accurately the knob is turned. Thus, we can accurately measure supination in the rat and use this to measure motor learning in health and after injury. We also show that this behavior relies strongly on the CST and allows an automated and quantitative assessment of CST function in the rat.

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Poster

072. Motor Deficits

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Topic: D.17. Voluntary Movements

Support: U.S. Agency for International Development (USAID), The Middle East Regional Cooperation Program (MERC)

Ministry of Science and Technology, Israel

BSF binational US Israel Science Foundation

Title: Locomotor adaptation in Cerebral Palsy patients is constrained by their increased performance variability

Authors: *F. MAWASE, L. SHMUELOF, S. BAR-HAIM, A. KARNIEL;
Ben-Gurion Univ., Beer Sheva, Israel

Abstract: Cerebral Palsy (CP) results from an insult or an injury to the developing brain before birth or in early childhood causing physical disability and a-typical motor patterns such as asymmetrical walking. Consequently, rehabilitation treatments for CP patients should address both the motor disability and the possible control impairments over the damaged system. To examine the interaction between these components in a rehabilitation-like treatment protocol, we studied the dynamics of step-asymmetry during a longitudinal locomotor adaptation task. Six

teenagers with CP were exposed to 30 sessions of adaptation to a novel split-belt treadmill over four months. Aged and gender matched controls were exposed to 10 sessions of adaptation. Adaptation rates and motor variability were computed throughout the adaptation sessions by analyzing the asymmetry and the variability of the center-of-pressure (COP). Compared to controls, CP patients showed slower adaptation (e.g., reduction in COP asymmetry) in the first session ($p<0.05$) and higher COP variability ($p<0.001$). By the last session, CP subjects adapted significantly faster ($p<0.01$) and showed a marked reduction in COP variability ($p<0.001$). To examine the improvement of CP patients at the control level, we modeled the data using a dual-state state-space model, assuming that locomotor adaptation is driven by a fast and a slow adaptation processes. Results showed that initially, CP subjects relied mainly on the slow stable process and the contribution of the fast process during initial adaptation was negligible. With practice, both the learning rate and the contribution of the fast liable process significantly increased. In addition to the changes in learning rates, the single subjects' fits to the models improved, pointing to the reduction in COP variability with training. To incorporate the improvement in adaptation with the changes in variability, we used a Bayesian state estimation model with a single state. Results showed that the improvement in learning can be explained by an increased sensitivity to prediction errors due to the reduction in observation noise, rather than by changes in state uncertainty. Our results suggest that the impaired adaptation of CP patients is primarily a result of their variable performance, and not of an impairment in state estimation. Thus, effective rehabilitation of CP patients should rely on two interacting processes: adaptation of a-typical motor patterns through shaping and reduction in variability through repetition training.

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Poster

072. Motor Deficits

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Topic: D.17. Voluntary Movements

Title: Transcranial direct current stimulation and upper extremity robotic therapy improves upper extremity function in an adult with cerebral palsy: a pilot study

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Abstract: Cerebral palsy is caused by a nonprogressive brain injury or stroke before birth or during the first two years of life. In unilateral spastic cerebral palsy (USCP), the damage has a unilateral predominance, resulting in weakness and motor skill deficits on one side of the body. People with USCP tend to under-use their paretic side, and do not develop robust motor control of the paretic side. As people with USCP age, motor deficits on the paretic side persist due to disuse of the paretic side. Few therapies exist for upper extremity rehabilitation in adults with USCP. The goal of the present study was to determine feasibility and efficacy of upper limb therapy in adults with CP. Upper extremity robotic therapy can improve arm movement deficits in adults after stroke. Transcranial direct current stimulation (tDCS) can augment the efficacy of robotic therapy when delivered immediately before training. We tested this same protocol in USCP. We hypothesized that tDCS plus upper extremity robotic training would be a safe, feasible protocol that improved upper extremity function. This pilot study is the first to our knowledge to test the efficacy of tDCS plus robotic therapy in an adult with USCP. Single-pulse transcranial magnetic stimulation (TMS) was used to map the motor representation of both hands. The participant received thirty-six sessions of therapy. During each therapy session, the participant received 20 min of 2mA anodal tDCS, immediately followed by robotic arm therapy. We applied anodal tDCS over the motor map of the paretic hand. During robotic therapy, the participant used his wrist and upper arm to follow a cursor in a center-out task for 1000 movements. We measured side effects of tDCS, kinematics of movements on the robot, and motor function of the affected upper extremity. Side effects of tDCS were mild transient scalp tingling and headache. Side effects dissipated within ten minutes of cessation of tDCS. No serious adverse events occurred. Therapy resulted in improved reaching accuracy and smoothness on the robotic task. These findings indicate that tDCS combined with robotic therapy can be used to safely improve motor deficits in adults with cerebral palsy.

Disclosures: **K.M. Friel:** None. **P. Lee:** None. **D. Gupta:** None. **A.R.P. Smorenburg:** None. **H. Kuo:** None. **D.J. Edwards:** None.

Poster

072. Motor Deficits

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Program#/Poster#: 72.13/HH14

Topic: D.17. Voluntary Movements

Support: NIH Grant R01NS079569

Title: Motor cortex inactivity during a key period of development permanently alters the organization of the rubrospinal system

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Abstract: Forelimb motor skills require two key inputs from the brain—the corticospinal (CS) system and the rubrospinal system (RS), the other major descending system for forelimb control—to act precisely on spinal motor circuits. The interplay of these two systems during development is poorly understood and knowledge may inform the pathophysiology of common developmental motor disorders, such as cerebral palsy. We previously showed in cats that disrupting activity of the CS system during a key developmental period (postnatal weeks, PW, 5-7) diminishes the cortical motor map and miswires connections with spinal circuits. To further understand how CS impairments affect motor system development we looked for changes in the RS system, which originates in the red nucleus (RN). Our hypothesis is that the RS motor map and spinal connections can compensate for impaired CS development. To determine the role of motor cortex activity on RS development we continuously infused muscimol (10mM, 0.5ul/hr) into the M1 forelimb area unilaterally via an osmotic minipump from weeks 5-7 in cats. We traced RS tract projections to the spinal cord using BDA or BDA-Alexafluor 488. In a terminal experiment at weeks 7-8 (previously reported for some findings; SFN 2012), or 12-14 (young adulthood) when the RS map has reached a mature organization, the RN motor maps were examined bilaterally using microstimulation. Motor cortex inactivation accelerated development of the RN motor map on the side in which M1 was inactivated (termed “inactive side”) at PW 7/8, and this persisted into young adulthood. Microstimulation thresholds in the inactive side to evoke contralateral forelimb movements were decreased compared to age-matched controls. Conversely, the RN map on the side in which M1 remained active (“active side”) had a paucity of effective sites and elevated thresholds at PW 7/8, suggesting a delay in RN map maturation. Remarkably, the RS never recovered; thresholds on the active side remained elevated and the number of effective sites were decreased compared with age-matched controls. Analyses of changes in RS tract axon terminations are ongoing. These results demonstrate that reducing M1 activity alters development of the RS system. The increased efficacy of RN output from the inactive side suggests competition between the developing CS and RS for access to spinal motor circuits during a key period of development. Harnessing this competition between CS and RS may lead to new therapeutic approaches to treat developmental motor disorders.

Disclosures: **P.T. Williams:** None. **D. Zuelke:** None. **S. Kim:** None. **A.P. Chang:** None. **J.H. Martin:** None.

Poster

073. Cortical Motor Planning

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 73.01/HH15

Topic: D.17. Voluntary Movements

Support: NHMRC Program Grant 1055084

Title: Antagonist muscle responses during sustained isometric maximal voluntary contraction

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Abstract: Agonist and antagonist muscles are often co-activated. For example, during a fatiguing submaximal contraction, descending drive increases to recruit more of the agonist muscle to maintain force. At the same time, antagonist muscle activity increases, as do the antagonist muscle's responses to cortical and subcortical stimulation (Levenez et al. 2008). During fatiguing maximal efforts, descending drive becomes suboptimal to produce maximal muscle force but it is not known whether it decreases in absolute terms (Gandevia et al. 1996). Here we examined activity and responses to stimulation in the antagonist muscle during a sustained knee extensor maximal voluntary contraction (MVC). Methods. Subjects (n=9) performed brief MVCs and then a fatiguing 2-min MVC of the knee extensors on 2 days. Surface EMG was recorded from an agonist muscle, vastus lateralis, and an antagonist, biceps femoris. On one day transcranial magnetic stimulation elicited motor evoked potentials (MEPs) during MVCs. On the other day, electrical stimulation over the thoracic spine to activate descending tracts elicited thoracic motor evoked potentials (TMEPs). Results. During the brief extensor MVCs, biceps femoris rms EMG was ~20% of maximal. During the sustained MVCs, knee extensor force fell by 69±9 %, vastus lateralis EMG fell by 45±39% and biceps femoris EMG fell by 50±19%. For the agonist muscle vastus lateralis, MEP area increased by 45% (42±18 to 61±2 mVms; $p<0.05$) from the brief MVCs to the end of the 2-min MVC whereas TMEP area did not change. In contrast, for the antagonist muscle biceps femoris, MEP area decreased 46% from 46±21 to 25±12 mVms ($p<0.01$) and TMEP area decreased 66% from 17±12 to 6±3 mVms ($p<0.05$). Conclusion. Thus, as previously seen, excitability of motor cortex related to the agonist muscle increased during a fatiguing maximal contraction despite the fall in agonist EMG. For the antagonist muscle, falls in the EMG, TMEP and MEP indicate that net excitatory drive to the motoneurones decreased. We propose that the fall in antagonist motoneurone activity suggests that descending drive to the agonist muscle is also reduced during a sustained fatiguing maximal effort. References Levenez et al. (2008) Cortical and spinal modulation of antagonist coactivation during a submaximal fatiguing contractions in humans. J Neurophysiol 99: 554-563.

Gandevia et al. (1996) Supraspinal factors in human muscle fatigue: evidence for suboptimal output from the motor cortex. J Physiol 490: 529-536.

Disclosures: **J.L. Taylor:** None. **C.J. McNeil:** None. **S.C. Gandevia:** None. **D.S. Kennedy:** None.

Poster

073. Cortical Motor Planning

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Topic: D.17. Voluntary Movements

Support: PVA Grant 2968

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Title: Interactions between I-waves in human motor cortex

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Abstract: Paired transcranial magnetic stimulation (TMS) applied over the motor cortex has been used to assess the temporal discharge of corticospinal volleys in surface electromyographic recordings in intact humans. Although indirect (I) wave volleys have been described at rest and during voluntary activity, little is known about the interactions between early and late volleys. Here, we used paired-pulse TMS over the hand representation of the motor cortex at a range of interstimulus intervals and intensities to assess the latency, amplitude, and duration of I-waves (I1, I2, and I3) in the first dorsal interosseous muscle across multiple trials. We demonstrate that I-waves occurred at consecutive intervals of around 1.5 ms (I1=1.37±0.10 ms, I2=1.33±0.14 ms, I3=1.56±0.18 ms) and that the amplitude and duration of the I3 (171±23 %, 0.45±0.14 ms) was lower than those of the I1 (234±75 %, 0.57±0.15 ms) and I2 (204±42 %, 0.60±0.20 ms). The variability between trials for latency (I1=0.03±0.09 ms, I2=-0.01±0.08 ms, I3=0.08±0.21 ms), amplitude (I1=-1.68±49.09 %, I2=12.64±29.51 %, I3=-1.78±21.05 %), and duration (I1=0.02±0.20 ms, I2=-0.04±0.27 ms, I3=-0.04±0.15 ms) was similar across I-waves. The amplitude but not latency and duration of successive I-waves correlated with that of the

preceding I-wave. I3 and I1 amplitudes were also correlated. Our findings provide evidence for a specific relationship between consecutive I-waves amplitude in humans, although a different configuration exists among late and early I-waves. Thus, our results argue against the possibility that the generation of I-wave periodicity involves a neural oscillator model, but instead it might result from a combination of neuronal elements with fixed and independent temporal characteristics.

Disclosures: **M.A. Savisky:** None. **J. Cirillo:** None. **M.A. Perez:** None.

Poster

073. Cortical Motor Planning

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 73.03/HH17

Topic: D.17. Voluntary Movements

Title: The influence of spontaneous movement tempo on motor resonance during action observation

Authors: *M. BOVE, G. LAGRAVINESE, A. BISIO, L. PERASSO, P. RUGGERI, L. AVANZINO;
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Abstract: Previous studies have shown that during action observation (AO) we use specialized motor representations to understand the observed actions. The higher is our motor familiarity with the observed action the more the “mirror system” is active. Thus, the observation of action involves matching to the individual’s motor repertoire. In this context, a question that remains still open is whether the temporal aspects of the subjective motor repertoire can influence the “mirror system” in the process of recognizing a certain movement. Our motor repertoire includes over-learned movements, that are well characterized in terms of temporal organization and it has been shown that, during the execution of a number of voluntary movements, healthy subjects have a common spontaneous movement tempo (SMT) ranging around 2Hz. To elucidate the influence of SMT in AO, in a first experiment we investigated the excitability of the left primary motor cortex (M1) during the observation of videos showing a right hand performing repetitive finger opposition movements at a rate similar to, lower or higher than the spontaneous one (i.e., 2Hz). As a result, we found that, during the 2Hz video presentation, left M1 excitability was increased more than during a video showing finger movements performed at a rate lower or higher than 2Hz. Starting from the notion that the motor evoked potential (MEP) facilitation is

considered evidence of motor resonance effects, here we demonstrated that motor resonance is influenced by the temporal properties of the observed movements (temporal motor resonance). To better address this last assumption we designed a second experiment aimed to (i) modify the SMT by a training based on AO and (ii) evaluate whether and how this training could affect the temporal motor resonance of the left M1. Here, we hypothesized that if the execution-observation matching system selectively recognizes the 2Hz rate as the SMT for finger movements, a training based on the observation of a 10-minutes video showing finger movements performed at a rate higher than 2Hz (3Hz video) could modify the SMT and consequently the temporal motor resonance. The results of this second experiment showed that training induced a shift of the SMT towards 3Hz and this was associated with changes in left M1 excitability during AO. Indeed, the MEPs collected during the 2Hz video presentation after the training were less facilitated than those collected in the same condition before it. Our results suggest that during AO the temporal properties of a movement are recognized and that the temporal motor resonance can be modulated by means of an AO training.

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Poster

073. Cortical Motor Planning

Location: Halls A-C

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Topic: D.17. Voluntary Movements

Title: Effects of concussion on motor cortex inhibition: A one-year follow-up

Authors: *A. L. YASEN, N. R. MILLER, L. F. MAYNARD, A. D. CHRISTIE;
Human Physiol., Univ. of Oregon, Eugene, OR

Abstract: In a previous study, we documented greater intracortical inhibition in acutely-concussed individuals, which did not recover to control levels two months post-injury. The time course of recovery of inhibition, therefore, remains unclear. As such, in the current study we re-evaluated motor cortex function in concussed participants at one year post-injury to determine recovery of cortical excitability and inhibition. Five concussed (20.2 ± 0.8 yr; 3 females) individuals, who had previously been tested at 72 hours and two months post-injury, returned for assessment one year following injury. Cortical excitability of the first dorsal interosseous (FDI) muscle was assessed through the resting motor threshold (RMT) using transcranial magnetic

stimulation (TMS). Intracortical inhibition was assessed in the FDI through the duration of the cortical silent period (CSP), obtained with a TMS pulse delivered at 120% RMT during an isometric contraction at 50% of maximum EMG activity. RMT values were higher at the 72-hour time point than at the two month time point (effect size (ES)=0.84) and the one year time point (ES=1.04). However, there was no difference in RMT between two months and one year post-injury (ES=0.05). As noted in our previous study, there was no difference in CSP duration in concussed individuals between 72 hours and 2 months post-injury (ES=0.37). However, at one year post-injury, CSP duration was shorter than both the 72-hour (ES=0.48) or two month (ES=0.94) time point. These results suggest that although cortical excitability is acutely affected by concussion, it appears to resolve within two months and is maintained at one year post-injury. Although inhibition of the motor cortex remains elevated up to two months post-injury, it appears that this enhanced inhibition is resolved within one year after injury.

Disclosures: **A.L. Yasen:** None. **N.R. Miller:** None. **L.F. Maynard:** None. **A.D. Christie:** None.

Poster

073. Cortical Motor Planning

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Topic: D.17. Voluntary Movements

Support: JSPS KAKENHI Grant Number 25233

Title: Neural network model including muscle synergies constructed based on redundant motor control simulation

Authors: *S. HAGIO[1,2], M. KOUZAKI[1];
[1]Grad. Sch. of Human and Envrn. Studies, Kyoto Univ., Kyoto, Japan; [2]Res. Fellow of the Japan Society for the Promotion of Sci., Tokyo, Japan

Abstract: Introduction The concept of muscle synergies has been proposed to simplify redundant motor control by low-dimensionally organizing functionally similar muscles (Hagio and Kouzaki, 2014; Tresch et al, 1999). However, muscle synergies remain no more than conjecture, and it is necessary to examine the interaction between synergies and neurons in the neural circuitry and the contributions of synergies to motor control, such as motor learning. The purpose of this study was to construct neural network model including muscle synergies.

Methods The neural network model including 3 intermediate layers was constructed based on the previously used model (Hirashima and Nozaki, 2012). We assumed isometric force-generation around right wrist on a horizontal plane (elbow and shoulder joint flexion angles: 90°and 30°, respectively). The first intermediate layer contained 1000 neurons in the primary motor cortex (M1). Each neuron received a desired shoulder flexion and/or elbow flexion torque vectors from the input layer with a synaptic weight (W_{neu}). These M1 neurons were connected to the second intermediate layer that indicated the muscle synergies with a uniformly distributed constant innervation. Therefore, we assumed that muscle synergies were intermediate neurons in the spinal cord: the connections between M1 neurons and muscle synergies were cortico-spinal pathway. The muscle synergies controlled 26 muscles spanning elbow and shoulder joints with a synaptic weight (W_{syn}) in the third intermediate layer. The muscle mechanical pulling vector was determined by physiological parameter of each muscle. An error back-propagation algorithm (Rumelhart et al., 1986) was successively used to modify W_{neu} and W_{syn}. The network was trained to produce appropriate output torque by randomly presenting 12 target torques, which were uniformly distributed on the two joint torque plane with the same intensity. The experiment was additionally conducted, which required the same task as above (5 repetitions in one direction). We then extracted muscle synergies by 8 recorded muscle activities using non-negative matrix factorization (Lee and Seung, 1999). **Result & Discussion** At least 4 synergies were required to appropriately produce the desired torques. The synaptic weights (W_{syn}) between synergy and muscle layers were similar to the extracted muscle synergy weightings in the experiment, when we assumed 4 synergies (the 26 muscles in the model were grouped into 8 groups). Therefore, we could construct the neural network model including muscle synergies, which demanded the similar muscle activations in the actual neural circuitry to achieve the desired torques.

Disclosures: **S. Hagio:** None. **M. Kouzaki:** None.

Poster

073. Cortical Motor Planning

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Topic: D.17. Voluntary Movements

Support: NSERC grant 222920-2010

CIHR MOP 84403

Title: Is there a link between pre-synaptic inhibition and muscles mechanics?

Authors: *F. CREVECOEUR, S. H. SCOTT;
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Abstract: A large literature documents the presence of inhibitory interneurons in the spinal cord forming axo-axonic connections with sensory afferents. This pre-synaptic inhibition is known to be active during voluntary movements and is hypothesized to filter relevant information during movement control. However, it also synapses onto motoneurons and reduces the gain of sensory feedback. Notably, recent work emphasizes that removing this set of interneurons results in oscillations during reaching but not during postural control in rodents (Fink et al., Nature, 508:43-48), suggesting that pre-synaptic inhibition plays a central role during control. However, this role remains unclear and the reasons why motor deficits were induced during reaching and not postural control remains to be elucidated. There is also a large literature illustrating that muscle exhibit dramatic changes in intrinsic elastic properties between posture and movement. Muscles generate high resistive force when stretched over a short range (high stiffness), which drops by an order of magnitude when muscles are stretched beyond ~2.5% of their initial length (low stiffness). This drop in stiffness also occurs when we generate movement. Here, we hypothesize that the pre-synaptic inhibition and changes in muscle stiffness are intimately linked, such that spinal feedback processes complement the mechanical properties of muscles. Specifically, high stiffness during posture can support higher direct feedback gains without compensating for temporal delays, whereas the presence of low stiffness during movement requires a reduction of these control gains to avoid oscillations or instability. This relationship also suggests that reducing spinal feedback during movement requires a greater reliance on internal models expressed by supra-spinal circuits in order to handle low stiffness and larger sensorimotor delays. Further, we present simulations highlighting two sources for the generation of oscillatory behavior when pre-synaptic inhibition is removed. First, oscillations can occur because spinal gains adequate during posture become too high during movement due to the change in muscles properties. A second possible source of oscillations upon removal of pre-synaptic inhibition is the mismatch between expected and actual spinal feedback, which can destabilize long-latency (cortical) control as a consequence of internal model error. To summarize, we suggest that control solutions of spinal and supra-spinal pathways are partially shaped by muscles thixotropic properties and account for changes in muscles biomechanics occurring between posture and movement tasks.

Disclosures: **F. Crevecoeur:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Queen's University. **S.H. Scott:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Queen's University.

Poster

073. Cortical Motor Planning

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Topic: D.17. Voluntary Movements

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Title: Common low-frequency dynamics in movement and sleep

Authors: *A. JACKSON, T. M. HALL, F. DE CARVALHO;
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Abstract: It has been known for over a century that upper-limb movements are often composed of discrete submovements, but the origin of this intermittency remains unclear. While neural correlates of submovement frequencies around 2-3 Hz can be found in the primary motor cortex (M1), the temporal profile of movement kinematics is usually assumed to be determined by extrinsic factors such as limb biomechanics and sensory feedback delays. However, another possibility is that movement intermittency arises from intrinsic rhythmicity in motor networks generating low frequencies in behavior. Delta activity recorded in the electroencephalogram during slow-wave sleep and from isolated cortical slices points to the existence of neural oscillators at frequencies similar to those found in behavior. However, to our knowledge the low-frequency dynamics of brain activity during movement and sleep have not been directly compared. We therefore used chronic multi-electrode arrays to record neural activity and local field potentials (LFPs) from M1 and ventral premotor cortex (PMv) in monkeys during an isometric movement task, natural sleep and ketamine sedation. We used principal component analysis to project the low-frequency LFP onto a plane, and observed cyclic trajectories in M1 that were phase-locked to each submovement. The areal velocity of trajectories increased for faster submovements, but the angular frequency remained constant at around 3 Hz. During sleep, LFP activity traversed cycles with the same frequency and direction of rotation (albeit with larger amplitude) and under ketamine sedation these were phase-locked to K-complexes occurring at the transition from down- to up-states of the cortex. Neural activity was locked to LFP cycles within the same cortical area under all behavioral conditions, and became synchronized across areas during sleep and sedation. Since the same cortical dynamics are observed during movement and in the absence of behavior during sleep and sedation, we suggest that the motor networks controlling the upper-limb possess intrinsic, low-frequency rhythmicity.

In the awake state, periodic descending drive from M1 constrains the temporal structure of tracking movements, while widespread synchronization across cortical areas generates the well-known delta rhythms associated with slow-wave sleep.

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Poster

073. Cortical Motor Planning

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Topic: D.17. Voluntary Movements

Support: Intramural Research Program of NIH/NINDS

Title: Distinct interneuronal networks influence excitability of the surround during movement initiation in humans

Authors: N. THIRUGNANASAMBANDAM[1], R. KHERA[1,3], H. WANG[1,4], S. KUKKE[1,5], *M. HALLETT[2];
[1]NINDS/NIH, Bethesda, MD; [2]Human Motor Control Sec, NINDS/NIH, BETHESDA, MD; [3]Univ. of Iowa Carver Col. of Med., Iowa City, IA; [4]Peking Union Med. Col. Hosp., Beijing, China; [5]Catholic Univ. of America, Washington, DC

Abstract: Surround inhibition (SI) is a feature of motor control for which the physiology is still unknown (Beck and Hallett, 2011). This phenomenon is impaired in patients with focal hand dystonia (Sohn and Hallett, 2004, Beck et al. , 2008) and hence it becomes relevant to study it more in detail. Several studies have demonstrated SI in the human motor system using transcranial magnetic stimulation (TMS) (Beck et al. , 2008, Sohn and Hallett, 2004, Kassavetis et al. , 2012). In all these studies SI was measured by applying a single suprathreshold TMS pulse during the movement initiation phase. The figure-of-eight coil was positioned such that it induced current along the postero-anterior (PA) direction in the brain. We were interested to study SI not just at a single intensity, but at intensities ranging from sub-threshold to supra-threshold levels. Also, we wanted to explore if SI could be elicited when the motor cortex was stimulated along other directions (antero-posterior (AP), latero-medial (LM)). Fifteen healthy volunteers participated in the study. Their task was to perform a brief isometric index finger flexion on hearing a tone. EMG was recorded from the first dorsal interosseous (active) and the abductor pollicis brevis (surround) muscles. Single-pulse TMS was applied at intensities ranging

from 20% to 100% of maximum stimulator output at 5% intervals either before the tone (rest) or after the tone ~50 ms before expected onset of muscle activity (SI). The MEP amplitudes were then plotted against stimulation intensities to obtain the MEP recruitment curves for the rest and SI conditions separately. The recruitment curves were plotted with the coil held in three directions for every subject. We found that SI can be elicited when the coil was positioned in the P-A direction. Stimulating the motor cortex along the A-P direction did not elicit SI, rather showed surround facilitation. LM stimulation elicited inhibition non-specific to the surround muscle. We can therefore conclude that SI can be elicited in those neuronal groups that can be stimulated best along the PA direction. The clinical relevance of surround facilitation needs further exploration.

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Poster

073. Cortical Motor Planning

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Topic: D.17. Voluntary Movements

Support: NS40412

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Title: Representing the dynamic effects of neural populations in the motor system

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Abstract: We have developed the theory of "Stochastic Dynamic Operators" in order to describe the effect of populations of neurons on dynamical motor behavior. SDOs are the equivalent of a state-dependent spike-triggered average, but they operate on probability distributions. As a result, SDOs have the following properties: Linearity: The effect of populations of neurons can be predicted by adding the operators for each neuron. Stability: Different stability types

including point attractors and cyclic pattern generators can be modeled. Interconnectivity: The effect of neurons on other neurons can be modeled using the same methods. Reflex Response: State-dependence of action means that reflexes can be easily described. Measurability: Quantitative predictions of operators can be derived from standard datasets. Controllability: Once estimated, operators can be selected to achieve real-time control, either by emulating control on a robot or by modulation of populations of neurons with known responses. We recorded from 8 well-isolated units on each of 6 tetrodes implanted in the lumbar spinal cord of decerebrate frogs during reflex wiping movements triggered by skin stimulation. EMG was recorded for 10 minutes from 10 hindlimb muscles. The position and velocity of the ankle were recorded. SDOs were calculated from the firing pattern of each cell and the immediately preceding and following kinematic variables (position and velocity) and EMG. SDOs were also calculated that related the firing of each cell to the preceding and following firing of the other recorded cells. We found subsets of cells that had different effects on the dynamics. Certain cells appear to cause a relatively pure translation, whereas others appear to stabilize at specific positions or velocities. Certain cells appear to either increase or decrease EMG, while others appear to stabilize EMG at intermediate values. When a cell was found whose firing was related to the modulation of other cells, the SDOs could be compared. In particular, we found examples where cell A appeared to modulate the firing of cells B and C, and the effect of cell A on EMG (estimated from its SDO) was well-predicted by the combined effect of cells B and C, consistent with cell A exerting its effect at least partially through modulation of cells B and C. Therefore stochastic dynamic operators appear to be a new and highly useful tool for understanding the effect of individual cells and populations of cells on movement. This theory extends the basic techniques of spike-triggered averaging and peri-stimulus-time histograms to be directly applicable to cells that have both sensory tuning and motor effects.

Disclosures: **T.D. Sanger:** None. **S.F. Giszter:** None.

Poster

073. Cortical Motor Planning

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ANR Grasp

Title: Latency of LFP beta power peak during movement preparation correlates with reaction time

Authors: *L. ZEHL[1], M. DENKER[1], S. GRÜN[1,2,3], A. RIEHLE[4,1,3], T. BROCHIER[4]; [1]Inst. of Neurosci. & Med. (INM-6), Inst. for Advanced Simulation (IAS-6), Jülich Res. Ctr. and JARA, Jülich, Germany; [2]Theoretical Systems Neurobio., RWTH Aachen Univ., Aachen, Germany; [3]RIKEN Brain Sci. Inst., Wako-Shi, Japan; [4]Inst. de Neurosciences de la Timone (INT), UMR 7289, CNRS – Aix Marseille Univ., Marseille, France

Abstract: Beta oscillations (15-30 Hz) are a prominent feature of neuronal population signals recorded in the sensorimotor cortex during motor behavior [rev.: Kilavik et al, Exp Neurol 245:15 (2013)]. They have been extensively studied in instructed delay tasks in which a cue provides prior information about the movement to be performed after a GO signal presented at the end of the delay. In such tasks, beta power often exhibits an initial peak of high power shortly after the cue presentation, then decreases during the delay and is lowest during movement execution. It was proposed that the pre-movement decrease in beta power is related to an increased activity in motor cortex related to the upcoming movement execution [e.g. Kilner et al, Eur J Neurosci 21:2547 (2005)]. Thus, we aim to test the hypothesis that the temporal profile of beta power modulations during movement preparation correlates with behavioral measures of the movement. To address this issue, we here analyze how reaction time (RT) as a behavioral measure relates to the modulations of motor cortical LFP beta power in monkeys trained to perform a delayed reach-to-grasp task. In this task, a cue instructs to use either a precision grip (PG) or a side grip (SG) to grasp an object. After a fixed delay of 1 s, the GO signal provides additional information about the object load and instructs the monkey to grasp the object, pull it and hold it in a narrow position window for 500 ms to receive a food reward. In the reverse task, the object load is provided first. Neuronal activity was recorded by using a 100 electrode "Utah" array, chronically implanted at the MI/PMd border. We quantify the beta power using the amplitude of the analytic signal of the beta filtered LFP and confirm that the averaged time course of beta power across trials shows the described temporal profile of power modulations. On a single trial basis, we find that the beta profiles indeed vary with RT. When the cue provides information about the grip, we observe that for short RTs (<200 ms) the initial power peak during the delay appears earlier than for long RTs (>400 ms), and find that the latency of the power peak significantly correlates with the RT. In addition, in trials with short RTs the power for PG is on average larger than for SG. In contrast, in trials with long RTs both grip types exhibit similar power profiles. Finally, in the reverse task, the profiles during the preparatory period do not correlate with the RT and are independent of the grip type. Based on these

findings, we suggest that the power peak during the delay period reflects processes which are functionally related to movement planning and affected by the characteristics of the upcoming movement such as the selected grip type.

Disclosures: **L. Zehl:** None. **M. Denker:** None. **S. Grün:** None. **A. Riehle:** None. **T. Brochier:** None.

Poster

073. Cortical Motor Planning

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NSF award number DMS-1225647

Career Award at the Scientific Interface to TJG from the Burroughs Welcome Fund

Title: Sequence generation within spatio-temporal cycles of inhibition

Authors: ***J. CANNON**, J. MARKOWITZ, N. KOPELL, T. GARDNER;
Boston Univ., Boston, MA

Abstract: Overview: We propose a theory for robust sequence generation in neuronal networks. In previous papers, rhythmic neural activity has been characterized as counterproductive for reliable sequence generation [1]. In our model, rhythm plays a key role in sequence generation. The model is based on two recent observations in songbird premotor cortex: (1) interneuron and principal cell activity are locked to different phases of the 25-35 Hz band of a stereotyped LFP [2], and (2) principal cells are strongly coupled via disynaptic inhibition [3]. In the model, long sequences of principal cells fire within the constraints of larger-scale inhibitory network dynamics. When principal cell activity reaches a cortical subregion, it triggers strong, locally diffuse feedback inhibition on a 30ms time scale. The feedback quickly suppresses local activity, but other cortical subregions are disinhibited at any given time, allowing activity to continue and propagate from one subregion to the next as each becomes disinhibited. Results: Due to this spatio-temporal coordination, the timing of sequence playback is robust to noise and to various

perturbations even in the absence of redundant feed-forward connectivity, making the model fundamentally distinct from the synfire chain. We draw on the theory of discrete dynamical systems to analytically demonstrate timing stability. We also present preliminary simulations that show that the proposed mechanism produces long, stereotyped sequences even when the connections between principal neurons are randomly generated. The model concisely accounts for the observed single-cell and network-level dynamics in songbird, and can be generalized to provide a similar explanation for traveling waves observed in primate motor cortex. [1] Aviel, Y., et al. On embedding synfire chains in a balanced network. Neural Computation 15, 1321:1340 (2003). [2] Markowitz, J. E., Guitchounts, G. & Gardner, T. J. Spatial organization of synchronous cell assemblies in HVC. Program No. 675.01/KKK59 Neuroscience 2013 Abstracts. San Diego, CA: Society for Neuroscience, 2013. [3] Kosche, G. & Long, M. A. Inhibitory coupling in a cortical premotor pattern-generating network. Program No. 782.03/MMM26 Neuroscience 2013 Abstracts. San Diego, CA: Society for Neuroscience, 2013.

Disclosures: **J. Cannon:** None. **J. Markowitz:** None. **N. Kopell:** None. **T. Gardner:** None.

Poster

073. Cortical Motor Planning

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Topic: D.17. Voluntary Movements

Support: the Japan Society for the Promotion of Science (#22500283)

Grant-in-Aid for Scientific Research on Innovative Areas(#22120504, #24120703)

Title: Spatiotemporal properties of current source density in the prefrontal cortices of behaving monkeys

Authors: *N. KAWAGUCHI[1,2], K. SAKAMOTO[3], K. YAGI[2], M. AOKI[1], H. MUSHIAKE[2,4]; [1]Dept. of Neurol., Tohoku University, Sch. of Med., Sendai, Miyagi, Japan; [2]Dept. of Physiol., Tohoku Univ. Sch. of Med., Sendai, Japan; [3]Tohoku University, Res. Inst. of Electrical Communication, Sendai, Japan; [4]The Core Res. for Evolutional Sci. and Technol. Program (CREST), JST, Tokyo, Japan

Abstract: Various types of information are integrated and processed in the prefrontal cortices (PFC), and thus exploring the input-output properties of the neuronal networks is fundamental to

understand mechanisms underlying the function of the PFC. However, it has been technically difficult to examine synaptic inputs into neuronal circuits of behaving animals. Recent development of multi-contact electrodes allow us to evaluate the synaptic inputs by current source density (CSD) analysis of local field potentials (LFPs) in addition to recording spikes. We conducted CSD analysis on LFPs recorded in the PFC during monkeys performing a shape manipulation task. We observed task-dependent spatiotemporal patterns of current sinks, which were considered to reflect the synaptic inputs to neurons adjacent to the recording sites. Specifically, the current sinks in the inferior convex of the PFC were observed during the delay period, whereas the current sinks in the principal sulcus were observed selectively during the sample cue and test cue periods. We found tangentially periodic patterns of current sinks, which appears to correspond to the columnar structures suggested in the PFC microcircuits. The input-output properties revealed by the CSD analysis will elucidate how the PFC plays executive roles in terms of local neural networks.

Disclosures: **N. Kawaguchi:** None. **K. Sakamoto:** None. **M. Aoki:** None. **H. Mushiake:** None. **K. Yagi:** None.

Poster

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Topic: D.17. Voluntary Movements

Support: NIH Grant K23NS073626

NIH Grant R01 NS048527

Title: Children with autism show decreased cortical activation with praxis movements

Authors: ***J. B. EWEN**[1], B. M. LAKSHMANAN[2], C. NETTLES[3], M. HALLETT, MD[4], N. E. CRONE[5], S. H. MOSTOFSKY[3];
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Abstract: Objective: While altered cerebral connectivity has long been a dominant systems-level theory in the pathophysiology of autism spectrum disorders (ASD), relatively little is known

about the role of altered local cortical functioning. We leveraged the ability of EEG to record oscillatory activity involved in cortical function: theta (3-7 Hz), alpha (8-12 Hz) and beta (13-30 Hz). Individuals with ASD have well documented deficits in complex motor control, such as imitation and praxis. Praxis competence correlates with social and communicative abilities in ASD. Because the motor network is particularly well mapped, examination of motor function in ASD provides a clear window into abnormalities of brain function. We hypothesized that children with ASD would demonstrate alterations of event-related power modulation associated with performance of a praxis task. Methods: In 17 children with ASD (ages 8-12y) and 25 typically developing (TD), age-/sex-matched controls, we recorded full-scalp EEG while the children performed a task in which they pantomimed the use of 12 different tools with their right hands. The task included a preparation phase and a gesture execution phase. Event-related desynchronization (ERD) refers to a task-related decrease in the power in a specific frequency band, whereas event-related synchronization (ERS) refers to an increase. We examined ERD/S in the three frequency bands, at left central and posterior (parietal/occipital) sites: regions known to be associated with praxis function. Results: Compared with controls, children with ASD demonstrated less ERD: 30% less left central beta ($p = 0.005$) and 43% less left posterior alpha ($p = 0.006$) ERD during the preparation phase, as well as 33% less posterior alpha ERD ($p = 0.048$) during the execution phase. Other comparisons did not show significant differences. It is meaningful that alterations of beta ERD were seen at central sites, given the known role of beta in motor function. Similarly, alpha ERD has a role in visual function.

Disclosures: **J.B. Ewen:** None. **B.M. Lakshmanan:** None. **C. Nettles:** None. **M. Hallett:** None. **N.E. Crone:** None. **S.H. Mostofsky:** None.

Poster

073. Cortical Motor Planning

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Topic: D.17. Voluntary Movements

Support: NICHD PO1 HD064653

Title: Development of functional mu-rhythm from infancy through adulthood, and relations to upper/lower alpha

Authors: *S. G. THORPE, E. CANNON, N. FOX;
Dept. of Human Develop. and Quantitative Methodology, Univ. of Maryland Child Develop. Lab., College Park, MD

Abstract: Previous EEG/MEG studies with infants and children have established that a developmental analogue of adult occipital alpha-rhythm is present in lower frequency bands which increase with age towards the 8 - 13 Hz band typically seen in adults. Additional studies have described a functionally distinct rhythm observable at central electrodes at frequencies overlapping the developing alpha band. This infant/child central rhythm has been shown to desynchronize during voluntary motor acts, and so shares some topographic and functional properties with adult mu-rhythm, with which it has been proposed to be analogous. In this study we characterize the evolving properties of mu and alpha rhythms in three subject populations consisting of 12-month old infants, 4-year old children, and mature adults. We show that EEG for all subject groups contains spectral peaks evident in both the lower and upper developing alpha band, and that spectral and topographic properties of functionally identified mu rhythm (characterized by desynchronization during voluntary reaching/grasping movement) strongly reflect those of upper alpha in all subject groups. We also investigate structural development of mu-rhythm via sLoreta source reconstructions and show that the distribution of cortical desynchronization observed during movement becomes more focal to central and parietal cortices with age. These results provide insight into the developmental trajectories of the upper and lower alpha bands, as well as compelling evidence that the status of infant/child central rhythm as analogue of adult mu should no longer be considered ambiguous.

Disclosures: **S.G. Thorpe:** None. **E. Cannon:** None. **N. Fox:** None.

Poster

073. Cortical Motor Planning

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Topic: D.17. Voluntary Movements

Support: MDC scholarship (Helmholtz Foundation)

Alexander von Humboldt-Stiftung post-doc scholarship

Title: Modulation of tactile sensory responses during a sensory-triggered decision task in mouse forepaw primary motor cortex

Authors: *L. ESTEBANEZ, J. POULET;
Max Delbrück Centrum, Berlin, Germany

Abstract: M1 fires action potentials during voluntary movement but also to sensory stimulation. Here we address the function of sensory responses in M1 by developing a sensory triggered reach-and-press task in the awake, head-restrained mouse. Mice were trained to respond to a brief vibrotactile stimulus of the forepaw with a quick reaching movement of the same paw (<500ms) from a "rest" sensor, positioned under the mouse, to a "reach" sensor, positioned in front of the mouse. A "no-reach" task was interleaved with the "reach" task where the mouse learnt to withhold reaching after the same stimulus in a different context. Mice learned to perform hundreds of accurate reaches in less than 10 days of training (two 20 min sessions a day). Extracellular polytrode recordings were performed in forepaw M1 layer V in parallel with forepaw camera tracking at 200Hz. During the task, a large proportion of neurons displayed short latency spiking following the vibrotactile stimulus onset. The strength of firing to the stimulus was often different in reach trials as compared to no-reach trials.

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Poster

074. Noninvasive Neurophysiology

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Topic: D.18. Brain-Machine Interface

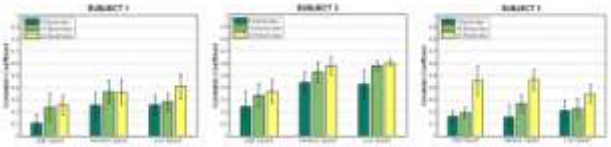
Support: Commission of the European Union under the BioMot project - Smart Wearable Robots with Bioinspired Sensory-Motor Skills (Grant Agreement number IFP7-ICT-2013- 10-611695)

Title: Analysis of electrode configurations for the decoding of knee angles from EEG signals during gait

Authors: *J. M. AZORIN, A. UBEDA, D. PLANELLES, A. COSTA, E. HORTAL, E. IAÑEZ;
Miguel Hernandez Univ. of Elche, Elche, Spain

Abstract: Although walking is automatically based on reflexes governed at the spinal level, there are evidences that suggest that the motor cortex is particularly active during specific phases

of the gait. In this work, we analyze the influence of walking speed by decoding knee angles from low frequency EEG components. Linear regression models are applied to show significant correlations between actual and decoded angles while different walking speeds are performed. To that end, three healthy subjects were asked to walk on a treadmill with three different speeds: 2, 3 and 4 km/h. Eight sessions were performed with one minute walking for each speed. Three different electrode configurations have been applied with 8, 16 and 32 electrodes distributed mainly over the motor cortex. Frontal electrodes were discarded and blinks were manually rejected. Afterwards, EEG signals and knee angles were simultaneously recorded. The EEG signals were filtered below 2 Hz and standardized. Knee angles were also standardized. The decoding method is based on a multidimensional linear regression. The decoding correlation coefficient was computed by applying an 8-fold cross-validation for each speed and electrode configuration. The results of the decoding performance show improvements when the number of electrodes increases (see Figure). The decoding performance is also affected by speed. For Subject 1 and 2, the decoding correlation coefficient significantly increases when the gait speed decreases. This is not so clear for Subject 3. However, future tests with more subjects will serve to generalize these preliminary results. This study has shown significant decoding correlations in the reconstruction of knee angles from low frequency EEG components. It has been also proved that the number of recorded electrodes affects the final decoding performance.



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Poster

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Topic: D.18. Brain-Machine Interface

Support: Deutsche Forschungsgemeinschaft (DFG)

Brain Products Munich

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Title: Brain communication in a completely locked-in-patient using an EEG system

Authors: *K. TAKANO[1,2], B. XIA[2], U. CHAUDHARY[2], G. GALLEGOS-AYALA[2,3,4], A. FURDEA[2], C. A. RUF[2], K. KANSAKU[1,5], H. FLOR[6], N. BIRBAUMER[2,7];
[1]Sys Neurosci Sect, Dept of Rehab for Brain Funct, Res. Inst. Natl. Rehab Cent,, Saitama, Japan; [2]Inst. of Med. Psychology and Behavioral Neurobiology, Univ. of Tuebingen, Tuebingen, Germany; [3]Grad. Sch. of Neural Information Processing, Univ. of Tuebingen, Tuebingen, Germany; [4]Escuela Superior Politecnica del Litoral (ESPOL), Ecuador, Ecuador; [5]Brain Sci. Inspired Life Support Res. Center, The Univ. of Electro-Communications, Chofu, Japan; [6]Central Inst. of Mental Health, Med. Fac. Mannheim, Heidelberg Univ., Mannheim, Germany; [7]Ospedale San Camillo, IRCCS, Venezia, Italy

Abstract: Amyotrophic lateral sclerosis (ALS) is a degenerative motor neuron disease causing paralysis and progressive degeneration of muscles, and it can lead to the completely locked-in state (CLIS) that is characterized by total paralysis. Brain-Computer Interfaces (BCIs) have been used to allow paralyzed people to regain basic communication. Gallegos-Ayala et al. presented the case where an NIRS BCI led to communication with a completely locked-in patient, and here we report the brain communication in the patient using an EEG system. A CLIS ALS patient participated (68 y.o., female). We asked her to answer yes or no questions. The questions presented as auditory sentences, which were recorded with the voice of her husband. We presented 10-20 auditory sentences in one trial. Intersentence interval (ISI) was 25s. Two - 5 trials were conducted in each day, and we performed the experiment in 34 days. Two-channel (P3, P4) EEG data were recorded, and these channels were referenced to Fpz and grounded to AFz. The signal was notch-filtered at 50Hz, and digitized (Sampling rate: 500Hz) with a BrainAmp MR (Brain Products GmbH, Germany). Non-adhesive solid-gel EEG electrodes were used (Toyama et al. 2012). Thirteen-second EEG data (from 2 to 15sec after the auditory presentation) was analyzed. The EEG data was high-path filtered at 2 Hz, and the filtered data was used to compute power spectrum. Window size was set at 2 sec (1.5 sec overlapped), and the imaginary part of the power spectrum was averaged to drive features. For offline classification, we used support vector machine (L1 regularized L2 loss), and used a leave-one-out cross-validation method in each trial. When we created a representative feature vector from all data for the classification, the mean accuracy was 53.9%. When we created a best feature vector in each day, the mean accuracy increased to 74.1%, which was sufficient for practical use (>70%). These results suggest that the EEG system is possibly useful for communication in CLIS patients.

Disclosures: **K. Takano:** None. **B. Xia:** None. **U. Chaudhary:** None. **G. Gallegos-Ayala:** None. **A. Furdea:** None. **C.A. Ruf:** None. **K. Kansaku:** None. **H. Flor:** None. **N. Birbaumer:** None.

Poster

074. Noninvasive Neurophysiology

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Topic: D.18. Brain-Machine Interface

Support: NIH NICHD Award PO1HD064653

Title: Decoding of goal-directed behaviors from scalp electroencephalography (EEG) in freely behaving infants

Authors: *Z. R. HERNANDEZ, T. TSE, J. L. CONTRERAS-VIDAL;
Electrical and Computer Engin., Univ. of Houston, Houston, TX

Abstract: The human mirror neuron system (MNS) is thought to be critical for perceiving and later learning and imitating actions performed by others. Human sensorimotor alpha-band electroencephalographic (EEG) desynchronization at central electrodes, observed both during execution and observation of goal-directed actions (i.e., mu suppression), has been considered an electrophysiological correlate of MNS function. However, current methods of investigating mu-rhythm in MNS development rely on constrained experimental paradigms that have yet to fully represent the behavior of infants in an ecological setting. It is therefore important to redefine a paradigm that instead studies the human NMS under conditions where infants are free to evoke an action or behavior at his/her will. Furthermore, a mechanistic relationship has yet to be determined between EEG mu-rhythm and MNS function, and the extent to which EEG can be used to infer intent during MNS tasks remains unknown. We present a novel methodology using active EEG and inertial sensors to record brain activity and movement from freely behaving 6-24 month old infants during interaction with an adult actor in the context of MNS tasks, including exploration, imitation, attentive rest, pointing, reaching-to-grasp, and reaching-to-offer an object. Multiple time lags of delta-band (1-4Hz) EEG were extracted as features to a machine learning algorithm that first reduced feature dimensionality (locality-preserving Fisher's discriminant analysis, LFDA) in order to construct a model (Gaussian mixture models, GMMs) for decoding each MNS task. Here, we present decoding results and analysis of potential motion artifacts that illustrate our approach and demonstrate the feasibility of EEG-based classification of freely occurring MNS behaviors displayed by infants. These results provide an alternative and novel paradigm and computational analysis to the mu-rhythm theory of MNS function and further indicate the informative nature of EEG in relation to the intentionality (goal) of each MNS task,

which may support action understanding and thus bear implications for advancing the understanding of MNS function.

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Poster

074. Noninvasive Neurophysiology

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Program#/Poster#: 74.04/II1

Topic: D.17. Voluntary Movements

Title: High Density Electroencephalography (EEG) correlates of pain- related changes in upper limb movements

Authors: *G. MISRA, E. OFORI, J. CHUNG, S. A. COOMBES;
Applied Physiol. and Kinesiology, Univ. of Florida, Gainesville, FL

Abstract: The experience of pain changes the way we execute movements. While much work has focused on mechanisms in the spinal cord and muscle, very little is known about how the brain controls movement in painful contexts. There is a long history of using Electroencephalography (EEG) recordings to study the brain oscillations associated with movements and the brain oscillations associated with pain processing. In the current study, we build on this literature by identifying brain oscillations that are associated with pain-related changes in upper limb movements. We employed high density EEG to examine electro-cortical dynamics while participants produced visually guided ballistic upper limb movements under two stimulation conditions: while feeling a pain-eliciting thermal stimulus on the bicep of the moving arm (PAIN) and while no stimulus was delivered/felt (CONTROL). Significant reductions in reaction time and significant reductions in error were found for the PAIN condition as compared to the CONTROL condition, revealing that movements were faster and more accurate when coupled with a pain-eliciting stimulus. Compared to the CONTROL condition, movements in the PAIN condition were associated with increased alpha de-synchronization in sensorimotor regions and increased alpha and beta synchronization in dorsolateral prefrontal regions. Our findings demonstrate that pain driven changes in movement are associated with cortical oscillations that vary systematically across region and frequency.

Disclosures: **G. Misra:** None. **E. Ofori:** None. **J. Chung:** None. **S.A. Coombes:** None.

Poster

074. Noninvasive Neurophysiology

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Program#/Poster#: 74.05/II2

Topic: D.17. Voluntary Movements

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Belgian Science Policy Office IAP P7/33

ESRC Professorial Fellowship

ESRC/ESF ECRP Research Project

ERC Advanced Grant (HUMVOL)

EU FP7 Project VERE, Work Package 1

Title: EEG correlates of a prospective sense of agency

Authors: *N. SIDARUS, P. HAGGARD;
Inst. of Cognitive Neuroscience, UCL, London, United Kingdom

Abstract: Sense of agency (SoA) refers to the feeling that we are in control of our own actions and, through them, of events in the outside world. One influential view claims that the SoA depends on a retrospective matching between the expected and actual outcome of an action. However, recent studies have revealed an additional, prospective component to the SoA, related to action selection. The present study aimed to clarify the neural mechanisms of this prospective mechanism by means of event-related potentials (ERPs). Participants responded to imperative left/right arrow stimuli that were preceded by either a compatible or an incompatible subliminal prime. After a variable delay, action outcomes were displayed, and subjective agency ratings were collected. Results show that compatibly primed actions led to a stronger SoA over action outcomes, relative to incompatibly primed actions. Target-locked lateralised readiness potentials were positively associated with subjective agency ratings in compatibly-primed trials. Action priming did not influence outcome-locked ERPs. Thus, replicating previous studies, we found that an unconscious influence on action selection processes can affect the conscious experience of agency. These findings also suggest that a metacognitive signal related to action selection fluency can prospectively inform the SoA. This signal is related to activity in the brain's motor

system. Furthermore, the influence of this prospective, fluency-based, component on SoA is independent from retrospective outcome monitoring.

Disclosures: **N. Sidarus:** None. **P. Haggard:** None.

Poster

074. Noninvasive Neurophysiology

Location: Halls A-C

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Program#/Poster#: 74.06/II3

Topic: D.18. Brain-Machine Interface

Title: Three-class classification of motor imagery EEG data including “rest state” using Common Spatial Pattern

Authors: ***T. SHIRATORI**[1], H. TSUBAKIDA[1], A. ISHIYAMA[1], Y. ONO[2];
[1]Waseda Univ., Tokyo, Japan; [2]Meiji Univercity, Kanagawa, Japan

Abstract: Our purpose is to develop the 3-class Brain Machine Interface (BMI) incorporating the classification of resting state using Electroencephalography (EEG). The 3 classes we defined in this experiment were (1) motor imagery of moving right hand, (2) motor imagery of moving left hand, and (3) rest state. Conventionally the most of BMI systems only accept EEG data when a subject performs some kind of task, such as motor imagery and gaze at visual stimuli. However, performing task causes fatigue of the subject. It is therefore important to develop classification algorithm for BMI system that utilizes rest state-EEG as one of the classes. Eight healthy young subjects (20-25 years old) participated in the study. We measured their EEG signals from 15 electrodes distributed over the scalp, sampled at 125Hz.Subjects looked at the computer screen in front of them during the whole experiment. The screen alternately showed an instruction (4s) and a fixation point (3s) for 60 times. An instruction of single state among the three states was displayed at the beginning of instruction display for 1s, and count-down displayed for 3s. After count-down subject performed the instructed state for 3s. Subject performed three kinds of states each for 20 times. We extracted feature vector using FIR filter and Common Spatial Filter (CSP) from EEG data, and made three 2-class classifiers (1-vs-2, 2-vs-3, and 3-vs-1) using Gaussian kernel Support Vector Machine (SVM). Majority voting among the classified results from 2-class classifiers decided the final output of the 3-class classifier. Mean accuracy rate of 2-class classifier was 69.1±7.6%(1-vs-2), 74.1±.5.0%(2-vs-3,), 69.4 ±6.0% (3-vs-1), the developed 3-class classifier was 54.8±8.8%.Result indicated that mean accuracy rate of 2-class incorporating resting state classifier is equivalent to 2-class motor

imagery classifier. Mean accuracy rate of 3-class classifier suggested that mean accuracy rate of proposal method is higher than our previous paper(42.9±0.72%).

Disclosures: **T. Shiratori:** None. **H. Tsubakida:** None. **A. Ishiyama:** None. **Y. Ono:** None.

Poster

074. Noninvasive Neurophysiology

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Program#/Poster#: 74.07/II4

Topic: D.18. Brain-Machine Interface

Support: NIH NINDS Award R01NS075889

Title: On the potential effects of motion artifacts during decoding of lower-body movement kinematics from scalp electroencephalography (EEG)

Authors: *J. L. CONTRERAS-VIDAL, Ph.D., K. NATHAN;
Electrical and Computer Engin., Univ. of Houston, Houston, TX

Abstract: Recent studies have questioned some conclusions drawn in previous studies on the delta-band EEG decoding of gait kinematics ([1] Castermans et al. Neurosci Lett., 2014, doi: 10.1016/j.neulet.2013.12.059) as well as the suitability of inferring movement kinematics using Wiener decoders ([2] Antelis et al. PLoS ONE, 2013, p. e61976). Unfortunately, there are severe methodological and theoretical flaws in [1]-[2] that in turn invalidate their conclusions. Here we will discuss these pitfalls: a) Distortion of Measurement: In [1], the authors fixed a piezoelectric accelerometer to a large rigid plate mounted on a three-point linkage that was firmly strapped on top of the EEG cap donned by the subject. This large setup, of unknown but significant mass, and in contact with the EEG electrodes most likely introduced distortions in the measurements by introducing mechanical artifacts (non-existing during proper measuring techniques) directly to the EEG electrodes therefore violating good and common measuring practice in instrumentation and measurement; b) Theoretical pitfalls: In [2] the authors claim that low-frequency sinusoidal signals lead to overly optimistic correlation results and that this assertion is used to explain the positive correlation coefficients around 0.3 in random-chance models with shuffled and synthetic data. What [2] does not consider is the length of the signals under consideration (see http://www.plosone.org/annotation/listThread.action?root=69163 for full discussion), which was very short, about 3.75s. Moreover, a peculiarity in the results of [2] is that there are not roughly an equal number of positive and negative correlations in their chance-

level models questioning further their claims. Also, [2] did not filter out very low frequency noise. EEG exhibits 1/f noise, and, with very few exceptions, in decoding studies EEG data are high-pass filtered upon acquisition (~0.05 to .3 Hz). It is unclear how neglecting to high-pass the EEG data would affect decoding results; hence, confounding the results of [2]. Finally, neither [1] and [2] used active EEG systems which help minimize motion artifacts while increasing the signal-to-noise ratio. We have redone [1] with an appropriate set-up and experimental protocol that follow good standard measuring practices that shows that EEG decoding is not significantly affected by head/body motion. This research will be discussed in this presentation.

Disclosures: **J.L. Contreras-Vidal:** None. **K. Nathan:** None.

Poster

074. Noninvasive Neurophysiology

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 74.08/II5

Topic: D.05. Visual Sensory-motor Processing

Support: NIH Grant P01HD064653.

Title: Simultaneous scalp EEG and multiunit recording from monkey ventral premotor cortex reveals the contribution of mirror neurons to alpha and beta desynchronization

Authors: M. BIMBI[1], *G. COUDE[1], F. FESTANTE[1], R. E. VANDERWERT[2], N. A. FOX[3], P. FERRARI[1];
[1]Univ. Parma, Dept Neurosci., Parma, Italy; [2]Harvard, Cambridge, MA; [3]Univ. of Maryland, College park, MD

Abstract: Most of our knowledge of mirror neurons comes from single unit recordings, made in premotor and parietal cortices of the macaque monkey. On the other hand EEG studies in humans have shown significant desynchronization in the alpha (9-13 Hz) and beta (15-25 Hz) bands during observation and execution of actions, thus providing an indirect signature of the activity of mirror neurons. However, it is not known whether the activity of mirror neurons contributes to the observed EEG desynchronization. Here we used simultaneous EEG and multiunit recording to understand the link between these two signals. We first performed a series of extracellular multiunit recordings in two macaque monkeys, using 16-channel Plexon electrodes. We identified premotor cortical area F5 and the subsector where mirror properties could be recorded. We then simultaneously recorded scalp EEG using a 7-channel lycra cap and

LFP with a 9-channel probe inserted in the contralateral hemisphere. The task carried out by the monkey consisted of the observation of a grasping action done by an experimenter in front of the monkey. The ERDs analysis showed the presence of both alpha and beta band desynchronizations, during grasping observation, in both frontal and central regions, but not in the occipital one. A coherence analysis between EEG and LFP signals showed moderate values in the alpha band for frontal and central EEG regions, while high coherence values were observed for two very narrow bands centered on 20 Hz and 22 Hz for all LFP channels recorded. There was no significant coherence between occipital EEG and any LFP channels. These data, for the first time, show that activity of mirror neurons in F5 contributes to the desynchronization of the scalp recorded EEG signal from frontal and central sites, especially in the beta band.

Disclosures: **M. Bimbi:** None. **G. Coude:** None. **F. Festante:** None. **P. Ferrari:** None. **R.E. Vanderwert:** None. **N.A. Fox:** None.

Poster

074. Noninvasive Neurophysiology

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 74.09/II6

Topic: D.18. Brain-Machine Interface

Support: MEXT/SRPBS (BMI)

MHLW grant (BMI)

MEXT grant (#23300151)

Title: Decoded neurofeedback training for MEG/SSVEF

Authors: ***H. ORA**[1,2], K. KANSAKU[1,2];
[1]Sys. Neurosci. Sect., Dept. of Rehab. for Brain Funct., Res. Inst. of Natl. Rehabil. Ctr., Tokorozawa, Japan; [2]Brain Sci. Inspired Life Support Res. Ctr., The Univ. of Electro-Communications, Chofu, Japan

Abstract: Decoded neurofeedback method was recently proposed to lead brain activity to a target state (Shibata, et al., 2011). While researchers utilized functional magnetic resonance imaging in the former studies, little is focused on magnetoencephalography (MEG). Real-time magnetoencephalography (rtMEG) is an emerging neurofeedback technology that could potentially benefit multiple areas of basic and clinical neuroscience, and in this study, we used an

rtMEG system to perform decoded neurofeedback training for MEG/steady-state visual evoked field (SSVEF). Five able-bodied participants (age 33.2 years old, 5 females) participated in this study. The visual stimuli were displayed on a screen in front of the participant, and consisted of a circular checkerboard patch on the left and a circular checkerboard patch on the right. The checkerboard patches flickered at 5 (left) or 6 (right) Hz. They were asked to attend to the left, right, or middle of the screen in a SSVEF task. The used MEG scanner was a 306-channel Elekta Neuromag system (Elekta Oy, Helsinki, Finland). We constructed a sparse multinomial logistic regression (SMLR) decoder from MEG signals during the SSVEF task in each participant. We then conducted 3-day MEG neurofeedback training. In a trial, a white fixation cross was turned green for 5 second, then a green solid circle, whose radius indicated a score of the SMLR decoder, was presented. Participants were asked to "somehow regulate your brain activity to make the green solid circle bigger while the fixation cross is green." During the trainings, any flickering visual stimuli was not presented. After the trainings, the SSVEF task was used again to evaluate the training effects. The SMLR decoder was able to classify the MEG signals into 3 attentional directions (left, right or middle) (88.2 %). The participants were able to increase decoder scores through the trainings ($p<0.05$). In the post-training SSVEF task, accuracy for the target orientation was higher than that for the non-target orientation ($p<0.05$). The results suggest that the decoded neurofeedback training was effective for MEG/SSVEF, and the method may enhance robustness of steady-state visual evoked potential (SSVEP)-based Brain-Computer Interface (BCI).

Disclosures: **H. Ora:** None. **K. Kansaku:** None.

Poster

074. Noninvasive Neurophysiology

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 74.10/II7

Topic: D.18. Brain-Machine Interface

Title: The performance of a SSVEP classifier under well-lit and dim-lit room environment

Authors: ***K. SAEKI**, Y. ONO, K. IKEMOTO, T. YOKOYAMA, T. URANO;
Sch. of Sci. and Technol., Meiji Univ. C/O Prof., Kanagawa, Japan

Abstract: Brain Computer interface (BCI) utilizes brain activity to operate external machine, which has a potential to allow people with severe motor disabilities to communicate. It is therefore important to investigate the usability of BCI system in the daily environment. We

investigated whether the difference in environmental brightness affect the performance of a steady-state visual evoked potential (SSVEP)-based BCI, which is one of the easy and promising techniques of BCI. Seven participants with normal or corrected-to-normal vision (21-35 years old, mean age 23.8) enrolled in the experiment. We recorded electroencephalogram (EEG) from the occipital area of O1, O2, and Oz according to the international 10-10 system. Participants watched one of the three white LEDs flickering at 6, 7, 8Hz arranged in front of them to enter a single command. Our in-house developed SSVEP-BCI system classified the EEG responses every 4s and gave feedback to them. Each participant was given a random sequence of 6 commands to complete, each of which corresponds to one of the three LED stimuli (For example, 6Hz>7Hz>8Hz>7Hz>6Hz>8Hz). The order of commands was randomized using Latin square. The total time required to complete the whole sequence was compared between when the BCI was operated in a well-lit environment (1455 lux) and in a dim-lit environment (4 lux). The mean durations to complete the sequence were 76.4±6.3s and 53.7±5.9s in a well-lit and a dim-lit room environments, respectively. The mean duration to enter single command was 12.7±1.1s and 8.9±0.9s in a well-lit and dim-lit room environments, respectively. Statistical analysis showed significant difference in these durations between both conditions (t-test, p=0.02). These results suggest that brightness of the environment influences the strength of SSVEP and affect the performance of SSVEP-based BCI system. Reduced contrast of stimuli might attenuate SSVEP response in a well-lit room. Enhancing local contrast of LED stimulus might improve the performance of the SSVEP-based BCI even in a well-lit room.

Disclosures: **K. Saeki:** None. **Y. Ono:** None. **K. Ikemoto:** None. **T. Yokoyama:** None. **T. Urano:** None.

Poster

074. Noninvasive Neurophysiology

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 74.11/II8

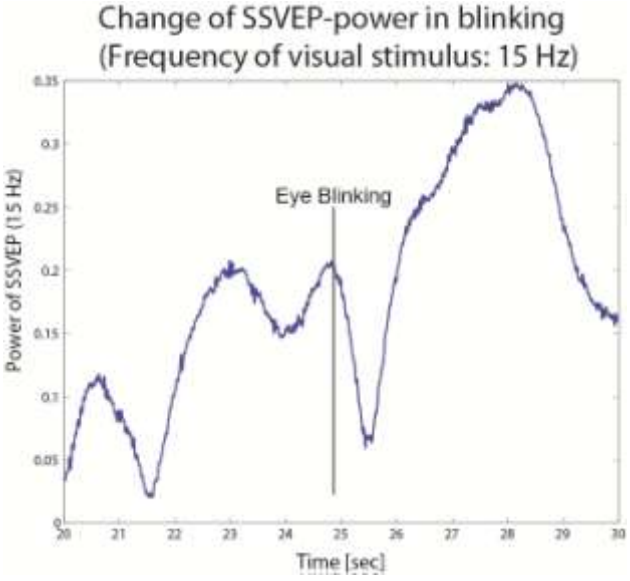
Topic: D.18. Brain-Machine Interface

Title: Changing power spectrum of SSVEP in blinking

Authors: ***A. FUNASE**, I. TAKUMI;
Dept. of Computer science, Nagoya Inst. of Technol., Nagoya, Japan

Abstract: Purpose: Recently, many researchers develop Brain-computer interfaces by EEG signals. Especially, we focus on the BCI based on the steady-state visual evoked potential

(SSVEP). The BCI based on the SSVEP is some advantages against another types of BCIs. SSVEP is generated by an external visual stimulus and SSVEP has the same frequency as frequency of an external visual stimulus. Therefore, it is easy to observe SSVEP. However, it is difficult for subjects to watch only external visual stimulus during an experiment; for example, eye blinking. Therefore, power spectrum of SSVEP is changing during an experiment. In this research, we focus on power spectrum of the SSVEP before eye blinking and after eye blinking. Experimental task: The visual stimulus for generating SSVEP is a matrix LED. The matrix LED is illuminated like a checker-flag. Flicking frequency of the matrix LED is 15 or 20 [Hz]. Subjects are watched this matrix LED during 120 [sec] and are recorded EEG signals. Electrodes are attached according to international 10-20 system method and the number of electrode is 19. Sampling frequency of EEG amplifier is 1000 [Hz]. Subjects are five males. Results and Discussion: The figure shows the power spectrum of SSVEP (Frequency of visual stimulus is 15 Hz). Horizontal axis indicates time and vertical axis indicates the power of SSVEP. In this figure, frequency of the SSVEP is 15 Hz. From this result, firstly, power of the SSVEP has fluctuation and power of the SSVEP is non-stationary signals. Therefore, in order to analyze SSVEP, it is important to decide suitable window-size for analysis. Secondly, we compare power of the SSVEP before eye blinking to after eye blinking. After eye blinking, power of the SSVEP decreases because subject cannot watch visual stimulus. Then, power of the SSVEP increases in this figure. The average of power of the SSVEP after eye blinking is larger than the average of power of the SSVEP before eye blinking. This tendency is observed in the case of all subjects' experiments. Therefore, in order to develop the BCI based on SSVEP, we should focus on analyzing the SSVEP after eye blinking.



Disclosures: **A. Funase:** A. Employment/Salary (full or part-time):; Nagoya Institute of Technology. **I. Takumi:** A. Employment/Salary (full or part-time):; Nagoya Institute of Technology.

Poster

074. Noninvasive Neurophysiology

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 74.12/II9

Topic: D.18. Brain-Machine Interface

Title: SSVEP-BCI system with an supervised classification algorithm

Authors: *K. IKEMOTO, T. URANO, K. SAEKI, Y. ONO;
Sch. of Sci. and Technol., Meiji Univ., Kanagawa, Japan

Abstract: Steady-state visual evoked potential (SSVEP) is one of the easiest and promising techniques of brain-computer interface (BCI). Conventional classification algorithm using linear discriminant analysis (LDA) based on spectrum power of electroencephalogram (EEG) has disadvantages of low accuracy rate and the requirement of training data sets. We therefore developed an unsupervised classification algorithm of SSVEP-based-BCI, which uses features both from spectrum power of raw EEG data and from averaged EEG signals. Thirty-two participants with normal or corrected-to-normal vision (aged 21.09±2.80, 27 males and 5 females) enrolled in the experiment. We recorded EEG at O1, O2, and Oz in the international 10-10 electrode system. Subjects gazed at each flickering LED at 6Hz, 7Hz, or 8Hz for 28 seconds individually four times per frequency. Each EEG data were divided into seven 4 s-fragments. The developed method consists of two methods, Spectrum Method and Average Method. The developed method adopts Spectrum Method in case that signal-to-noise ratio of the spectrum power is large enough, and otherwise adopts Average Method. Spectrum Method detects the frequency which gives the maximum difference of spectrum power between maximum (±0.1Hz) and median (±0.8Hz) of each frequency band corresponding to the flickering light stimuli. Average Method detects the frequency which gives the largest peak-to-peak amplitude of visual evoked potential by dividing and averaging the raw EEG data with different cycles corresponding to the flicker frequencies. Both methods are applied to the data at each electrode and majority voting among the results at three electrodes determine the final output of the classifier. The mean accuracy in 3-class classification in the offline analysis was 75.1±3.1% with the developed algorithms, which was significantly larger than that using conventional Linear Discriminant Analysis (68.5±3.7%, $p<0.01$). In order to confirm the developed algorithm can achieve accurate performance online, the proposed algorithm was implemented as online BCI system working on a MATLAB Simulink. Seven participants with normal or corrected-to-normal vision (aged 21.43±1.19, 6 males and 1 females) enrolled in the experiment. Three LDAs flickering at 6Hz, 7Hz, or 8Hz were arranged on a breadboard and participant gazed at one of

them for four seconds 12 times per each frequency. The mean accuracy was 74.6±6.1%, which is comparable to the accuracy in the offline analysis. These results suggest that our proposed algorithm successfully implemented as online programs.

Disclosures: **K. Ikemoto:** None. **T. Urano:** None. **K. Saeki:** None. **Y. Ono:** None.

Poster

074. Noninvasive Neurophysiology

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 74.13/II10

Topic: D.18. Brain-Machine Interface

Support: BFNT grant

Title: EEG-based classification of video quality perception using steady state visual evoked potentials (SSVEP)

Authors: ***L. ACQUALAGNA**[1], S. BOSSE[2], A. K. PORBADNIGK[3], G. CURIO[4], K. R. MÜLLER[3], T. WIEGAND[2], B. BLANKERTZ[3];
[1]TU Berlin, Berlin, Germany; [2]Fraunhofer Inst. for Telecommunications, Berlin, Germany; [3]Technische Univ. Berlin, Berlin, Germany; [4]Charité, Berlin, Germany

Abstract: Current methods for video quality assessment mostly rely on behavioral ratings that are affected by subjective factors and have a high inter-subject variability. Recent studies investigate neural correlates of the perception of video quality to establish a direct measure. This approach capitalizes on the cognitive P3 component of the EEG. However, the P3 is not directly linked to the sensory process of the stimuli and requires a high amount of trails to provide a distinctive signal. Our approach exploits SSVEPs as EEG features to classify perceived quality changes. Besides, we correlate our results with the Mean Opinion Score (MOS) values that the subjects gave during the conventional behavioral assessment, using the Degradation Category Rating. Sixteen people participated in the experiment. Six gray-level texture images were chosen as the basis for stimuli generation and presented in six levels of distortion (D1, ⋯, D6), introduced by coding the textures using the HM10.0 test model of the High Efficiency Video Coding (H.265) standard. The textures were grouped in 51 videos, 117 s long. In each video, they were presented in random order alternating between distorted and undistorted images with a stimulus onset asynchrony of 333 ms. This succession of quality changes gave the flickering effect that elicits SSVEPs whenever the threshold of sensory processing is exceeded. In the data

analysis, we calculated spatio-temporal features and used Linear Discriminant Analysis with shrinkage of the covariance matrix to detect SSVEP modulations. In ten-fold cross-validation, we obtain mean classification accuracy (AUC values) of 0.84±0.1 for D6, 0.75±0.12 for D5, 0.61±0.1 for D4, and chance level for the first three levels of quality change (which were around or below perception threshold). Classification accuracy significantly correlated with MOS values for all the subjects ($p<0.01$). These results show that SSVEPs are significantly modulated by the quality changes above perception threshold and can be related to the behavioral measurements and therefore give rise to a graded neural index of video quality change perception.

Disclosures: **L. Acqualagna:** None. **S. Bosse:** None. **T. Wiegand:** None. **A.K. Porbadnigk:** None. **B. Blankertz:** None. **K.R. Müller:** None. **G. Curio:** None.

Poster

074. Noninvasive Neurophysiology

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 74.14/II11

Topic: D.18. Brain-Machine Interface

Title: A monolithic portable fNIRS system with 1cm spatial resolution for functional brain imaging

Authors: ***J. KIM**[1], J. CHOI[2], M. CHOI[2], H. WON[2], G. HWANG[2], H. BAE[2];
[1]KAIST, [2]Electrical Engin., KAIST, Daejeon, Korea, Republic of

Abstract: Functional near-infrared spectroscopy (fNIRS) is an effective and non-invasive functional brain imaging method. Neuronal activities of the brain are strongly coupled with the hemodynamics in the local cerebral cortex. Such hemodynamics can be extracted by using the fact that the absorption spectra of oxy hemoglobin (HbO) and deoxy hemoglobin (HbR) differ in the near-infrared region of the spectrum. The fNIRS has an apparent advantage over functional magnetic resonant imaging (fMRI) in terms of cost and portability. However, the inherent limitation of the spatial resolution of the fNIRS restricts its widespread use in clinical applications. In addition, the conventional fNIRS devices have yet to utilize its potential for portability to the fullest extent. In this paper, a monolithic high resolution portable fNIRS system is proposed. Variety of innovations at the algorithmic, architectural, and circuit levels are applied to overcome aforementioned technical limitations. Each individual laser sources are modulated with their own Walsh codes for uncompromised temporal resolution and a matched filtering scheme is incorporated at the receiver to maximize the electrical signal-to-noise ratio (SNR). In

addition, multi-input-multi-output (MIMO) scheme is employed to enhance the spatial resolution without increasing hardware complexity. Furthermore, in order to enhance the portability of the device to the fullest extent, the receiver containing variable amplifiers, analog-to-digital converters, and multiplexers is monolithically integrated and fabricated in a 0.35um 1P4M CMOS process. The IC occupies 5 x 2 mm2 and consumes 3.2mW per channel. To the best of author's knowledge, the proposed system achieves the highest level of spatial resolution and portability among recently published fNIRS systems.

Disclosures: **J. Kim:** None. **J. Choi:** None. **M. Choi:** None. **H. Won:** None. **G. Hwang:** None. **H. Bae:** None.

Poster

074. Noninvasive Neurophysiology

Location: Halls A-C

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Program#/Poster#: 74.15/II12

Topic: D.18. Brain-Machine Interface

Support: The Ministry of Internal Affairs and Communications entitled, 'Novel and innovative R&D making use of brain structures'

JSPS Grant 26730146

Title: Decoding daily behaviors from NIRS signatures by using a portable NIRS device in the daily-life environment

Authors: ***T. OGAWA**[1], P. K. GUPTA[2], K. YANO[2], J. A. ABDUR-RAHIM[2], H. MORIOKA[2,3,4], J.-I. HIRAYAMA[2], S. YAMAGUCHI[5], A. ISHIKAWA[5], Y. INOUE[5], M. KAWANABE[2], S. ISHII[2,3];
[1]Seika-cho, [2]Dynamic Brain Imaging, ATR, Kyoto, Japan; [3]Syst. Science, Grad. Sch. of Informatics, Kyoto Univ., Kyoto, Japan; [4]JSPS, Tokyo, Japan; [5]Shimadzu Corp., Kyoto, Japan

Abstract: Thanks to the development of technologies to miniaturize the sensor device (EEG, NIRS, etc.) and wireless communication, portable/wearable devices are recently available. A challenging issue within the field of neuroimaging is elucidation of natural brain processing in less-controlled environment. Participants are allowed to be released from tight experimental conditions. To examine "natural behavior" signatures based on such portable devices, however, , there are two main problems, i) how to analyze recorded signals with external/internal artifacts in

the real environment, ii) how to detect spontaneous behavior events from the signals without cue stimulus. To this end, we developed a wearable multi-channel fNIRS-EEG system (kNIRS-EEG). In the unique experimental environment that mimics in a living house, called BMI (Brain Machine Interface) house, we developed a system to record NIRS-EEG and environmental data simultaneously. We examined NIRS-EEG recordings from both single-participant and dual-participants simultaneous sessions. The participants were instructed to perform sequential daily-life and free moving behaviors. In off-line analysis, we labeled each behavior a tag and registered it to a "Brain-log database". After each segment of 8ch NIRS signals was associated with the behavioral tag that signifies the class of daily-life behaviors (i.e. operating TV/AC, reading etc.), we attempted to decode each behavior from the NIRS data to discriminate daily behavior. We applied SVM for the decoding and evaluated the classifier and timing of the decodable period. Our preliminary results found that the classification accuracy rose after the onset, in particular, by using SVM. This results suggested that we could extract some behavior-related information from the recorded signal even if it contains motion artifacts. It is plausible to decode a subject's action and/or intention based on single-trial NIRS signals using subject behavior as a label. In addition, we would discuss relationship between environmental sensor information and NIRS information.

Disclosures: The Disclosure Block has exceeded its maximum limit. Please call Tech support at (217) 398-1792 for more information.

Poster

074. Noninvasive Neurophysiology

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 74.16/II13

Topic: D.18. Brain-Machine Interface

Title: Ultrasound modulation in frog nerve

Authors: *D. W. GULICK, B. C. TOWE;
BME, Arizona State Univ., Tempe, AZ

Abstract: We looked for ultrasound (US) effects on *ex vivo* bullfrog sciatic nerve, using pulses similar to US brain stimulation. We saw no clear effect. We first applied electrical stimulation to elicit a half-maximum compound action potential (CAP), then added US pulses to look for a change in the CAP amplitude [1]. Because many nerve fibers are near threshold, any US effect on the membrane or ion channels should increase or decrease the CAP. We looked for an effect

under conditions similar to recent brain stimulation results (sub-MHz US, ~10 W/cm2 power, ~100 ms pulse) [2]. The US appeared to have no strong direct effect on the CAP. Our observation is compatible with human results that US stimulates sensory receptors but not nerves in passing [3]. There are several reasons we might not have seen the nerve effects reported by others: Mihran et al. used shorter pulses with much higher peak power [1]. Colucci et al. used higher average power, causing heating [4]. We did find an apparent artifact by which US affected the CAP in our setup. The artifact appeared to be caused by US radiation pressure rippling the water around the stimulation electrodes, because it could be blocked by placing plastic wrap or tissue paper over the nerve. It is unclear if this artifact may appear in any other work. It occurs only when the nerve is stimulated at the water surface - if the stimulation site is in air or is submerged, the water level would not affect the stimulation current. We also tested the combination of US and direct current (DC) on the nerve. US and DC together had no effect beyond that of the DC alone. This suggests that any synergy between US and DC might be specific to the brain, rather than a general physical effect [5]. [1]R. T. Mihran, F. S. Barnes, and H. Wachtel, “Temporally-specific modification of myelinated axon excitability *in vitro* following a single ultrasound pulse,” Ultrasound in Medicine & Biology, 1990. [2]S. S. Yoo, A. Bystritsky, J. H. Lee, Y. Zhang, K. Fischer, “Focused ultrasound modulates region-specific brain activity,” Neuroimage, 2011. [3]L. R. Gavrilov, E. M. Tsirulnikov, and I. I. Davies, “Application of focused ultrasound for the stimulation of neural structures,” UMB, 1996. [4]V. Colucci, G. Strichartz, F. Jolesz, and N. Vykhodtseva, “Focused Ultrasound Effects on Nerve Action Potential *in vitro*,” UMB, 2009. [5]T. Wagner, “Apparatus for stimulation of biological tissue" - European Patent 2550992, 2013.

Disclosures: **D.W. Gulick:** None. **B.C. Towe:** None.

Poster

074. Noninvasive Neurophysiology

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Program#/Poster#: 74.17/II14

Topic: D.18. Brain-Machine Interface

Support: GE Healthcare

NIH 1 R01 EB019005-01

Stanford Interdisciplinary Graduate Fellowship

Bio-X Neuroventures

Bruce E. and Doris A. Nelson Bioengineering Fellowship

Title: Ultrasound neuromodulation frequency dependence is not fully explained by changing sonication duration

Authors: *P. P. YE[1], J. BROWN[2], K. B. PAULY[3];
[1]Stanford Univ., Palo Alto, CA; [3]Radiology, [2]Stanford Univ., Stanford, CA

Abstract: Ultrasound neuromodulation is known to be capable of stimulating neural activity but response efficacy is highly dependent upon sonication parameters. Previous work has shown a strong dependence on the carrier frequency, where lower frequency sonication requires significantly less intensity to achieve the same success rate for eliciting a motor response [King et al., 2013]. Understanding the cause of this frequency dependence could cast light on the underlying mechanisms of ultrasound neuromodulation which currently remain unknown. We performed experiments to examine whether controlling for sonication duration could account for all or some of the frequency dependence. Six mice were sonicated with ultrasound transcranially at four carrier frequencies at various intensity levels, while keeping the number of cycles constant (40,000 cycles) and again while keeping sonication duration constant (80 ms). The mice were lightly anesthetized at 0.34% isoflurane at 1 liter per minute O2 throughout the experiment. Electromyograms were measured in the forelimbs and processed to calculate the success rate of eliciting muscle contractions. Our data suggests a frequency dependence where lower frequencies require reduced intensities to achieve the same success rates whether or not sonications used constant cycles or constant durations. Success rates were greater with longer duration sonications at 300 kHz, and success rates were lower with shorter duration sonications at 600 kHz ($p = 0.03$, one-tailed Wilcoxon signed rank test). By directly comparing constant cycle and constant duration sonications on the same mice, we show that keeping sonication duration constant only partially explains the observed frequency dependence. Future work examining other frequency-dependent factors such as standing waves and focal spot size may be necessary to fully explain the frequency dependence of ultrasound neuromodulation.

Disclosures: **P.P. Ye:** None. **J. Brown:** None. **K.B. Pauly:** None.

Poster

074. Noninvasive Neurophysiology

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 74.18/II15

Topic: D.18. Brain-Machine Interface

Title: fMRI BOLD response in humans using transcranial focused ultrasound

Authors: *J. MUELLER, W. LEGON, W. TYLER;
Virginia Tech. Carilion Res. Inst., Roanoke, VA

Abstract: Previous research has demonstrated transcranial focused ultrasound (tFUS) in humans to affect the amplitude of somatosensory evoked potentials, as well as broad spectrum EEG power (Legon et al. 2014). Here, we test the effect of tFUS for induction of a blood oxygen level dependent (BOLD) response in humans using functional magnetic resonance imaging (fMRI). Participants (N = 6) were fitted with one of two different 0.5 MHz ultrasound transducers having an axial and lateral resolution of 30 mm. An event-related design was used where 90 US stimuli were delivered every 12-14 seconds. Each stimulus was created using a two-channel function generator where channel 1 served as the trigger for channel 2. Channel 1 had a pulse repetition frequency (PRF) of 1kHz and total number of pulses of 500 for a 0.5 sec duration. Channel 2 had an acoustic frequency of 0.5 MHz of 180 cycles for a duration of 0.36 msec. This corresponded to an intracranial peak-to-peak pressure intensity of Isppa = 5.9 W/cm2. Group whole head and region of interest (ROI) analyses were conducted, as well as on a subject by subject basis. ROI analysis was concentrated on areas of the brain within the focal axes of the ultrasound beam and surrounding beam penumbra. Preliminary results indicate tFUS to have variable effects upon BOLD response between subjects. There was no statistically significant area of activation on the group level. However, individual participant analysis revealed 3 of 6 participants demonstrated a focal increase in BOLD response within the US beam maxima. 2 of 6 participants showed no discernible BOLD activation pattern to US stimulation and 1 participant demonstrated a combination of increased and decreased BOLD response in the general area of the US beam axes. These results, though variable, suggest that focused ultrasound can induce detectable BOLD signature changes in humans but that refinement of US stimulus parameters is likely needed and optimization of signal to noise ratios is necessary to induce a stable effect across subjects.

Disclosures: **J. Mueller:** None. **W. Legon:** None. **W. Tyler:** A. Employment/Salary (full or part-time):; Thync Inc.

Poster

075. Gonadotropin-Releasing Hormone and Hpg Control

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 75.01/II16

Topic: E.01. Neuroendocrine Processes

Support: CONACyT (grant 81898)

DGAPA-PAPIIT (grant IN-220014-3)

Title: At diestrus-2 muscarinic receptor type 1 (M_1R) of the left or right ovary participates in the progesterone (P_4) and 17β-estradiol (E_2) secretion on proestrus day

Authors: *M. B. CRUZ, A. FLORES, R. DOMÍNGUEZ;
Investigacion Y Posgrado, Univ. Autonoma De México, Mexico DF, Mexico

Abstract: Previously we have shown that at 13:00 h of diestrus-2 the injection of pirenzepine (PZP) (a blocker of M_1R) on the left and right ovaries resulted in the blockade of ovulation (87.5% of the treated animals) at the predicted estrus day (Cruz et al., 2013). LHRH injection induced ovulation in rats treated with PZP. The injection of estradiol benzoate (EB) did not induce the ovulation in the rats treated with PZP in the left ovary, while ovulation occurred in almost all rats with PZP injected in the right one, after EB treatment. These results suggested that at diestrus-2 the stimulation of M_1R on the left or right ovary regulates the GnRH secretion of proestrus day, as a result of the modifications on the surge E_2 secretion. The aim of present study was to analyze the effects of the blockade of M_1R at diestrus-2 on P_4 and E_2 serum levels measured at 11:00 and 15:00 h of the predicted proestrus day. Groups of cyclic rats were anaesthetized at 13:00 h on diestrus-2 with ketamine-xylazine and micro-injected in the left or right ovary with 0.5 μL of sterile water or PZP (19ng/0.5 μL), and sacrificed at 11:00 or 15:00 h of the predicted day of proestrus. Groups of untouched control cyclic rats were sacrificed at same times. The vehicle injection into the left or right ovary resulted in lower P_4 serum levels at 11:00 h of the proestrus day (left ovary: 8.2±0.9, right ovary: 7.2±1.5 vs. control: 19.4±2.1, $p<0.01$) and did not modify the E_2 levels. The PZP injection in either ovary increased the P_4 levels in comparison with animals injected with vehicle (left ovary: 19.7±2.3 vs. 8.2±0.9, right ovary: 33.7±4.0 vs. 7.2±1.5, $p<0.01$. E_2 serum levels were higher in those rats injected with PZP in the left ovary than those injected with the vehicle (90.1±9.4 vs. 65.7±5.2, $p<0.05$). in those animals sacrificed at 15:00 h vehicle injection on either ovary did not modify the P_4 levels, while E_2 levels were lower than control (left ovary: 84.7±10.1, right ovary: 92.3±6.3 vs. 138.2±8.9, $p<0.01$). Present results suggest that at diestrus-2 the stimulation of the M_1R on the ovaries regulates in an inhibitory way the P_4 and E_2 secretion at proestrus day. Based on these results and the asymmetric effects of the EB on ovulation on PZP-treated rats (Cruz et al., 2013), we suggest that the stimulation of the M_1R on the left ovary participated in the regulation of the positive feed-back estrogen effects.

Disclosures: **M.B. Cruz:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); CONACyT (Grant 81898) and DGAPA-PAPIIT (IN-220014-3). **A.**

Flores: C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); CONACyT (Grant 81898) and DGAPA-PAPIIT (IN-220014-3). **R. Domínguez:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); CONACyT (Grant 81898) and DGAPA-PAPIIT (IN-220014-3).

Poster

075. Gonadotropin-Releasing Hormone and Hpg Control

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 75.02/II17

Topic: E.01. Neuroendocrine Processes

Support: ZIA NS002824-24

Title: Unraveling the cellular mechanisms triggering GnRH release

Authors: *S. S. CONSTANTIN, S. WRAY;
NINDS / NIH [C], Bethesda, MD

Abstract: Fertility relies on communication between gonadotropin-releasing hormone (GnRH) neurons, pituitary gonadotrophs and gonads, a “ménage à trois”, where all information is conveyed through discrete secretory patterns. For ~40 years, the critical role of pulsatile release of GnRH in the portal blood system has been known. Chaotic GnRH release occurs in polycystic ovary syndrome, one of today’s leading causes of infertility. However, the cellular mechanisms that drive secretion and pulsatility in GnRH neurons still remain unknown. At the single cell level, electrical activity in GnRH soma show no obvious pattern that could trigger a pulse of secretory activity at their terminals. The goal of this study is to unravel GnRH secretion to better understand the etiology of reproductive pathologies. Since GnRH neurons release peptide into the blood stream, their terminals are not classical synapses. In addition, GnRH is packaged in dense core vesicles (DCV) in contrast to synaptic vesicles (SV). Thus, two approaches are being used to identify mechanisms underlying GnRH secretion using primary cultures. First, the secretory apparatus of GnRH neurons is being characterized by immunocytochemistry, using an extensive list of vesicle and synaptic active zone markers. However, as SV and DCV emerge from the Golgi apparatus, they share many markers, making it difficult to distinguish between DCV and SV at the light microscopic level and only helping to identify putative secretion sites. Thus, an assay for GnRH secretion is being developed. Since GnRH neurons lack a postsynaptic partner, secretion must be assessed directly on GnRH neurons or indirectly on their physiological partners, gonadotrophs. FM1-43, a dye commonly used to study exocytosis, was used for direct

measurements. A range of field stimulations was tested to determine the protocol to optimally label vesicles. FM1-43 labeling was confirmed by post-hoc immunocytochemistry for GnRH. To ensure GnRH was effectively released, the same stimulation will be applied to GnRH cultures while their media is dialyzing on gonadotrophs. Gonadotroph responses will be monitored via calcium imaging, an increase in calcium will unequivocally indicate release of GnRH. Finally, the same stimulation will be applied to GnRH neurons and activity monitored via calcium imaging, allowing, for the first time, one to record a cellular event concomitant with secretory activity in GnRH neurons.

Disclosures: **S.S. Constantin:** None. **S. Wray:** None.

Poster

075. Gonadotropin-Releasing Hormone and Hpg Control

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 75.03/II18

Topic: E.01. Neuroendocrine Processes

Support: Health Research Council New Zealand 11/404

Health Research Council Programme New Zealand 12/670

Title: Defining the neuronal network mediating impaired progesterone negative feedback in a mouse model of polycystic ovarian syndrome

Authors: A. M. MOORE, M. PRESCOTT, *R. E. CAMPBELL;
Univ. Otago, Ctr. Neuroendocrinol, Dunedin, New Zealand

Abstract: Polycystic ovarian syndrome (PCOS) is the most common cause of female infertility worldwide. In this syndrome, an increase in luteinising hormone (LH) pulse frequency is suggestive of changes in gonadotropin-releasing hormone (GnRH) neuron regulation by progesterone negative feedback. Previously, we have shown in a mouse model of PCOS generated by prenatal androgen (PNA) exposure that progesterone receptor (PR) expression is reduced in the arcuate nucleus (ARC) and that GnRH neurons receive a greater number of GABAergic inputs, which may contribute to modified GnRH neuron activity. We therefore sought to test the hypothesis that impaired progesterone negative feedback may result from reduced progesterone sensitivity in ARC GABAergic afferents that project to GnRH neurons. To achieve this, we assessed the negative feedback effects of progesterone in vehicle-treated control

(n=8) and PNA-treated (n=7) mice by measuring LH before and after ovariectomy and after implanting a progesterone pellet. We then identified the percentage of progesterone sensitive ARC GABAergic neurons by performing immunohistochemistry for PR on brain slices collected from vesicular GABA transporter (vGaT)-cre/tdTomato control (n=6) and PNA-treated (n=5) mice. Finally, we injected a Cre-dependent adenovirus expressing farnesylated enhanced green fluorescent protein (EGFPf) into the ARC of vGaT-cre control (n=5) and PNA-treated (n=6) mice to address whether progesterone sensitive ARC GABAergic neurons project to GnRH neurons. Compared with controls, PNA-treated mice exhibited a blunted postcastration rise in LH ($p<0.01$) and an absence of LH suppression by progesterone. PR colocalization with tdTomato positive-GABAergic neurons was significantly decreased in the ARC of PNA-treated mice compared with controls ($p<0.05$), suggestive of decreased progesterone sensitivity of ARC GABAergic neurons in PNA-treated mice. Remarkably, ARC GABAergic projections were found to contact 67.5±9.5% and 62.5±10.9% of GnRH neurons from control and PNA-treated mice, respectively. Interestingly, the density of ARC GABAergic fibres apposing the primary dendrite of GnRH neurons was significantly increased in PNA-treated mice compared to controls ($p<0.05$), in line with our previous findings. Finally, PNA-treated mice exhibited a significant decrease in the percentage of EGFPf-positive GABAergic neurons colocalised with PR in the ARC ($p<0.01$) compared with controls. Together, these data describe a novel, robust GABAergic input to GnRH neurons that originates in the ARC and may underpin decreased progesterone feedback to GnRH neurons in PCOS.

Disclosures: **A.M. Moore:** None. **R.E. Campbell:** None. **M. Prescott:** None.

Poster

075. Gonadotropin-Releasing Hormone and Hpg Control

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 75.04/II19

Topic: E.01. Neuroendocrine Processes

Support: UCLA Office of the Vice Chancellor for Research (NW)

UCLA Office of the Dean of the School of Medicine (NW)

National Institutes of Health (DP)

Title: Morphological and physiological interactions between GnRH3 and hypocretin/orexin neuronal systems in zebrafish

Authors: *Y. ZHAO[1], C. SINGH[2], M.-C. LIN[1], D. PROBER[2], N. WAYNE[1];
[1]Dept. of Physiol. David Geffen Sch. of Med., UCLA, LOS ANGELES, CA; [2]Div. of Biol., Caltech, Pasadena, CA

Abstract: Hypocretin/orexin (Hcrt) is a neuropeptide produced in the dorsal and lateral hypothalamus, and is best known for regulating arousal and food intake. Recent evidence indicates that it is also involved in reproduction as an inhibitor of hypothalamic gonadotropin-releasing hormone (GnRH) neurons. However, little work has been done on the anatomical and functional relationships between these two neuronal circuits. In the present experiments, we combined optical imaging and electrophysiology to explore the interaction and regulation between these two neural circuits in the hypothalamus. First, we generated a dual transgenic zebrafish line hcrt:RFP; GnRH3:EMD in which Hcrt and GnRH3 neurons are genetically labeled with red and green fluorescent proteins, respectively. Using confocal microscopy, this animal model allows us to study the development and interactions of these two neuronal systems from the embryonic stage (20 hours post-fertilization, hpf) to larval stage (8 days post-fertilization, dpf) *in vivo*, as well as in the excised adult brain. Similar to our published work, genetically labeled GnRH3 neurons started emerging in the hypothalamus around 20 hpf, gradually increasing in number and were located in the periventricular area throughout the hypothalamus. In contrast, genetically labeled Hcrt neurons were expressed in two bilateral clusters in the dorsal hypothalamus starting around 25 hpf. By 8 dpf, both Hcrt and GnRH3 neuronal processes projected widely throughout the brain. Furthermore, we observed close apposition of Hcrt and GnRH3 neurons in the hypothalamus, accompanied by focal co-localization of these two neuropeptides expression. Second, using *in situ* hybridization, we found that the hcrt receptor is expressed in hypothalamic GnRH3 neurons (5 dpf), indicating that Hcrt can directly regulate GnRH3 neurons. To elucidate the role of Hcrt in regulating GnRH3 neuron activity, we recorded the electrical activities of GnRH3 neurons using whole-cell and loose-patch electrophysiology and studied responses to Hcrt application with and without the Hcrt receptor antagonist almorexant. Similar to findings with hypothalamic GnRH neurons in mice, Hcrt significantly inhibited action potential firing frequency of GnRH3 neurons in adult zebrafish, and this effect was abolished with almorexant pretreatment. In summary, this study reveals that Hcrt neurons in zebrafish may play an important role in regulating reproduction through anatomical and physiological interactions with the GnRH3 neuronal system.

Disclosures: **Y. Zhao:** A. Employment/Salary (full or part-time):; ucla. **C. Singh:** None. **M. Lin:** None. **D. Prober:** None. **N. Wayne:** None.

Poster

075. Gonadotropin-Releasing Hormone and Hpg Control

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Program#/Poster#: 75.05/II20

Topic: E.01. Neuroendocrine Processes

Support: JSPS (24870007) to SK.

JSPS (26840111) to SK.

SUNBOR to SK.

JSPS(20247005) to YO.

Title: Hypothalamic NPY neurons decrease their npya expression after food depletion

Authors: *S. KANDA, S. KITAHARA, Y. OKA;
Dept Biol. Sci., Dept Biol Sci, Grad Sch. Sci, Univ. Tokyo, Tokyo, Japan

Abstract: Neuropeptide Y is supposed to be one of the most potent orexigenic peptides, and their expression in hypothalamus is increased after food restriction in mammals to regulate their energy condition. Moreover, it was recently reported that NPY directly inhibits firing activity of GnRH1 neurons in mice (Roa and Herbison, 2012). Thus, the NPY neurons are considered to regulate energy homeostasis as well as reproduction in concert in accordance with energy conditions. In spite of the importance of the NPY neurons in the energy homeostasis in mammals, neither the localization nor their physiological functions has been fully understood in vertebrate species other than mammals. Here, we used a small teleost, medaka, in which one can easily apply genetic manipulations as well as electrophysiology, and we detailed NPY neuron distribution by *in situ* hybridization and analyzed changes in *npy* expressions in accordance with the energy conditions. Here we showed that *npya* is expressed in many hypothalamic nuclei, optic tectum, and telencephalon, while its paralogs, *npyb*, which is considered to be duplicated in the 3R whole genome duplication, is expressed only in limited areas of telencephalon. Then, we examined differences in their expression levels between the well-fed group and the starvation group. Among all NPYa and NPYb neurons, the number of *npya* mRNA positive neurons that are distributed in areas ranging from nucleus ventralis tuberis (NVT), nucleus recessus lateralis (NRL) and nucleus anterior tuberis (NAT) specifically decreased in one day to two week-starvation groups. From these results, it was suggest that NPYa neurons in NVT, NRL, and NAT are sensitive to energy conditions, and they probably have functions related to food intake, although there has been no clear demonstration of NPY neuronal functions in teleosts. In addition, we examined co-expression of *npy* and *agrp* mRNA, which has been demonstrated in mammalian species. Surprisingly, there was no *npy* and *agrp* co-expressing neuron in medaka hypothalamus, although they were closely distributed. In summary, we found similarities and differences between mammals and teleosts. As in mammals, the hypothalamic *npya* expression in medaka varies in accordance with the energy status. However, contrary to the situation in

mammals, the *npya* expression decreases after food restriction in hypothalamic nuclei, and there was no colocalization with *agrp* expression. Further comparison between mammals and teleosts may provide clues to understanding the differences in energy balance strategies between poikilothermic and homoeothermic animals as well as general mechanisms common to both.

Disclosures: **S. Kanda:** None. **S. Kitahara:** None. **Y. Oka:** None.

Poster

075. Gonadotropin-Releasing Hormone and Hpg Control

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 75.06/II21

Topic: E.01. Neuroendocrine Processes

Support: NSF CAREER Award 1253126 to SR

Title: Impact of bisphenol A on the developing GnRH3 neural system and locomotor behavior in Japanese medaka

Authors: *T. INAGAKI[1,2], E. K. LEE[2], S. RAMAKRISHNAN[1,2];
[1]Biol., [2]Neurosci. program, Univ. of Puget Sound, Tacoma, WA

Abstract: Bisphenol A (BPA), a xenoestrogen, disrupts normal brain function and behavior mediated by neuroendocrine systems. Gonadotropin-releasing hormone (GnRH) neurons in the brain play a crucial role in reproductive physiology and behavior in most vertebrates. In fish, three types of GnRH neurons, GnRH1 at the preoptic area (POA), GnRH2 at the midbrain, and GnRH3 at the terminal nerve (TN), have been identified. While it is known that GnRH3 neurons project widely throughout the brain and spinal cord, and that neuromodulatory roles in reproductive behavior have been suggested, their functions remain unclear. Aside from reproduction, recent studies have also alluded to a role for GnRH in locomotion, with GnRH receptors found in the spinal cord. Here we examine effects of chronic exposure to low dose BPA through development on GnRH3 neuronal systems, and locomotor activity in transgenic Japanese medaka with GnRH3 neurons tagged with green fluorescent protein (GFP). Fertilized medaka eggs were collected daily, and placed individually in 12- or 24-well plates. Embryos in BPA groups were chronically exposed to 200 ng/ml of BPA starting on 1 day post-fertilization (dpf), through hatch until 10 days post hatch (20 dpf). To assess effects of chronic BPA exposure on GnRH3 neurons, brain images were taken from live embryos in eggs at 1, 2, 3 and 4 dpf, and intensity of GnRH3-GFP neurons were analyzed using an upright epifluorescence microscope.

At 3 dpf, GnRH3-GFP neurons in BPA-treated embryos showed 23.7% increased fluorescence in the terminal nerve population and 14.3% reduced intensity in the trigeminal population when compared to vehicle treated control embryos. Hatched larvae were kept in glass petridishes with continued exposure to 200 ng/ml BPA. Locomotor activity of larvae was observed at 20 dpf (~10 days post hatch) and analyzed using Noldus Ethovision XT. Head-growth, body sizes, times to hatching were also measured. We found that BPA exposed larvae (n=12 each group) showed significantly decreased locomotory activity both in total distance covered (88.36 ± 28 mm vs. 176.2±14.6 mm controls, $p<0.012$) and velocity of movement (2.53 ±0.6 mm/s vs. 4.74± 0.72 mm controls $p<0.013$). There were no significance differences in head-growth, body sizes and times to hatching between BPA and the control groups. This study will allow us to understand BPA effects on GnRH3 neuron development and how it may affect locomotion. (Supported by NSF Career Award 1253126 to SR).

Disclosures: **T. Inagaki:** None. **E.K. Lee:** None. **S. Ramakrishnan:** None.

Poster

075. Gonadotropin-Releasing Hormone and Hpg Control

Location: Halls A-C

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Program#/Poster#: 75.07/II22

Topic: E.01. Neuroendocrine Processes

Support: JSPS 238950 to TK

JSPS 20247005 to YO

Title: Dopaminergic regulation on gonadotoropes in medaka

Authors: ***T. KARIGO**, Y. MOCHIZUKI, Y. OKA;
Dept Biol Sci, Grad Sch. Sci, Univ. Tokyo, Tokyo, Japan

Abstract: The inhibitory functions of dopamine (DA) on the hypothalamic-pituitary-gonadal (HPG) axis regulation have been suggested in some mammalian species (ewe, Goodman et al., 2012; mice, Liu and Herbison, 2013). Also in teleost fish, the inhibitory effect of DA on reproduction has been studied especially in goldfish. DA has been suggested to inhibit GnRH release from the hypothalamus and GTH release from the pituitary (Yu and Peter, 1990, 1992). We have already shown the involvement of DA on the HPG axis regulation at the levels of both GnRH1 neuron and gonadotropes in medaka (Karigo et al, SfN 2013 abstract). We found that

TH immunoreactive (ir) neurons project to the ventral preoptic area GnRH neurons, which in turn directly project to the pituitary, and also directly project to LH and FSH cells in the pituitary. We also showed that DA significantly inhibits the neural activities of GnRH1 neurons. However, we have not examined the physiological effects of DA on the gonadotropes. To examine such effects, we here performed the following experiments. First, by using retrograde axonal tracing experiments with biocytin, we identified some groups of pituitary projecting TH-ir neurons in the hypothalamic nucleus, nucleus ventralis tuberis (NVT) and nucleus anterior tuberis (NAT). Next, we analyzed the distribution of DA receptors in the brain and pituitary. DA receptors are generally known to be classified into two families and five subtypes; D1-like receptor family (D1R and D5R) and D2-like receptor family (D2R, D4R and D5R). We found eight subtypes of DA receptors from medaka genome database; two D1Rs, two D2Rs, one D3R, two D4Rs, and one D5R. As Fontaine et al. (2013) reported in zebrafish that they have three types of D2 receptors, D2a, D2b, and D2c, and LH cells express all three, we first focused on D2 receptors, D2-1R and D2-2R, in medaka. We performed *in situ* hybridization of D2-1R and D2-2R and found that D2-1R is mainly expressed in the brain, while D2-2R is strongly expressed in the pituitary. We performed double labeling of LH or FSH cells and D2-2R mRNA and found that some of the LH and FSH cells express D2-2R. The two types of gonadotropes, LH and FSH cells, are separated into two different cell populations in teleosts, and we have already generated transgenic medaka lines that express Ca^{2+} indicator separately in those cells (Karigo et al., 2014). We are currently analyzing the effects of DA on LH and FSH cells by Ca^{2+} imaging using those transgenic medaka lines. LH and FSH cells tend to show different Ca^{2+} responses to DA applications. Here we suggest characteristics of complex dopaminergic regulations on the HPG axis.

Disclosures: **T. Karigo:** None. **Y. Mochizuki:** None. **Y. Oka:** None.

Poster

075. Gonadotropin-Releasing Hormone and Hpg Control

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 75.08/II23

Topic: E.01. Neuroendocrine Processes

Support: LSU-HHMI grant for Undergraduate Research

Title: Immunocytochemical localization of gonadotropin-releasing hormone (GnRH) neurons in the brain of the American alligator, alligator mississippiensis

Authors: K. HUANG[1], Y. GAO[2], *R. TERUYAMA[1];
[1]Dept of Biol. Sci., Louisiana State Univ., Baton Rouge, LA; [2]Dept. of Neurosci., Johns Hopkins Univ., Baltimore, MD

Abstract: The gonadotropin-releasing hormone (GnRH) system plays a fundamental role in coordinating reproductive physiology and behavior of animals. The present study was conducted to localize GnRH neurons in the alligator brain. Because nomenclature for specific neural structures is not determined in the alligator brain, we first constructed a stereotaxic atlas of the alligator hypothalamus. Six alligators were deeply anesthetized and perfused with 0.9% saline, which was followed by buffered 4% paraformaldehyde fixative. Animals were decapitated and the heads were stored overnight in the same fixative then transferred to 20% sucrose in PBS overnight for cryoprotection. The top of the skull was removed to expose the brain, and the head was then mounted on a modified monkey stereotaxic apparatus with the mouth placed 45° below the horizontal axis of the instrument. A portion of the brain that containing the hypothalamic region was blocked in the stereotaxic apparatus. Each block was then sectioned using a cryostat or a freezing microtone. Brain sections obtained from the cryostat were stained with Nissl stain and used for construction of a stereotaxic atlas. Nomenclature for specific neural structures was selected from several stereotaxic atlases for avian brains based on similarities in staining characteristics and relative locations of the specific structures. Brain sections obtained from a freezing microtome were used for immunocytochemical localization of GnRH using the free-floating incubation technique. GnRH immunoreactive structures were then mapped in the atlas. Groups of GnRH immunoreactive neurons were found along the midline in the preoptic area close to the third ventricle and in the lateral septal area close to the lateral ventricle. Dendritic processes of some of the GnRH immunoreactive neurons appeared to penetrate the ependymal cell layers of the lateral and the third ventricle indicating that some GnRH neurons are "the cerebrospinal fluid contacting neurons". These results demonstrate that the distribution of GnRH neurons in the alligator is similar to that found in avian brains.

Disclosures: **K. Huang:** None. **Y. Gao:** None. **R. Teruyama:** None.

Poster

075. Gonadotropin-Releasing Hormone and Hpg Control

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Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

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Topic: E.01. Neuroendocrine Processes

Support: HRC Grant 11/404

Title: Revealing synaptic inputs along the full extent of gonadotropin-releasing hormone (GnRH) neuron processes using an *in vivo* viral-mediated cell filling technique

Authors: *S. YIP, R. CAMPBELL;
Ctr. for Neuroendocrinology, Dept. of Physiol., Univ. of Otago, Dunedin, New Zealand

Abstract: Gonadotropin-releasing hormone (GnRH) neurons are critical for the regulation of fertility. The morphology of GnRH neurons is unique in that some neurons extend one or two lengthy dendrites millimeters away from the somata and, intriguingly, elaborate into axons in the median eminence (ME). Much of our understanding of the synaptic regulation of GnRH neurons has been limited to investigating GnRH somata and proximal dendrites scattered around the rostral forebrain due to limitation of the length of dendritic that can be imaged. An *in vivo* cell-filling approach has overcome this limitation and enabled us to investigate the density of synaptic inputs throughout the rostral to caudal extent of GnRH neuron processes. Adult female GnRH-Cre mice (n=4) were stereotaxically injected with 100nl (5.1x1011 pfu/ml) of a Cre-dependent recombinant adenovirus containing a farnesylated enhanced green fluorescent (EGFPf) gene into the rostral preoptic area (rPOA) and animals were perfused 5 days later. Half of each fixed brain was cut into 30μm sagittal sections and immuno-labelled for GFP, to identify the full extent of GnRH neuron and synapsin (a pre-synaptic marker). Adenoviral-mediated cell filling was restricted to a small number of GnRH neuron exclusively. The rostral to caudal extent of GFP-positive fibre projections was divided into 3 zones (approximately 1000μm each) between the rPOA and the ME. The number of GFP-positive fibres were counted in each zone and their morphologies were characterised as type 1 smooth fibre or type 2 fibre with swellings. The total density of synapsin puncta in close apposition to GFP-positive fibres were then evaluated for each fibre type from each zone using confocal microscopy. Our results showed that approximately 2 to 15 GnRH neurons, restricted to the rPOA were filled in each animal. Both fibre types were found in equal number throughout the rostral to caudal zones investigated. Evaluation of synapsin close appositions to GFP-positive GnRH processes found a total synapsin density of 0.27±0.02, 0.33±0.06 and 0.24±0.01 puncta per μm for type 1 fibres and 0.24±0.05, 0.28±0.04 and 0.22±0.03 puncta per μm for type 2 fibres from zones 1, 2 and 3 respectively. These data indicate that the lengthy processes of rPOA GnRH neurons possess synaptic input, equally distributed throughout their entire length, regardless of the distance from the somata. Thus this study has opened a new window to explore the hierarchy of synaptic inputs and their function related to plasticity in GnRH neurons, for example, during different stages of reproductive cycle and in states of infertility.

Disclosures: **S. Yip:** None. **R. Campbell:** None.

Poster

075. Gonadotropin-Releasing Hormone and Hpg Control

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Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 75.10/II25

Topic: E.01. Neuroendocrine Processes

Support: University of Otago

NZ HRC

Title: Optogenetic activation of mouse GnRH neurons *in vivo*

Authors: P. CAMPOS, B. HYLAND, *A. E. HERBISON;
Ctr. for Neuroendocrinology, Univ. of Otago, Dunedin, New Zealand

Abstract: The gonadotrophin-releasing hormone (GnRH) neurons represent the key output cells of the neuronal network controlling the hypothalamic pituitary-gonadal axis and thus fertility in all mammalian species. The patterns of neuronal activity by which GnRH neurons generate pulses of GnRH and thus luteinizing hormone (LH) are unknown. To date, the scattered distribution of the GnRH cell bodies remain the main limitation to investigating the cellular events that lead to pulsatile secretion of LH. The ability to remotely manipulate the neuronal activity of GnRH neurons in a selective manner *in vivo* would facilitate our understanding of the patterns of GnRH neuron activity required to generate pulses of LH. We have used a Cre-dependant adeno-associated virus (AAV) in transgenic GnRH-Cre mice to target channelrhodopsins (ChR2) to the GnRH neuronal phenotype. Taking advantage of the retrograde transport properties of AAVs, we injected AAV into the median eminence of GnRH-Cre mice to target expression of ChR2 to only hypophysiotropic GnRH neurons. Immunofluorescence studies showed that ≈93% of all ChR2-expressing cells in the brain expressed GnRH. First, to characterize the ability of ChR2 to modulate GnRH neuron excitability we prepared acute brain slices from AAV-injected GnRH-Cre mice and undertook cell attached recordings of ChR2-expressing GnRH neurons. Blue light stimulation using 5 ms pulses at 10Hz and 30 Hz for up to 5 minutes induced action potential firing with a response fidelity of 85% and 72% respectively. Second, we stimulated the GnRH neurons *in vivo* in AAV injected GnRH-Cre female ovariectomized mice by placing an optic fiber in the rostral preoptic area and applying different patterns of activation in order to elicit LH secretion. Activation of GnRH neurons for 5 minutes at 1 Hz and 5 Hz was found to have no effect on LH secretion. Blue-light stimulation at 10Hz ($p=0.0024$) and 30Hz ($p=0.0008$) produced significant pulse-like increases in LH secretion. Synchronized burst firing has been suggested to underlie GnRH pulsatility. As such, GnRH neurons were synchronously activated for 5 minutes to fire in bursts using the burst patterning previously detected for GnRH neurons *in vivo*. This was found to have no effect on LH secretion.

Together, these studies are highlighting the patterns of GnRH neuron electrical activity required to generate pulsatile LH secretion.

Disclosures: **P. Campos:** None. **A.E. Herbison:** None. **B. Hyland:** None.

Poster

075. Gonadotropin-Releasing Hormone and Hpg Control

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Topic: E.01. Neuroendocrine Processes

Support: NSF Grant IOS-1052288

Title: GnRH-(1-5)-mediated regulation of the extracellular milieu in the migration of GN11 cells

Authors: ***D. O. LARCO**[1], S. MANI[2], T. WU[1];
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Abstract: Gonadotropin-Releasing Hormone (GnRH) neurons originate outside the central nervous system (CNS) in the nasal placode where their migration to the basal forebrain is dependent on the integration of multiple signaling cues during development. The proper migration and establishment of the GnRH neuronal population within CNS is critical for normal pubertal onset and reproductive function. The endopeptidase EP24.15 is expressed along the migratory path of GnRH neurons and cleaves the full-length GnRH to generate the metabolite GnRH-(1-5). Using the GN11 cell model, which is considered an immature GnRH neuronal cell line, we recently demonstrated that GnRH-(1-5) inhibits cellular migration in a wound closure assay by binding the orphan G protein-coupled receptor 173 (GPR173). GnRH-(1-5) activating GPR173 initiates the formation of a complex with Beta-arrestin 2 and phosphatase and tensin homolog to subsequently inhibit the signal transducer and activator of transcription 3 (STAT3) pathway to regulate migration. In this study, we screened for genes and secreted factors implicated cell migration that are regulated in response to GnRH-(1-5) in GN11 cells. To do so, we used a PCR array (Qiagen) to measure changes in 84 genes in cells treated with GnRH-(1-5) for 30 min. Acute GnRH-(1-5) treatment significantly decreased members of the cytokine signaling pathway such as IL-1alpha (0.62 relative to 1.0) and IL-4Ralpha (.73 relative to 1.0), which correlates with our previous finding that GnRH-(1-5) inhibits the normally cytokine-activated STAT3 pathway. We also measured members of the transforming growth factor beta

family and found that GnRH-(1-5) had no effect, indicating a cytokine-specific mechanism of regulation. These results suggest that GnRH-(1-5) may modulate the response of migrating GnRH neurons to external cues present in the extracellular milieu (ECM). Therefore, we examined the effect of GnRH-(1-5) on the migration of GN11 cells in the presence of an *in vitro* ECM using a matrigel invasion assay (BD biosciences). Interestingly, GnRH-(1-5) (100 nM) significantly ($p < 0.05$) inhibited the ability of GN11 cells to migrate through matrigel environment relative to control cells. Collectively, our studies demonstrate that the downstream mechanism of GnRH-(1-5) binding GPR173 to decrease cell migration and invasion is ECM-dependent and likely involves changes in the response and sensitivity of GnRH neurons to external cues.

Disclosures: **D.O. Larco:** None. **S. Mani:** None. **T. Wu:** None.

Poster

075. Gonadotropin-Releasing Hormone and Hpg Control

Location: Halls A-C

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Program#/Poster#: 75.12/II27

Topic: E.01. Neuroendocrine Processes

Support: NIH HD027305

Title: Estradiol and progesterone regulation of Jak/Stat signaling in hypothalamic neuronal cells

Authors: H. L. ADAMS[1], K. A. INTLEKOFER[2], *S. L. PETERSEN[3];
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Abstract: Progesterone (P_4) acts rapidly to maximize estradiol (E_2)-dependent gonadotropin-releasing hormone (GnRH) neuronal activity and luteinizing hormone (LH) surge release. Subsequently, P4 inhibits the daily LH surge initiated by E_2. Regulation of the surge is most likely through neurons in the anteroventral periventricular nucleus (AVPV), a preoptic region that communicates steroid signals to GnRH neurons. Our previous studies localized expression of a non-classical progestin receptor, progesterone receptor membrane component 1 (Pgrmc1), as well as its interacting partners, to the AVPV. Little is known about Pgrmc1 signaling in the brain; therefore, to identify pathways through which it may act, we used mouse N42 hypothalamic cells that model AVPV neurons. We previously performed microarray analyses with and without Pgrmc1 silencing and probed the resulting data sets with Ariadne Pathway

Studio™. Four out of the five pathways identified as significantly regulated by Pgrmc1 involved signal transducer and activator of transcription (Stat). To determine whether Stat signaling molecules may play a role in mediating steroid hormone effects, we first verified that *stat3, stat5a, stat5b, janus kinase 1 (jak1) and jak2* genes were all expressed in N42 cells. We then examined effects of E_2 and P_4 on expression of each of these genes using QPCR. We found that Stat3 and Stat5A mRNAs were significantly increased by 10nM E_2, but not by P_4 alone. In contrast, there were no steroid effects on Stat5B, Jak1 or Jak2 mRNA levels. To determine whether Pgrmc1 regulated Stat3 signaling, we silenced the gene in N42 cells transfected with a Stat3 reporter construct. Although P_4 failed to regulate Stat3 mRNA levels, we found that Stat3 promoter activity was repressed by Pgrmc1. These findings suggest that E_2 stimulation of Stat3 expression and P_4 blockade of Stat3 signaling may explain differential effects of these steroids on GnRH and LH surge release. Studies in progress are testing whether Pgrmc1 gene targets previously identified using microarrays are regulated by STAT3.

Disclosures: **H.L. Adams:** None. **K.A. Intlekofer:** None. **S.L. Petersen:** None.

Poster

075. Gonadotropin-Releasing Hormone and Hpg Control

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Topic: E.01. Neuroendocrine Processes

Support: This work was supported by CONACYT fellowship program 257136.

Title: Effects of the blockade of DA1 ovarian receptors on spontaneous ovulation and steroids hormones synthesis

Authors: *B. VENEGAS MENESES[1], M. GARCIA[1], J. L. MORAN[1], C. MORAN[1], A. HANDAL[1], N. H. ROSAS-MURRIETA[2], R. DOMINGUEZ[3];
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Abstract: There is evidence that the ovarian innervation participates in the regulation of gonadotropin effects on the ovaries. The participation of the DA2 receptors in the regulation of the gonadotropin secretion and ovulatory process have been described. The effect of the peripheral blockade of dopaminergic system on ovulation varies during the estrous cycle and

presents some type of circadian variations. The aim of this work was to evaluate the effects of local blockade of DA1 ovarian receptor at 8:00, 13:00 or 20:00 h on dioestrous-1 on spontaneous ovulation, the synthesis of steroid hormones: progesterone (P4) and estradiol (E2) on estrous predicted. Cyclic rats of the CIIZV strain, maintained under controlled light/dark cycle (lights on 05.00-19.00) were used. On diestrous-1 at 8:00, 13:00 or 20:00 h, the animals were laparotomized dorsolaterally and the bursas of the ovaries were injected with 20 µL per ovary, SCH-23390 [5 µg/µL] or with vehicle. An untouchedcontrol group was included. The animals were sacrificed at 9:00 h of the next predicted estrous day. The blood of the trunk was collected, centrifuged and stored at -20 °C until hormones were measured in the serum by RIA. At autopsy, the oviducts were dissected and the number of ova present was counted with the aid of a dissecting microscope. The vehicle injection did not modify the spontaneous ovulation on D1. The blockade of DA1 receptor in ovaries on D1 blocked the spontaneous ovulation in all animals. The P4 and E2 levels in non-ovulating rats resulting by the injection with SCH not modify The E2 levels in non-ovulating rats resulting by the blockade of the DA1 ovarian receptors at 08:00, 13:00 or 20:00 h on D1 were higher than in control animals. Most of the differences were not significant because the high dispersion of data resulted in higher levels than control group. Based on the results suggest that blockade of ovulation can be the result of increased blood concentrations of P4 and E2.

Disclosures: **B. Venegas Meneses:** None. **J.L. Moran:** None. **C. Moran:** None. **A. Handal:** None. **M. Garcia:** None. **N.H. Rosas-Murrieta:** None. **R. Dominguez:** None.

Poster

075. Gonadotropin-Releasing Hormone and Hpg Control

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Program#/Poster#: 75.14/II29

Topic: E.01. Neuroendocrine Processes

Support: ZIA NS002824-24

Title: Examination of hypothalamic POMC cells in GnRH deficient mice

Authors: *C. TAYLOR-BURDS, B. POPE, S. WRAY;
CDNS, NIH/NINDS, Bethesda, MD

Abstract: Recently, selective ablation of the transcription factor NSCL-2 under the Gonadotropin releasing hormone-1 (GnRH) promoter was found to reduce the number of GnRH

neurons in the preoptic area and proopiomelanocortin (POMC) neurons in the Arc in male mice (Schmid et al., 2013). GnRH neurons located within the brain are integral components of the hypothalamic-pituitary-gonadal axis, and as such, control reproduction and puberty. It is well established that metabolic state can alter or disrupt reproductive function and puberty onset. This interaction has been associated, in part, with POMC neurons in the arcuate nucleus (Arc), key players in energy homeostasis. GnRH neurons contact POMC Arc neurons (and vise versa) and GnRH1R is expressed in neurons within the Arc, enabling direct interactions to occur between these two systems. However, it is also known that the GnRH promoter is transiently expressed in many cells during development. Thus, it is unclear whether the loss of POMC cells after ablation of NSCL-2 under the GnRH promoter was due to 1) direct reduction of neuroendocrine GnRH neurons or 2) indirect developmental changes associated with loss of the NSCL-2 in transient expressing non-neuroendocrine GnRH cells. To determine if GnRH signaling can alter expression of, or number of POMC hypothalamic neurons, we are utilizing the HPG mouse which lacks GnRH peptide. Preliminary data indicate that the number of POMC ARC is similar in brains from WT and HPG adult males. These findings suggest that loss of GnRH itself does not reduce POMC expression or the number of POMC Arc cells. Further experiments will determine whether age differences or sex differences occur in the POMC ARC cells in HPG compared to WT mice.

Disclosures: **C. Taylor-Burds:** None. **B. Pope:** None. **S. Wray:** None.

Poster

075. Gonadotropin-Releasing Hormone and Hpg Control

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Program#/Poster#: 75.15/II30

Topic: E.01. Neuroendocrine Processes

Support: ZIA NS002824-24

Title: Environmental pollutant BPA inhibits GnRH neuronal activity

Authors: ***U. KLENKE**, S. WRAY;
CDNS/NINDS/NIH, Bethesda, MD

Abstract: Reproduction is dependent on a functional hormonal cascade between hypothalamus, pituitary and gonads. However, peripheral feedback of estrogens coming from the gonads towards the hypothalamus is critical for proper timing of reproduction. Bisphenol-A (BPA), an

environmental pollutant with estrogenic actions, can disrupt this feedback and lead to infertility in both humans and animals. Although the hypothalamic neurons secreting gonadotropin-releasing hormone (GnRH) are potential targets for endocrine disruptors, to date direct effects of BPA on GnRH neurons have not been shown. Our study investigated the effects of BPA on GnRH neuronal activity using an explant model. In this *in vitro* model, large numbers of primary GnRH neurons are maintained that express many of the receptors found *in vivo* and exhibit oscillations in intracellular calcium that correlate with electrical activity. Single-cell RT-PCR analysis confirmed GnRH neurons express estrogen receptor (ER) β, GPR30 and estrogen related receptor γ, all potential targets for BPA. Calcium imaging was used to assay BPA effects on GnRH neuronal activity. Exposure to BPA (50 μM) significantly decreased GnRH calcium activity by 20%. Notably, population analysis revealed that only 20% of GnRH neurons responded to BPA, but that these had a decrease in activity of >50%. Blockage of GABAergic and glutamatergic input did not abrogate the inhibitory BPA effect, suggesting direct regulation of GnRH neurons by BPA. Perturbation studies of the signaling pathway revealed that both ERβ and GPR30 are involved in the BPA-mediated inhibition of GnRH neuronal activity. These data show that the endocrine disruptor BPA can directly affect a subpopulation of GnRH neurons via ERβ and GPR30. These results provide the first evidence of a direct effect of BPA on GnRH neurons.

Disclosures: **U. Klenke:** None. **S. Wray:** None.

Poster

075. Gonadotropin-Releasing Hormone and Hpg Control

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Program#/Poster#: 75.16/JJ1

Topic: E.01. Neuroendocrine Processes

Support: ZIA NS002824-24

Title: Are GnRH neurons affected by prenatal exposure to the endocrine-disrupting-compound BPA?

Authors: *H. STONER, U. KLENKE, S. WRAY;
NIH/NINDS, Bethesda, MD

Abstract: Bisphenol-A (BPA) is an estrogenic endocrine disrupting compound (EDC) used in production of polycarbonate plastics, including food and drink storage containers. There is a

growing concern over the effects of BPA on the development of the reproductive axis, which is estrogen sensitive. Gonadotropin-releasing hormone-1 (GnRH) neurons are an integral component of the reproductive axis and express estrogen receptors. As BPA is an estrogenic EDC, the reproductive axis - and possibly the GnRH neuron population - may be vulnerable to disruption. This study investigated the effects of prenatal BPA exposure on GnRH neuronal development. GnRH neurons migrate from the nasal placode to the brain between embryonic day (E) 11.5-16.5. Once within the brain, GnRH neurons are distributed bilaterally throughout the forebrain. BPA (50 mg/kg/day) or vehicle solution (castor oil) was delivered to pregnant dams for five days (E10.5-14.5) via gavaging. Embryos (E14.5, E16.5), as well as brains of prenatally exposed adult males and females (postnatal day (PN) 36) were collected and fresh-frozen. Tissue was serially sectioned, stained for GnRH, and the location and number of GnRH-positive neurons quantified. Preliminary analysis indicates that at E14.5, and in adults, BPA does not change the total number of GnRH neurons but alters the distribution of the cells. At E14.5, BPA decreased the number of GnRH neurons located in the forebrain junction and increased the number in the brain, suggesting accelerated migration. In adult brains (PN36, male), a difference between the distribution of GnRH neurons in BPA-treated versus control mice was detected, with GnRH cells located more caudally in treated mice. Whether the change in GnRH cell location after BPA exposure alters reproductive function is currently being examined.

Disclosures: **H. Stoner:** None. **U. Klenke:** None. **S. Wray:** None.

Poster

076. Kisspeptin and Related Systems

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Program#/Poster#: 76.01/JJ2

Topic: E.01. Neuroendocrine Processes

Support: NIH Grant HD41469

Title: Fast glutamatergic transmission to hypothalamic kisspeptin neurons is regulated in an estrous cycle-dependent manner

Authors: *L. WANG[1], S. M. MOENTER[1,2,3];
[1]Mol. and Integrative Physiol., [2]Intrnl. Med., [3]Obstetrics & Gynecology, Univ. of Michigan, Ann Arbor, MI

Abstract: Gonadotropin-releasing hormone (GnRH) neurons of the hypothalamus form the final common pathway for central control of reproduction. Estradiol, via estrogen receptor alpha (ERα), is a major hormone providing feedback to GnRH and subsequent luteinizing hormone (LH) release. Relatively low levels of estradiol through most of the reproductive cycle provide negative feedback (-FB) regulation of the hypothalamus. During the preovulatory stage, estradiol levels rise and estradiol action switches from negative to positive feedback (+FB) to initiate the GnRH/LH surges, causing ovulation. Blockade of ionotropic glutamate (iGlu) receptors in the hypothalamus blocks the LH surge *in vivo*. Since GnRH neurons receive limited iGlu input and are under indirect ERα regulation, we tested if iGlu input to afferents of GnRH neurons are regulated by estradiol. Kisspeptin-producing neurons in the anteroventral periventricular nucleus (AVPV) and arcuate nucleus (ARC) are probable estradiol-sensitive GnRH afferents. Kisspeptin increases GnRH neuron activity. Estradiol increases kisspeptin gene expression in AVPV and decreases it in ARC. This evidence led to a model that kisspeptin AVPV neurons mediate estradiol +FB actions and ARC neurons mediate -FB actions. We hypothesize that iGlu input to these two kisspeptin populations is differentially regulated by estradiol during the cycle. To begin to test this, we studied presynaptic iGlu input to kisspeptin neurons in adult female C57BL/6-Tg Kiss1-hrGFP mice in the afternoon of proestrus (+FB, high estradiol level) and diestrus (-FB, low estradiol level). Spontaneous excitatory postsynaptic currents (EPSCs) mediated by AMPA receptors were recorded via whole-cell voltage-clamp. Membrane potential was held at -66mV to isolate AMPA-mediated EPSCs; blockade of AMPA receptors eliminated all PSCs (n=4). AVPV kisspeptin neurons exhibit increased EPSC frequency during +FB compared to -FB (3.8±0.5 n=11 vs 1.4±0.3 Hz n=12; $p<0.001$). In contrast, ARC kisspeptin neurons exhibit decreased EPSC frequency during +FB (1.5±0.2 n=9 vs 2.8±0.4 Hz n=10; $p<0.01$). EPSC amplitude was unaffected by cycle stage, suggesting no change of postsynaptic receptor expression or affinity. Altered EPSC frequency suggests that glutamatergic neurons presynaptic to kisspeptin neurons are also under cyclic estradiol regulation, changing either activity or the number of synapses. Future studies will help distinguish between these mechanisms. Our observations further imply that while kisspeptin neurons are involved in estradiol -FB and +FB during the cycle, upstream Glu neurons also appear to be critical elements of the feedback circuits.

Disclosures: **L. Wang:** None. **S.M. Moenter:** None.

Poster

076. Kisspeptin and Related Systems

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Topic: E.01. Neuroendocrine Processes

Support: NIH Grant HD34860

Title: Kisspeptin stimulates gonadotropin-releasing hormone (GnRH) release detected by fast-scan cyclic voltammetry (FSCV) via mechanisms requiring both mobilization of intracellular Ca2+ stores and its influx through Ca2+-permeable membrane channels

Authors: *K. M. GLANOWSKA[1], S. M. MOENTER[2];
[1]Neurosci. Grad. Program, Univ. of Virginia, Charlottesville, VA; [2]Mol. and Integrative Physiology, Intrnl. Med. and Obstetrics and Gynecology, Univ. of Michigan, Ann Arbor, MI

Abstract: GnRH release is the final output for central control of fertility. Kisspeptin increases GnRH release in the median eminence (ME) for control of reproduction, and in the preoptic area (POA), where GnRH is a neuromodulator. GnRH release evoked by local thapsigargin differs between these regions, being action potential (AP)-dependent in ME but not POA. We tested the mechanisms of GnRH release evoked by locally-applied kisspeptin in the ME vs POA. FSCV was used to detect GnRH release in brain slices from adult male GnRH-GFP mice. FSCV electrodes were placed to detect GnRH release near neuron terminals in the ME or between GFP-identified GnRH neuron processes in the POA. To study the role of intracellular Ca^{2+} stores, xestospongin C (XC) was used to block IP_3-mediated Ca^{2+} release. XC (local, near kisspeptin application) blocked kisspeptin-induced GnRH release in 5 of 6 ME slices, but 0 of 5 POA slices. To test if XC failed to block GnRH release in the POA because its targets are more distal than kisspeptin receptors, XC was bath-applied; this blocked GnRH release in 7 of 7 slices. This suggests different arrangement of kisspeptin receptors relative to intracellular stores in POA vs ME processes and/or differences in local networks in these regions. We next tested if kisspeptin-evoked release in the ME is AP-dependent. Surprisingly, kisspeptin evoked release in 8 of 9 slices after blocking APs with TTX. Thapsigargin mobilization of intracellular Ca^{2+} failed to evoke GnRH release when APs were blocked, suggesting kisspeptin evokes GnRH release via additional mechanisms. Kisspeptin depolarizes GnRH neuron membrane potential, which could open voltage-gated Ca^{2+} channels. To test if extracellular Ca^{2+} influx contributes to kisspeptin action in the ME, we tested kisspeptin-evoked GnRH release in the presence of the Ca^{2+} channel blocker Cd^{2+} plus TTX. This combination successfully blocked kisspeptin-evoked GnRH release in 4 of 4 slices, indicating that kisspeptin stimulation of GnRH release requires both entry of extracellular Ca^{2+} and mobilization of intracellular Ca^{2+} stores. Both mechanisms appear to be required since inhibition of either prevents GnRH release. The ability of kisspeptin to induce GnRH release in the ME in the absence of APs indicates local interactions among kisspeptin and GnRH neuronal processes in this region could stimulate GnRH release without involving the somatic regions of GnRH neurons. This suggests the hypothesis that tight coupling between action potential generation and hormone release may not occur in GnRH neurons. Further, the

variations in GnRH release between the POA and ME suggest functional differences in GnRH processes in these two regions.

Disclosures: **K.M. Glanowska:** None. **S.M. Moenter:** None.

Poster

076. Kisspeptin and Related Systems

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Topic: E.01. Neuroendocrine Processes

Support: NIH Grant HD41469

Title: Estradiol modulates the number of GABAA receptors binding GABA at arcuate nucleus kisspeptin (KNDy) neuron synapses

Authors: *R. A. DEFAZIO[1], C. F. ELIAS[1,2], S. M. MOENTER[1,2,3];
[1]Mol. and Integrative Physiol., [2]Obstetrics and Gynecology, [3]Intrnl. Med., Univ. of Michigan, Ann Arbor, MI

Abstract: KNDy neurons are components of the central circuitry controlling fertility. Estradiol feedback alters the postsynaptic response of KNDy neurons to GABA. Specifically, estradiol reduces the amplitude of action potential-independent (miniature) postsynaptic currents (mPSCs). $GABA_A$ receptors ($GABA_A$Rs) are ligand-gated ion channels and changes in mPSC amplitude typically depend on postsynaptic factors such as receptor (i.e., channel) number, conductance and affinity. To identify which of these contribute to estradiol-induced changes in mPSC amplitude, we used non-stationary noise analysis (NSNA), which allows conductance and channel number to be estimated from the variance of the current during the decay phase of individual mPSCs. Briefly, mPSCs occurring in isolation were aligned and averaged to generate the mean mPSC (I_{mean}). I_{mean} was scaled to the peak of each individual event, subtracted from it and the difference was squared to calculate variance. Variances of individual mPSCs were averaged and plotted as a function of I_{mean}. This plot was fitted with a parabola: variance = $i*I_{mean} - I_{mean}^2/N$, where 'i' is the current via an individual channel (unitary current), and 'N' is the number of $GABA_A$R channels activated by synaptic GABA release. Unitary current was divided by driving force to yield unitary conductance. To validate the method, we recorded mPSCs from cortical pyramidal neurons (n=2) at holding potentials of -60mV and -80mV. Consistent with previous studies, mean unitary conductance was 24.2 pS, regardless of holding potential, and the

mean number of channels was 23.3. To assess the role of estradiol on KNDy neuron $GABA_A$Rs, recordings were obtained from ovariectomized (OVX) mice ± estradiol (E). Estradiol did not alter unitary conductance (OVX 46.4±6.6 vs OVX+E 54.5±5.3 pS; $p>0.3$, n=6 cells/group). In contrast, estradiol decreased the number of activated channels (OVX 44.9±5.4 vs OVX+E 25.1±3.1; $p<0.02$). Changes in the number of postsynaptic $GABA_A$Rs activated by synaptic GABA release can be due to changes in number of available receptors or to changes in receptor affinity. We tested this with diazepam, which increases affinity of $GABA_A$Rs for GABA and thus increases mPSC amplitude if there are unbound postsynaptic $GABA_A$Rs. Diazepam increased mPSC amplitude in 5 of 5 recordings, suggesting that $GABA_A$Rs are not saturated by synaptic GABA release. NSNA confirmed diazepam increased the number of channels activated by GABA by 26% without changing conductance ($p<0.03$, n=5). We conclude estradiol reduces $GABA_A$R mPSC amplitude by decreasing the number of receptors activated by GABA, at least in part via a decrease in the affinity of the $GABA_A$R.

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Poster

076. Kisspeptin and Related Systems

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Program#/Poster#: 76.04/JJ5

Topic: E.01. Neuroendocrine Processes

Support: HD04612

5T32HD007228

Title: Membrane-initiated signaling mediates estradiol-induced kisspeptin in immortalized anterior hypothalamic kisspeptin neurons

Authors: *M. A. MITTELMAN-SMITH[1], P. E. MICEVYCH[2];
[1]Neurobio., [2]David Geffen Sch. of Med., UCLA, Los Angeles, CA

Abstract: Kisspeptin neurons in the anteroventral periventricular region of the hypothalamus (AVPV) are hypothesized to mediate estrogen positive feedback on gonadotropin release. This estrogen feedback is a critical precursor to the LH surge underlying ovulation. However the nature of estrogen signaling in these kisspeptin neurons remains unknown. Because dissection of intracellular signaling pathways in kisspeptin neurons is difficult to achieve *in vivo*, we used an

immortalized cell line isolated from adult female mouse hypothalamus (mHypoA-51, CELLutions Biosystems, ON, Canada) as a model for AVPV kisspeptin neurons. Immunocytochemistry and western blot revealed expression of kisspeptin, estrogen receptor alpha and progesterone receptor (PR) in mHypoA-51 neurons. A 48-hour treatment with 1 nM 17B-estradiol (E2) induced significant increases in kisspeptin mRNA and protein. Similar results were observed when cells were treated with membrane-impermeable estradiol (E-6-BSA) for 48 hours, suggesting that upregulation of kisspeptin expression is mediated by E2 membrane-initiated signaling. PR mRNA and protein were also increased following a 48-hour E2 treatment. However, no induction of PR mRNA was observed following 48 hours of E-6-BSA, suggesting the involvement of a classical nuclear mechanism for PR induction. Data presented here suggest that membrane-initiated estrogen signaling may play an important role in kisspeptin signaling underlying the LH surge. Supported by HD04612 and 5T32HD007228

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Poster

076. Kisspeptin and Related Systems

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Topic: E.01. Neuroendocrine Processes

Support: NIH Grant RO1HD042635

Title: AVPV kisspeptin neurons mediate neuroprogesterone induction of the luteinizing hormone surge

Authors: *L. K. PAASKE[1], T. CHUON[1], P. MICEVYCH[2], K. SINCHAK[1];
[1]Biol. Sci., California State University, Long Beach, Long Beach, CA; [2]Dept Neurobiology, David Geffen Sch. of Med. at UCLA, Lab. of Neuroendocrin, UCLA, Los Angeles, CA

Abstract: On the afternoon of proestrus, estradiol positive feedback stimulates the de novo synthesis of neuroprogesterone in hypothalamic astrocytes, which is needed to trigger the luteinizing hormone (LH) surge in the rat. Given that diagonal band of Broca (DBB) gonadotropin-releasing hormone (GnRH) neurons do not express progesterone receptors (PR), the actions of neuroprogesterone must be mediated by PR-expressing interneurons that synapse on DBB GnRH neurons that mediate the release of pituitary LH. Anteroventral periventricular nucleus (AVPV; aka RP3V) kisspeptin neurons co-express PR, and directly activate DBB GnRH

neurons to induce the LH surge. Therefore, we hypothesized that these AVPV kisspeptin neurons mediate induction of the LH surge by neuroprogesterone. To demonstrate that kisspeptin release is downstream of neuroprogesterone synthesis in this neurocircuit, progesterone synthesis was inhibited with aminoglutethimide (AGT; 1 mg/kg; s.c.) in ovariectomized (ovx) and adrenalectomized (adx) Long Evans rats and either kisspeptin (20 nmol/µl) or saline was infused into the DBB. Trunk blood was collected 53 hours post-EB injection, and serum LH levels analyzed by sandwich ELISA kit (Shibayagi, via BioVendor). AGT inhibition of neuroprogesterone synthesis blocked the EB-induced LH surge. Compared with saline controls, DBB infusion of kisspeptin and subcutaneous progesterone each restored the LH surge (SNK $P < 0.05$). In a second set of experiments, DBB infusion of kisspeptin receptor (GPR54) antagonist (kisspeptin-234) blocked EB- and kisspeptin-induced LH surges compared to saline infused rats (t-test $P < 0.05$). Finally, we tested whether kisspeptin synthesized in the AVPV was necessary for the LH surge. Kisspeptin asODNs were infused into the 3V at the level of the AVPV in EB and oil treated animals. Blocking kisspeptin mRNA translation in EB-primed rats blocked the LH surge compared to rats infused with scrambled ODNs (SNK $P = 0.011$). These results are consistent with the hypothesis that estradiol induces AVPV synthesis of kisspeptin, which estradiol-induced neuroprogesterone releases to activate DBB GnRH neurons to trigger the LH surge.

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Poster

076. Kisspeptin and Related Systems

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Support: University of Strasbourg

CNRS

Région Alsace

ANR 07-BLAN-056

Title: A Kiss-Clock times estrous cycle

Authors: *D. CHASSARD, I. BUR-PIVERT, J. MENDOZA, V. SIMONNEAUX;
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Abstract: Ovulation is triggered by a LH surge occurring at a specific time on the day of the proestrus, typically at the end of the day in nocturnal rodents. The LH surge timing depends on an interaction between an oestrogenic and a circadian signal. The kisspeptin (Kp) neurons in the anteroventral periventricular nucleus (AVPV) have been reported to regulate the GnRH-induced release of LH in female. Here we have investigated whether Kp neurons could be the site where both oestrogenic and circadian signals are integrated in female mice. Kp content displays a marked and transient decrease at late day, 2 hours before the LH surge on the day of proestrus, but not in diestrus, indicating that a daily regulation of Kp secretion occurs only when estrogen level is elevated. It has been reported that a circadian signal (vasopressin) originating from the master clock in the suprachiasmatic nuclei (SCN) activates Kp neurons in the AVPV. Additionally we investigated whether these AVPV Kp neurons could host a “peripheral” circadian clock. Our data show that AVPV Kp neurons express a daily rhythm of the clock protein PER1 with a 4h phase delay compared to the PER1 rhythm in the SCN. Furthermore, isolated AVPV explants from PER2-luciferase mice display marked circadian oscillations of bioluminescence activity with a circadian period of 23,31 ± 0,49h. This period is shorter than the circadian period in the SCN (25,09 ± 0,61h). In conclusion, our data demonstrate that AVPV Kp neurons display a estrogen-dependent daily activity probably driven by a endogenous Kiss-Clock, acting in synergy with the SCN clock to time the the preovulatory LH surge.

Disclosures: **D. Chassard:** None. **I. Bur-Pivert:** None. **J. Mendoza:** None. **V. Simonneaux:** None.

Poster

076. Kisspeptin and Related Systems

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 76.07/JJ8

Topic: E.01. Neuroendocrine Processes

Support: JSPS (24870007) to SK

JSPS (26840111) to SK

SUNBOR to SK

JSPS (20247005) to YO

Title: Multidisciplinary analyses of gpr54-EGFP transgenic medaka reveal novel functions of the kisspeptin neuronal system

Authors: *M. NAKAJO, T. KARIGO, S. KANDA, Y. OKA;
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Abstract: Kisspeptin-Gpr54 system has recently been considered as the key player of HPG-axis regulation in mammals. Despite the importance of hypothalamic kisspeptin neurons in mammalian reproduction, a growing body of evidence in non-mammalian vertebrates suggests important non-reproductive functions of kisspeptins. For instance, GnRH neurons do not express gpr54 mRNA and adjacent non-GnRH cells do so in some teleosts (Escobar et al., 2013; Grone et al., 2010; Kanda et al., 2013). Furthermore, it has been recently reported by electrophysiology and calcium imaging studies in medaka that kisspeptin does not affect GnRH neurons nor gonadotrophs (Karigo et al., 2012). In addition to these previous results, the complete lack of kisspeptin genes in avian species may even suggest that kisspeptin is not essential for reproduction in non-mammalian species in general. We have been searching for novel functions of Kisspeptin-Gpr54 system by using the transgenic medaka whose gpr54-1 expressing cells are labeled specifically by GFP (Kanda et al., 2012). Here, GFP-labeled Gpr54-1 cells are mainly located in some ventral telencephalic nuclei (Vd/Vs/Vp), preoptic area (POA), nucleus preopticus pars magnocellularis (POm), and nucleus posterioris periventricularis (NPPv). First, we performed electrophysiological experiments in POA Gpr54-1 cells and examined effects of Kiss1 peptides on them. Loose cell patch clamp recordings showed that the neuronal activities of these cells were persistently up-regulated by Kiss1 peptides perfusion (1μM) by ~5-fold on average. This result suggests that kisspeptin may stimulate POA Gpr54-1 cells to release unidentified neurotransmitter(s) to activate downstream neural circuitry. Therefore, we performed deep sequencing in harvested GFP positive cells. Among ~4000 candidate genes from the deep sequencing, we performed a detailed analysis of co-expression of some highly expressed neurotransmitters and Gpr54-1 by dual labeling of GFP immunohistochemistry and *in situ* hybridization. We identified that some neuropeptides that are suggested to be related to endocrine functions and feeding behaviors are expressed in Vd/Vs/Vp and POA Gpr54-1 neurons. Besides, detailed immunohistochemical analysis of GFP-labeled axons also suggested that POA Gpr54-1 cells heavily project to the pituitary, whereas Vd/Vs/Vp cells project to the medulla oblongata via hypothalamus. Thus, our present strategy using the receptor-expressing transgenic animals is expected to provide various novel insights into the kisspeptin functions that are common to and have been conserved in many vertebrate species.

Disclosures: **M. Nakajo:** None. **T. Karigo:** None. **S. Kanda:** None. **Y. Oka:** None.

Poster

076. Kisspeptin and Related Systems

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Topic: E.01. Neuroendocrine Processes

Support: JSPS (268599) to M.H.

JSPS (24870007) to S.K.

SUNBOR to S.K.

JSPS (20247005) to Y.O.

Title: Electrophysiological analyses of sex steroid-sensitive Kiss1 neurons in a seasonal breeder, medaka

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Abstract: Kisspeptin neurons express sex steroid receptors and show drastic changes in their Kisspeptin gene expression in accordance with the serum sex-steroid concentration in various vertebrates, including mammalian and non-mammalian species. Thus, in vertebrates, Kisspeptin neurons are suggested to modulate various neuronal activities according to reproductive status. However, in spite of the importance of their reproductive state-dependent regulation in vertebrates, there has been no physiological analysis of Kisspeptin neurons in seasonally breeding animals. Here, by using a kiss1-EGFP transgenic line of a seasonal breeder, medaka, we performed electrophysiological analyses of sex steroid-sensitive Kiss1 neurons in a hypothalamic nucleus (Kanda et al., 2008), nucleus ventralis tuberis (NVT). By whole-cell and on-cell loose-patch recordings, we found that NVT Kiss1 neurons in a breeding condition exhibit a variety of spontaneous firing patterns; burst, regular, irregular, and silent patterns. We also found that NVT Kiss1 neurons show different firing patterns according to the different average resting membrane potential (RMP), and continuous current injections caused shifts in the firing pattern among silent, irregular, and regular. These results suggest that RMP plays an important role in determination of firing pattern in NVT Kiss1 neurons, as demonstrated in other neurons (Augustinaite and Heggelund, 2007). On the other hand, the burst firing could not be induced from other firing patterns by a current injection, which suggests that the burst firing in NVT Kiss1 neurons requires some additional acute/chronic modulations, such as synaptic inputs and/or changes in the intrinsic ion channel properties. Finally, we analyzed the neuronal activities of NVT Kiss1 neurons between breeding and non-breeding conditions. NVT Kiss1 neurons in the non-breeding conditions showed significantly lower neuronal activities than those

in the breeding conditions. Thus, the results suggest that sex steroid-sensitive Kiss1 neurons in NVT change their neuronal activities in accordance with the reproductive state, which likely reflects the serum sex steroid levels.

Disclosures: **M. Hasebe:** None. **S. Kanda:** None. **H. Shimada:** None. **Y. Akazome:** None. **Y. Oka:** None.

Poster

076. Kisspeptin and Related Systems

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Topic: E.01. Neuroendocrine Processes

Support: JSPS (24870007) to SK

JSPS (26840111) to SK

SUNBOR to SK

JSPS (20247005) to YO

Title: Kisspeptin increases the spontaneous firing rate of vasotocin (VT) neurons in the brain of medaka

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Abstract: The central regulatory mechanism of reproduction, HPG axis, is comprised of various “key players” including neuropeptides and hormones such as gonadal sex steroids. Among them, a hypothalamic neuropeptide kisspeptin has been the focus of intensive research in this decade. In mammals, accumulating evidence suggests that kisspeptin neurons receive estrogen feedback signals from the gonads and regulate GnRH neurons. In most nonmammalian vertebrates, however, kisspeptin neurons do not necessarily regulate GnRH neurons directly. Given that birds have lost their kisspeptin genes during evolution and can still do without any kisspeptin genes for reproduction, the essential nature of kisspeptin for reproduction may be restricted to mammalian species, and kisspeptin may not be essential for reproduction in nonmammalian species. This prompted us to focus on the possible non-reproductive function(s) of vertebrate kisspeptin neuronal systems by using medaka brain. Medaka has several experimental advantages for the

study of neuronal regulatory mechanisms of reproduction; molecular genetic tools are available, and their small and transparent brain allows us to perform physiological analyses by using whole brain *in vitro* preparations in which the synaptic connections and other regulatory mechanisms are kept intact. Based on our previous finding that vasotocin (VT; ortholog of mammalian vasopressin) neurons express kisspeptin receptor, gpr54-2 mRNA, we generated transgenic medaka expressing DsRed under the control of VT 5' flanking region, and the effects of kisspeptin on VT neurons in the magnocellular preoptic area (POm) were analyzed by loose patch voltage clamp recoding. Here we examined the effects of Kiss2 peptide, because its affinity to Gpr54-2 is higher than that of Kiss1 peptide. We found that the firing rate of VT neurons transiently increased after five minute-administration of 1μM synthetic Kiss2 (12 amino acid residues with its C-terminus amidated) in about 80% of male madaka. On the other hand, Kiss2 increased only 20% of female VT neuron activity, probably due to a lower receptor expression rate in the breeding female (Kanda et al., 2013). Taken together with previous reports, it is suggested that kisspeptin may control behaviors or homeostasis such as osmoregulation by directly regulating VT neurons, although the functional meaning of sexual difference remains to be elucidated. Given that intracerebroventricular administration of KP10 (synthetic Kiss1) increases plasma vasopressin levels in rats (Scott and Brown, 2011), the regulation of VT neuron may be a widely conserved common function of vertebrate kisspeptins.

Disclosures: **Y. Shikano:** None. **S. Kanda:** None. **Y. Oka:** None.

Poster

076. Kisspeptin and Related Systems

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Topic: E.01. Neuroendocrine Processes

Support: R01 AG-032315

Title: 17-beta-Estradiol reduces the dendritic spine density of KNDy neurons in the arcuate nucleus of ovariectomized Tac2-EGFP transgenic mice

Authors: M. CHOLANIAN[1], S. J. KRAJEWSKI-HALL[1], N. T. MCMULLEN[2], *N. E. RANCE[1];
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Abstract: Neurons in the arcuate nucleus that coexpress kisspeptin, neurokinin B, and dynorphin (KNDy neurons) play an important role in the regulation of reproduction. Estradiol markedly decreases NKB and kisspeptin gene expression, as well as the electrical excitability of KNDy neurons (Cholanian et al., Endocrinology 2014). Here we examined whether the dendritic architecture of KNDy neurons is also altered by estradiol. Tissue slices from the arcuate nucleus of ovariectomized (OVX) and OVX plus 17β-estradiol-treated (OVX + E2) Tac2-EGFP (Tachykinin 2-enhanced green fluorescent protein) mice were used to target and fill KNDy neurons with biocytin. Biocytin-filled KNDy neurons were visualized with anti-biocytin immunohistochemistry and manually digitized using an image combining computer microscope equipped with Neurolucida software. KNDy neurons exhibited two to three primary dendrites with few branches. Spiny and spine-free (smooth) KNDy neurons were present in both OVX and OVX + E2 groups. The axons of KNDy neurons originated from the cell body or proximal dendrite and branched extensively within the arcuate nucleus. In addition to their local projections within the nucleus, the axons of KNDy neurons terminated in the adjacent median eminence, ependymal layer of the third ventricle and outside of the arcuate nucleus. Quantitative analysis revealed that treatment of OVX Tac2-EGFP mice with physiological levels of E2 decreased the cell size and dendritic spine density of KNDy neurons. These results provide the first detailed description of morphological features of arcuate KNDy neurons. In parallel with its effects on neuropeptide gene expression and electrical excitability, E2 treatment of OVX Tac2-EGFP mice induces structural changes of the dendrites of KNDy neurons.

Disclosures: **M. Cholanian:** None. **N.E. Rance:** None. **S.J. Krajewski-Hall:** None. **N.T. McMullen:** None.

Poster

076. Kisspeptin and Related Systems

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Program#/Poster#: 76.11/JJ12

Topic: E.01. Neuroendocrine Processes

Support: HL074927

HL51971

P20GM104357

American Heart Association

Title: Early onset of reproductive senescence programmed by intrauterine growth restriction in the female rat is accompanied by altered KNDy cell peptide expression and hypertrophy in the arcuate nucleus of the hypothalamus

Authors: *C. FERGANI[1], S. INTAPAD[2], S. ROLLINS[1], M. N. LEHMAN[1], B. T. ALEXANDER[2], L. M. COOLEN[2];
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Abstract: Low birth weight is associated with earlier age at menopause suggesting that early life events program reproductive senescence; yet, the mechanisms involved remain unknown. Placental insufficiency in the rat results in low birth weight indicative of intrauterine growth restriction (IUGR) and the development of persistent anestrous and age-dependent hypertension by 12 months (m) of age. KNDy (kisspeptin/neurokinin B/dynorphin) cells in the arcuate nucleus (ARC) of the hypothalamus play a key role in steroid feedback control of gonadotropin-releasing hormone neurons necessary for the maintenance of estrous cyclicity. Therefore, we hypothesized that an earlier onset of reproductive senescence in IUGR females would be accompanied by alterations in kisspeptin and/or NKB peptide expression in the ARC. Reduced uteroplacental perfusion was used for induction of IUGR. Sprague-Dawley pregnant rats were anesthetized at day 14 of gestation and silver clips were placed around the lower abdominal aorta above the iliac bifurcation and around both branches of the ovarian artery. Offspring from control (sham) pregnant and reduced uterine perfusion pregnant rats were monitored for estrous cycles. Based on vaginal smears, at 12 m of age, control animals cycled regularly whereas IUGR females exhibited persistent anestrous. At 18 m of age, estrous cyclicity had ceased in both control and IUGR groups. Next, brains were fixed (n=5-9/group) and hypothalamic sections processed for kisspeptin or NKB. Numbers as well as size of immunopositive cells were measured in the ARC. At 12 m of age, IUGR females had significantly fewer kisspeptin and NKB cells compared to controls. At 18 m of age, there were no differences in NKB cell number between groups; however, the numbers of NKB cells in controls was reduced compared to the 12 m old control females. Analysis of NKB cell size showed that NKB soma area and perimeter were significantly increased in IUGR females compared to controls at 18 m, but not 12 m of age. These data support the hypothesis that IUGR programs early onset of reproductive senescence, possibly via a reduction in kisspeptin and NKB peptide in ARC, and perhaps reduced responsiveness to steroid negative feedback. Furthermore, the NKB cell hypertrophy observed in 18 m old IUGR females closely resembles findings in long term ovariectomized monkeys and post-menopausal women, and may therefore be indicative of ovarian failure. Early menopause is a risk factor for increased cardiovascular outcomes. Thus, the possibility that the earlier onset of reproductive senescence is a major risk factor for the development of hypertension in middle-aged IUGR female offspring remains to be explored.

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Poster

077. Neural Control of Cardiovascular Function I

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Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 77.01/JJ13

Topic: E.04. Autonomic Regulation

Support: Postdoctoral National Program (PNPD) of Coordination for the Improvement of Higher Education Personnel (CAPES).

Title: Glutamatergic neurotransmission of paraventricular nucleus of the hypothalamus modulates cardiovascular responses evoked by hemorrhage in rats

Authors: *C. BUSNARDO[1], C. C. CRESTANI[2], A. FASSINI[1], L. B. M. RESSTEL[1], F. M. A. CORRÊA[1];
[1]Pharmacol., Univ. of São Paulo, Ribeirão Preto, Brazil; [2]Dept. of Natural Active Principles and Toxicology, Sch. of Pharmaceut. Sciences, UNESP, Araraquara, Brazil

Abstract: Introduction: The glutamatergic neurotransmission of paraventricular nucleus of the hypothalamus (PVN) has been implicated in several aspects of cardiovascular control. Moreover, using the technique of immunohistochemistry to detect Fos protein, a marker of neuronal activation, it was shown that there was a greater increase in the number of Fos-immunoreactive neurons in the parvocellular region of PVN, known to contain neurons that connect with autonomic regions of the brain stem and spinal cord after hemorrhagic hypotension. Therefore, the present work studied the possible involvement of PVN glutamatergic neurotransmission in the mediation of hemorrhage-induced cardiovascular changes. Methods: Guide cannulas were implanted into the PVN of rats. Catheters were introduced into right and left femoral artery for blood pressure recordings and for bleeding. We submitted rats to hemorrhage and studied the effect of the microinjection of the selective NMDA glutamate receptor antagonist LY235959 (LY, 2nmol/100nL) or the selective non-NMDA glutamate receptor antagonist NBQX (2nmol/100nL) into the PVN on mean arterial pressure (MAP) and heart hate (HR) responses induced by hemorrhage. Results: Microinjection of LY into the PVN did not affect baseline MAP or HR (before LY: MAP = 95±1 mmHg, HR = 365±1.4 bpm; after: MAP = 95±0.3 mmHg, t = 0.3, P = 0.7, HR = 372±3.2 bpm, t = 2, P = 0.06, n = 5). Two-way ANOVA indicated a significant effect of LY on cardiovascular responses to hemorrhage [ΔMAP: $F(1,136) = 16.2$, $P<0.0001$; ΔHR: $F(1,136) = 73.1$, $P < 0.0001$], a significant effect over time [ΔMAP: $F(16,136) = 3$, $P = 0.0008$; ΔHR: $F(16,136) = 10.3$, $P<0.0001$], and an interaction between treatment and

time ΔMAP: F(16,136) = 2.1, P = 0,01; ΔHR: F(16,136) = 6, P < 0.0001]. Microinjection of NBQX into the PVN did not affect baseline MAP or HR (before NBQX: MAP = 93±0.5 mmHg, HR = 379±1.3 bpm; after: MAP = 92±0.7 mmHg, t = 1.3, P = 0.2, HR = 377±2.8 bpm, t = 0.5, P = 0.6, n = 5). Two-way ANOVA indicated a significant effect of NBQX on cardiovascular responses to hemorrhage [ΔMAP: F(1,136) = 20.8, P<0.0001; ΔHR: F(1,136) = 13.7, P = 0.0003] and a significant effect over time [ΔMAP: F(16,136) = 17.3, P<0.0001; ΔHR: F(16,136) = 28.7, P<0.0001]. Conclusion: These results show that local PVN glutamatergic neurotransmission is involved in the neural pathway of cardiovascular responses to hemorrhage. Financial **Support:** Postdoctoral National Program (PNPD) of Coordination for the Improvement of Higher Education Personnel (CAPES).

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Poster

077. Neural Control of Cardiovascular Function I

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 77.02/JJ14

Topic: E.04. Autonomic Regulation

Title: Surface sugars in the nucleus of the solitary tract alter neuronal excitability to modify blood pressure and the baroreflex

Authors: *P. BOKINIEC[1], L. BOU-FARAH[1], S. MCMULLAN[1], N. H. PACKER[2], A. K. GOODCHILD[1];
[1]Australian Sch. of Advanced Med., Macquarie Park, Australia; [2]Dept. of Chem. and Biomolecular Sci., Macquarie Univ., Macquarie Park, Australia

Abstract: Background- The surface sugar polysialic acid, most commonly attached to NCAM has a restricted abundance in areas of the adult brain primarily associated with synaptic plasticity. It has been associated with BDNF and GluN2B activity. The nucleus of the solitary tract (NTS) has high expression levels of PSA in adult and receives and integrates sensory afferent traffic arising from diverse sources including baroreceptors, chemoreceptors etc. Although stimulation of afferent nerves decreases the expression of PSA (Bouzioukh et.al., 2001), the functional role of PSA in the NTS has not been determined. Our aim therefore was to determine in the NTS the role of PSA in the control of cardiovascular homeostasis. Methods- *In vivo* electrophysiological recordings of blood pressure (BP), heart rate (HR) and splanchnic

sympathetic nerve activity (sSNA) were made in the urethane anaesthetised, paralysed and ventilated male Sprague-Dawley rats. The effects of microinjecting enzymes within the NTS which cleave different sugar residues (Neuraminidase [NEU], β -Galactosidase [β-GAL] and Peptide-N-Glycosidase F [PNGase-F]) as well as those which inhibit endogenous cleavage of PSA (N-Acety-2,3-dehydro-2 deoxyneuraminic acid[NADNA]). The neuroanatomical and molecular localisation of PSA within the NTS is also been determined. Finally whole cell patch clamp recordings were made within the medial NTS of P16 Sprague-Dawley pups. Slices were either incubated in ACSF or ACSF containing NEU after which cells were subjected to a voltage step protocol. Results- NEU (n=8) compared to vehicle (n=7) evoked significant increases in BP (22.4±5 vs 3.3±1mmHg), HR (41±10 vs -18±4bpm) and sSNA (32±12.6 vs -10±6%). Furthermore, sympathetic baroreceptor reflexes were significantly attenuated (n=3). In contrast, β-GAL (n=3) or PNGase-F (n=3) or NADNA (n=3) failed to evoke any changes in BP, HR, sSNA or reflex function. At the cellular level, NEU significant enhanced currents generated by depolarizing voltage steps above 0mV (n=15 vs 8 control, $p<0.001$). PSA-NCAM is also not associated with glia, and shares close appositions with synaptophysin throughout the rostrocaudal extent of the NTS. Conclusions- Our data indicate that PSA exhibits tonic inhibitory effects within the NTS, and alters neuronal signalling which regulates baroreceptor mediated reflex activity. These results suggest that cleavage of PSA alters transmission. Hypotheses currently being tested at the cellular level explore associations of PSA with BDNF and/or the Glu2NB receptor.

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Poster

077. Neural Control of Cardiovascular Function I

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Topic: E.04. Autonomic Regulation

Support: NIH Grant HL028785

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Title: Connectome of the Rostral Ventrolateral Medulla Catecholaminergic (RVLM-CA) neurons of the mouse determined using a modified rabies virus

Authors: *R. L. STORNETTA[1], K. E. VIAR[2], P. G. GUYENET[2];
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Abstract: The projections of the RVLM-CA neurons have been mapped in rodents, but their inputs are not entirely defined. Whether different groups of RVLM-CA neurons have different inputs and outputs is also unknown. We sought answers to these questions by using the glycoprotein-deleted rabies virus pseudotyped to recognize an avian receptor, TVA (SADΔG-B19-eGFP-EnvA) (Wickersham et al., Neuron 53:639-647, 2007). Both TVA and rabies glycoprotein were introduced selectively into the RVLM-CA neurons of DβH-Cre mice with a unilateral injection of 2 AAV5 Cre-dependent viral vectors encoding TVA-mCherry or rabies-glycoprotein, respectively. After 4-6 weeks, the modified rabies virus was injected into the hypothalamus (n=13) or upper thoracic spinal cord (n=4), both known projection fields of RVLM-CA neurons. Ten days later, brain and spinal cord were examined by immunocytochemistry to detect the presence of TVA (mCherry), tyrosine hydroxylase (TH) and GFP. Most TVA expressing cells were RVLM-CA neurons (~270/ animal, 94% TH+). Approximately 54 "seed" cells (mCherry+ GFP+TH) / mouse were found in the RVLM. These cells were infected with rabies virus via their TVA+ terminals and were extensively GFP-labeled throughout their entire structure. The seed cells were located in more rostral VLM following spinal cord injections and in more caudal VLM after hypothalamic injections consistent with the location of hypothalamic vs. spinal projecting RVLM-CA cells reported in rats. The axonal terminals of the seed cells were observed in DMV, LC, Sp5, raphe pallidus, NTS, PBN, and contralateral RVLM consistent with known projections from the RVLM-CA cells in mice and rats. There was no discernible difference in the terminal fields of the seed cells labeled from hypothalamic vs. spinal cord rabies injections. GFP+ cells without TVA were considered to be presynaptic to the seed cells. These neurons were located within the LTF and medullary and pontine reticular formation, NTS, spinal trigeminal nucleus, raphe pallidus and magnus, PAG, PBN, and deep cerebellar nuclei. Again, there was no discernible difference in the location of the first order afferents to RVLM seed cells labeled following hypothalamic or spinal cord injections of rabies virus. In summary, the populations of mouse RVLM-CA neurons projecting to the hypothalamus or the spinal cord are located at different positions in the rostro-caudal RVLM. These neurons receive input from the same brain regions and innervate a common set of targets via their axonal collaterals. Thus, the spinal- vs. hypothalamic-projecting RVLM-CA neurons cannot be discriminated on the basis of their anatomical connections.

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Poster

077. Neural Control of Cardiovascular Function I

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Topic: E.04. Autonomic Regulation

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Title: Liver cirrhosis differentially modulates neuronal excitability in cardiac sympathetic and parasympathetic ganglia

Authors: *C.-K. LEE[1], S.-W. JEONG[1], K.-H. PARK[2];
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Abstract: Patients with liver cirrhosis frequently experience cardiac autonomic dysfunctions such as augmented cardiac sympathetic and reduced cardiac vagal functions which are highly associated with mortality. Until now, however, the cirrhosis-induced autonomic imbalance has not been studied at cellular and molecular levels. Here we tested whether liver cirrhosis differentially modulates excitability of cardiac sympathetic and parasympathetic neurons. In this regard, cirrhotic rats were produced by bile duct ligation (BDL) or intraperitoneal injection of thioacetamide (TAA, 200 mg/kg). Three week after BDL or chemical injection, development of liver cirrhosis and blunted arterial baroreflex were evaluated. Action potentials (AP) were recorded in single neurons isolated from the stellate ganglia (STG) and the intracardiac ganglia (ICG) using the gramicidin-perforated patch-clamp technique. Both types of neurons mostly show tonic firing. In response to current injection (1X, 2X, and 3X), the frequency of action potentials (AP) was significantly increased in the STG neurons, while decreased in the ICG neurons of cirrhotic rats when compared with sham control. Liver cirrhosis altered rheobase and AP duration in the opposite direction in the STG and the ICG without affecting other parameters including input impedance, resting membrane potentials, AP amplitude, and afterhyperpolarization amplitude/ duration. Real-time PCR analysis and voltage-clamp recordings revealed that expression and activities of A-type K^+ and N-type Ca^{2+} channels were decreased, respectively in the STG and the ICG neurons of cirrhotic rats. Taken together, our data suggest that BDL- and TAA-induced liver cirrhosis causes the imbalance between cardiac sympathetic and parasympathetic activities through down-regulation of A-type K^+ and N-type Ca^{2+} channels. Therefore, liver cirrhosis-blunted baroreflex may arise from impaired functions of the autonomic motor limbs in the reflex arc in addition to the afferent limb (baroreceptor neuron) dysfunction.

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Poster

077. Neural Control of Cardiovascular Function I

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Program#/Poster#: 77.05/JJ17

Topic: E.04. Autonomic Regulation

Title: Cardiovascular autonomic function in hypertensive subjects

Authors: *R. K. GOIT[1], B. H. PAUDEL[2];
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Abstract: Objective: The aim of the study was to compare heart rate variability (HRV) and vibration perception threshold (VPT) of hypertensive subjects with control. Research design/ Methods: The study was conducted on 50 hypertensive subjects and 50 controls. The short term HRV and VPT were assessed in both the groups. Results: All the time domain measures, standard deviation of all RR intervals (SDNN) [26 (15.5-35) vs 36 (30-40.25) ms, P=0.002], the square root of the mean of the sum of the squares of differences between adjacent RR intervals (RMSSD) [25.9 (11.95-40.45) vs 36.65 (27.05-44.13) ms, P=0.002], and percentage of consecutive RR intervals that differ by more than 50 ms (pNN50) [3.5 (0.23-21.83) vs 16.4 (4.45-27.63) %, P=0.002] were significantly less in hypertensive subjects. In frequency domain measures, low frequency (LF) [115.5 (83.75-140.75) vs 141 (104.25-249.75) ms2, P=0.021], high frequency (HF) [114.5 (74.5-179) vs 182.5 (104.25-247) ms2, P=0.006], HF [33.3 (24.52-53.22) vs 56.8 (43.02-69.17) nu, P=0.002], LF [45.2 (35.4-57.02) vs 49.8 (36.97-69.55) nu] and LF/HF [0.85 (0.5-2.02) vs 0.98 (0.65-1.62) %] were significantly less in hypertensive subjects. However, VPT was comparable between the groups. Conclusion: The hypertensive subjects had reduced cardiovascular autonomic activity.

Disclosures: **R.K. Goit:** None. **B.H. Paudel:** None.

Poster

077. Neural Control of Cardiovascular Function I

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 77.06/JJ18

Topic: E.04. Autonomic Regulation

Support: Heart and Stroke Fdn. of Ontario

Title: Gender differences in the distribution of Urocortin-1 immunoreactivity in brainstem autonomic nuclei

Authors: *J. CIRIELLO[1], M. M. CAVERSON[2];
[2]Schulich Sch. of Med. and Dent., [1]Univ. Western Ontario, London, ON, Canada

Abstract: Urocortin-1 (Ucn-1), a neuropeptide closely related to the hypothalamic hormone corticotropin-releasing factor, has been shown to be associated with stress and feeding behaviors and to have significant gender differences. Additionally, central administration of Ucn-1 has been shown to elicit transient changes in arterial pressure and heart rate in the male rat. We investigated whether estrogen (E; 17β-estradiol) treatment (9 weeks) affected the expression of the Ucn-1 in brainstem autonomic nuclei in female Wistar rats. Experiments were done in age matched adult males (controls), ovariectomized (OVX) only and OVX+E (30 pg/ml plasma) treated females, perfused transcardially with Zamboni's fixative. Brainstem sections (40 μm) were cut and processed immunohistochemically for Ucn-1. In the male, moderate Ucn-1 fiber labeling was found in the nucleus of the solitary tract (NTS) and throughout the rostral ventral lateral medulla (RVLM). Additionally, a few Ucn-1 immunoreactive neurons were observed in nucleus ambiguus (Amb). In OVX+E females, fewer labeled fibers were found within NTS compared to males, although RVLM was more densely innervated by Ucn-1 fibers. Furthermore, in the OVX+E female Ucn-1 labeled neurons were found not only within Amb, but also in NTS. In the OVX only animals, moderate to dense Ucn-1 fiber labeling was observed in NTS and throughout RVLM compared to males. Furthermore, numerous Ucn-1 labeled neurons were found within Amb, NTS, dorsal motor nucleus of the vagus, hypoglossal nucleus, RVLM, magnocellular reticular nucleus and nucleus raphe pallidus. These data suggest not only that gender differences exists in the distribution of Ucn-1 within brainstem autonomic areas, but that the circulating level of E may play an important role in the function of these autonomic structures during stress responses. Supported by Heart and Stroke Foundation of Ontario

Disclosures: **J. Ciriello:** None. **M.M. Caverson:** None.

Poster

077. Neural Control of Cardiovascular Function I

Location: Halls A-C

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Program#/Poster#: 77.07/JJ19

Topic: E.04. Autonomic Regulation

Support: HL098351

DA08259

AG016765

HL096571

AG059850

T32 DA007274

Title: Sex differences in NMDA receptor trafficking in angiotensin receptor type 1A containing neurons in the mouse hypothalamus following slow-pressor angiotensin infusion

Authors: *J. MARQUES LOPES[1], M.-K. LYNCH[1], T. VAN KEMPEN[1], E. M. WATERS[2], C. IADECOLA[1], V. M. PICKEL[1], T. A. MILNER[1,2];
[1]Div. of Neurobio., Weill Cornell Med. Col., New York, NY; [2]Lab. of Neuroendocrinology, The Rockefeller University, NY

Abstract: In mice, slow-pressor angiotensin II (AngII) infusion induces hypertension in males but not in young (2 mo) females [Xue et al., 2005]. Renin-angiotensin system over-activity, up-regulation of post-synaptic NMDA receptor (NMDAR) function, and increased reactive oxygen species (ROS) production in the hypothalamic paraventricular nucleus (PVN) are hallmarks of AngII-induced hypertension [Veerasingham and Raizada, 2003; Wang et al., 2013]. We hypothesize that differential AngII-induced changes in post-synaptic NMDAR density and trafficking, and in ROS production in PVN angiotensin type 1a receptor (AT1aR)-expressing cells could be associated with sex differences in hypertension. Moreover, given our recent results showing protection from AngII-induced hypertension associated with decreased NMDAR density in estrogen receptor (ER) β-containing PVN dendrites of young female mice [Marques-Lopes et al., 2014], we assessed PVN AT1aR and ERβ co-localization. AngII (600ng/kg/min) or saline were infused for 14 d in 2 mo male and female transgenic mice expressing enhanced green fluorescent protein (EGFP) in AT1aR-containing cells (N=8-10/group). In males, AngII increased systolic blood pressure, and ROS production in the PVN at baseline and following NMDA in males but not females. Immunoelectron microscopy showed that AngII increased cytoplasmic and total NR1 density in AT1aR-EGFP dendrites in both males and females. However, unlike in females, AngII enhanced plasmalemmal and near plasmalemmal NR1 density in AT1aR-EGFP dendrites in males. Sexual dimorphism also was observed in morphology of AT1aR-EGFP dendrites. In males, AngII decreased area and diameter in small (<

1μm) and large (> 1μm) dendrites. Conversely, in females, AngII increased dendritic area of small dendrites and decreased diameter of large dendrites. Fluorescence microscopy revealed that AT1aR and ERβ do not co-localize in the PVN. The data suggest that increased post-synaptic plasmalemmal and near plasmalemmal NMDAR density, and augmented ROS production in AT1aR-containing PVN neurons are associated with AngII-induced hypertension in male mice. The observed AngII-evoked sex differences in PVN AT1aR neurons may be due to estrogen-mediated decreased excitatory input arising from other brain areas, including circumventricular organs.

Disclosures: **J. Marques Lopes:** None. **M. Lynch:** None. **T. Van Kempen:** None. **E.M. Waters:** None. **C. Iadecola:** None. **V.M. Pickel:** None. **T.A. Milner:** None.

Poster

077. Neural Control of Cardiovascular Function I

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 77.08/JJ20

Topic: E.04. Autonomic Regulation

Support: Basic Science research Program through the National Research Foundation (NRF) funded by the Ministry of Education, Science and Technology (2013R1A1A2013424).

Title: Liver cirrhosis-blunted baroreflex is associated with down-regulation of voltage-gated sodium channels in aortic baroreceptor neurons

Authors: C.-K. LEE[1], K.-H. PARK[2], *S.-W. JEONG[1];
[1]Dept. of Physiology, Brain Res. Group, [2]Dept. of Pathology, Brain Res. Group, Yonsei Univ. Wonju Col. of Med., Wonju, Gangwon-Do, Korea, Republic of

Abstract: Arterial baroreflex is a central mechanism for maintenance of cardiovascular homeostasis. Some evidence has shown that liver cirrhosis in humans and experimental animal models is associated with attenuated baroreflex sensitivity (BRS). To date, however, neural mechanisms underlying the liver cirrhosis-blunted baroreflex remain unknown. In the present study, we hypothesized that liver cirrhosis would attenuate excitability of aortic baroreceptor neurons located in the nodose ganglia. To address the hypothesis, cirrhotic rats were produced by bile duct ligation (BDL) or intraperitoneal injection of thioacetamide (TAA, 200 mg/kg). Three week after BDL or chemical injection, expression of cirrhosis markers including α-smooth muscle actin, collagen, and transforming growth factor-β was significantly increased in the liver.

These findings were consistent with histological examination of the liver tissues acquired from BDL and TAA groups. At the same period of time, BRS was significantly impaired in cirrhotic rats. Using the gramicidin-perforated patch-clamp technique, action potentials (AP) were recorded in Di-I-labeled baroreceptor neurons from sham control and cirrhotic rats. As results, excitability of tonic (A-type) and phasic (C-type) neurons was significantly attenuated with increased rheobase and decreased AP amplitude in cirrhotic rats. Consistent with the findings, TTX-sensitive and TTX-insensitive sodium currents were significantly decreased in both types of the baroreceptor neurons. RT-PCR and immunoblotting analyses revealed that expression of $Na_v1.7$, $Na_v1.8$, and $Na_v1.9$ was down-regulated in the baroreceptor neurons from the cirrhotic rats. Taken together, these data suggest that BDL- and TAA-induced liver cirrhosis blunts arterial baroreflex through attenuating excitability of the aortic baroreceptor neurons. The ionic mechanisms underlying the hypoexcitability may include down-regulation of voltage-gated sodium channels.

Disclosures: **C. Lee:** None. **S. Jeong:** None. **K. Park:** None.

Poster

077. Neural Control of Cardiovascular Function I

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 77.09/JJ21

Topic: E.04. Autonomic Regulation

Support: JSPS Grants 23500524, 26350508

Title: A stress-quantification gadget: Detrended fluctuation analysis of heartbeats, from crustacean animal models to humans

Authors: *T. YAZAWA;
Tokyo Metropolitan Univ., Hachioji, Japan

Abstract: Heartbeats are controlled by the nerves (ANS). In hermit crabs (*Aniculus aniculus*) both, the acceleratory nerves (CA) and the inhibitory nerves (CI) fired at about 0Hz - 5Hz and 0Hz - 60Hz, respectively. When I experimentally approached the crabs, the activity of CI dropped and CA remained, i.e., the heart rate was increased by stress. I defined this “unhappy” state into an “acute-stressful” state. In turn, CI was active a lot, while resting in a “peaceful” state: A brief-period-slowdown in rate was observed, which repeated itself regularly. I called this an ANS-induced slowdown. The period length of slowdown varied, from 1 to 5 min. I called this

state a “stress-free” state. Meantime, a lobster (*Homarus americanus*, naturally lives in clean water) died of an illness, because it was forced to live in an aquarium, together with dirty shrimps (*Squilla mantis*), naturally living in a muddy sea. The poor lobster got sick and maintained a steady heart rate, but never showed a slowdown for two weeks, before dying. So, it died of an environmental crisis. I thus knew that I can tell whether a crab is happy or nervous by the EKGs. These crustaceans were found to be a miraculous specimen for studying stress-quantification. As a quantification tool, a detrended fluctuation analysis (DFA) was used. Our modified DFA (mDFA) checked power-law characteristics of the heartbeat-intervals data. I studied both crustaceans and humans to determine whether mDFA could be a useful tool, a gadget, for the evaluation of the subject’s quality of an illness and/or a normal healthy state. Heartbeats of stress-free lobsters (*Panulirus japonicus*) exhibited a normal scaling exponent (*SI*) of 1 ($SI = 0.99 \pm 0.38$), and stressful lobsters (*P. japonicus*) exhibited a lower scaling exponent of 0.5-0.7 ($SI = 0.55 \pm 0.21$). Interestingly, human hearts reflect a mental condition, as in a chronic job stress, for example, President of Univ., $SI = 0.84$; Dean, $SI = 0.72$; Teaching Only Prof., $SI = 0.98$, etc.). The perceived level of wellness varies among the subjects, because there are no two individuals existing, who are physiologically identical. Present case studies have shown, how the wellness of subjects can be evaluated by using EKGs. DFA is a new tool, to quantify the degree of wellness and the transition from sickness to wellness and vice versa. The exponent reflects the ANS functions.

Disclosures: T. Yazawa: None.

Poster

077. Neural Control of Cardiovascular Function I

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 77.10/JJ22

Topic: E.04. Autonomic Regulation

Support: ULUDAG UNIVERSITESI BILIMSEL ARASTIRMA PROJELERI OUAP(T)-2012/16

Title: Central administration of nesfatin-1 reverses hypotension in hemorrhagic shock

Authors: *M. S. YILMAZ[1], B. ALTINBAS[2], C. SEVIM[1], G. L. ERKAN[2], G. GUVENC[2], N. GUNGOR[1], M. YALCIN[2], V. SAVCI[1];
[1]Uludag Univ. Fac. of Med., Bursa, Turkey; [2]Uludag Univ. Vet. Fac., Bursa, Turkey

Abstract: Nesfatin-1 has been identified as one of the most potent centrally acting anorexigenic peptides, and it has also been shown to play important roles in the control of cardiovascular function. Intracerebroventricularly (i.c.v.) injected nesfatin-1 induced an increase in blood pressure in conscious rats. The hypertensive effect of nesfatin-1 is thought to be mediated via the activation of sympathetic nerves through acting on melanocortin-3/4 receptors. It was further shown that the hypertensive effect of nesfatin-1 is mediated via acting on hypothalamus oxytocin receptor, which is thought to be downstream of the melanocortin system. Another recent study has also revealed that nesfatin-1 modulates blood pressure through directly acting on peripheral arterial resistance. The present study was thus undertaken to investigate the possible protective effects of nesfatin-1 against hemorrhagic shock. Male Sprague-Dawley rats were used in the study. In normotensive animals, after the connection of arterial catheter to the transducer, baseline blood pressure and heart rate measurements were recorded. Rats were allowed to be stabilized for 15 min. At the end of this period, i.c.v. injections were made. In hemorrhaged animals 20 min after hemorrhage; saline (5 μL) or nesfatin-1 (100 pmol) was injected i.c.v. just after the hemorrhage. Mean arterial pressure (MAP) and heart rate (HR) were evaluated through the experiment. For measurement of plasma catecholamine levels, blood samples (100 μl) were withdrawn from arterial catheter. In normotensive rats, blood samples were collected just before and 20, 40 and 60 min after saline or nesfatin-1 injections. In hypotensive rats, blood samples were withdrawn before and 20 min after hemorrhage and 20,40 and 60 min after saline or nesfatin-1 injections. In normotensive animals; nesfatin-1 slightly increase MAP but HR was not significantly increased by nesfatin-1 administration. Treatment with nesfatin-1 reversed hypotension in hemorrhaged rats. Nesfatin-1 caused an increase in plasma catecholamine levels in normotensive rats. Hemorrhage, itself, caused significant increases in plasma levels of catecholamines. Intracerebroventricular administration of nesfatin-1 produced additional increases in plasma catecholamine levels. Our results provide that nesfatin-1 increases blood pressure and reverses hypotension in hemorrhagic shock and increases in plasma catecholamine levels mediate this effect.

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Poster

077. Neural Control of Cardiovascular Function I

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 77.11/JJ23

Topic: E.04. Autonomic Regulation

Support: FAPESP

CNPq

Title: Arterial pressure, baroreflex, chemoreflex and sodium excretion in rats treated with chronic infusion of aldosterone into the 4th ventricle

Authors: ***S. GASPARINI**, M. R. MELO, G. M. F. ANDRADE-FRANZÉ, P. J. RUCHAYA, M. BASSI, J. V. MENANI, E. COLOMBARI;
Sao Paulo State University. Dep. of Physiol. and Pathology, Araraquara, Brazil

Abstract: Recent results have shown that aldosterone infused into the 4th ventricle (4th V) produces intense sodium intake and positive sodium balance. In the present study, we investigated whether aldosterone infused into the 4th V modifies arterial pressure, baroreflex, chemoreflex and renal sodium excretion. Male Holtzman rats (n = 4-7/group) with stainless steel cannulas implanted into the 4th V were used. Arterial pressure was recorded continuously for 14 days by telemetry and baroreflex sensitivity and chemoreflex were tested on the 7th day of aldosterone (100 ng/µl/h) infusion into the 4th V. Chemoreflex was tested with intravenous potassium cyanide (40 µg/rat) and baroreflex sensitivity with intravenous phenylephrine (40 µg/kg body wt) and sodium nitroprusside (30 µg/kg body wt). In rats that had access to 1.8% NaCl, chronic aldosterone infusion into the 4th V impaired baroreflex (-2.8 ± 0.5, vs. vehicle: -5.1 ± 0.9 bpm/mmHg) and the bradycardic response to chemoreflex activation (-30 ± 12, vs. vehicle: -101 ± 15 bpm), without changing mean arterial pressure in the light (101 ± 3, vs. vehicle: 104 ± 6 mmHg) or dark period (110 ± 5, vs. vehicle: 113 ± 6 mmHg). In rats that had no access to 1.8% NaCl, the infusion of aldosterone into the 4th V did not modify sodium excretion (0.7 ± 0.1 to 0.9 ± 0.1 mEq/24 h, vs. vehicle: 0.1 ± 0.2 to 0.7 ± 0.1 mEq/24 h). The present results show that chronic infusion of aldosterone into the 4th V reduces baroreflex sensitivity and the bradycardic responses to chemoreflex activation, without changing arterial pressure. In addition, the results suggest that sodium intake induced by aldosterone infused into the 4th V is not dependent on the natriuresis.

Disclosures: **S. Gasparini:** None. **M.R. Melo:** None. **G.M.F. Andrade-Franzé:** None. **P.J. Ruchaya:** None. **M. Bassi:** None. **J.V. Menani:** None. **E. Colombari:** None.

Poster

077. Neural Control of Cardiovascular Function I

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 77.12/JJ24

Topic: E.04. Autonomic Regulation

Title: Stress evaluation using changes in pupillary diameter of human eyes

Authors: *N. BINTI ALUWI[1], Y. ONO[1], N. HARA[2,3];
[1]Sch. of Sci. and Technol., Meiji Univ., Kanagawa, Japan; [2]Dept. of Orthoptics and Visual Sci., Intl. Univ. of Hlth. and Welfare, Tochigi, Japan; [3]Dept. of Ophthalmology, Kanagawa Dent. Col. Yokohama Dent. and Med. Clin., Yokohama, Japan

Abstract: Information on the autonomic nervous system can be derived both from heart rate variability (HRV) and pupil size, however their functional relationship needs to be elucidated. We have carried out two types of cognitive and behavioral tasks that modify autonomic balance to test the significance in the changes of the pupillary diameter and its correlation with the HRV in healthy young adults. We adopted Verbal Fluency Task (VFT) and Eye Massage to simulate stressful and less stressful condition, respectively. Participants performed VFT of phonemic category in which they have to say as many words as possible that begin with a specific letter in a given time (60s) followed by rest (60s) for 3 times while their pupil diameter and electrocardiogram were continuously measured. For eye massage task, pupil diameter was measured for 5 minutes each before and after subjects put on the eye massager (mild periocular mechanical stimulation with warm steam) while electrocardiogram were continuously measured throughout the experiment. The WEB-1000 multi-telemetry system (Nihon Kohden Co. Ltd., Japan) was adopted to obtain ECG signals in the lead-II configuration. Pupil diameter was continuously recorded using an infrared electronic pupillometer (IRISCORDER DUAL, Hamamatsu Photonics, Japan). The mean pupil diameter and LF/HF of HRV during VFT showed concurrent increase compared to those during rest period. The mean pupil diameter significantly decreased at post eye-massage period compared to that at pre eye-massage. The mean HF and LF/HF for post eye-massage were significantly changed from pre massage with increased HF and decreased LF/HF respectively. These results suggest that there is a common tendency between change in pupil diameter and both the sympathetic and parasympathetic component of heart rate variability. Correlation between pupil diameter and sympathetic/parasympathetic marker of HRV might reveal the potential of evaluating human stress by monitoring the changes in pupil size.

Disclosures: **N. Binti aluwi:** None. **Y. Ono:** None. **N. Hara:** None.

Poster

077. Neural Control of Cardiovascular Function I

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

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Topic: E.04. Autonomic Regulation

Support: The Methodist Hospitals Professor of Neuroscience

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Research to Prevent Blindness (MECF)

The University of Tennessee Neuroscience Institute (CL)

Title: Characterization of the central neurons responsible for parasympathetic regulation of choroidal blood flow in rat eye using pseudorabies virus

Authors: *C. LI[1], M. E. C. FITZGERALD[1,4], N. DEL MAR[1], S. CUTHBERTSON[1], M. S. LEDOUX[1,2], S. GONG[1], P. RYAN[3], A. REINER[1];
[1]Anat. & Neurobio., [2]Neurol., [3]Microbiology, Immunology, and Biochem., Univ. of Tennessee Hlth. Sci. Ctr., Memphis, TN; [4]Biol., Christian Brothers Univ., Memphis, TN

Abstract: Parasympathetic control of choroidal vasodilation is mediated by the superior salivatory nucleus (SSN) of the pons via its projection to pterygopalatine ganglion (PPG) neurons innervating choroidal blood vessels. The central neuronal cell groups upstream from the SSN neurons controlling choroidal blood flow (ChBF) have, however, been uncertain. We therefore made restricted injections of minute amounts of the Bartha strain of the retrograde transneuronal tracer pseudorabies virus (PRV) into choroid in rats in which the superior cervical ganglion had been completely excised (to prevent labeling of sympathetic circuitry). In this manner, transneuronal transport was confined to higher order neurons controlling choroidal SSN. Several neuronal populations showed prominent PRV+ immunolabeling following restricted intrachoroidal injections, in addition to the part of ipsilateral SSN previously shown by us to contain the preganglionic neurons innervating choroidal PPG neurons. The major higher order neurons included preoptic hypothalamus, the paraventricular nucleus (PVN) of the hypothalamus, the central gray, raphe magnus (RaM), the A5 cell group, the nucleus of the solitary tract (NTS), the rostral ventrolateral medulla (RVLM), and the caudal spinal trigeminal nucleus. Detailed mapping and fluorescent double-labeling was carried out to characterize the location and neurochemical phenotype of PRV+ neurons in several of these cell groups. In the PVN, PRV+ neurons were localized to its parvocellular subdivision, and they did not

immunolabel for oxytocin, vasopressin, or neuropeptide Y. In RaM, PRV+ neurons co-contained serotonin. Consistent with a serotonergic input from RaM to choroidal SSN, SSN was found to be rich in 5HT2A receptors, and serotonergic terminals were seen to make direct contact with choroidal SSN neurons. PRV+ neurons in NTS were located in its baroreceptive dorsal, intermediate and solitary tract subdivisions. Many contained calretinin but few contained tyrosine hydroxylase (TH). By contrast, many PRV+ RVLM neurons contained TH. The neuron types of PVN, RaM, NTS and RVLM projecting to choroidal SSN are responsive to systemic blood pressure signals and/or are involved in systemic sympathetic blood pressure regulation. Our results thus indicate that the brain sites responsible for parasympathetic control of ChBF are also involved in sympathetic control of the systemic vasculature. This implies that the blood pressure, salinity and volume signals that drive sympathetic constriction of the systemic vasculature may also drive parasympathetic vasodilation of the choroidal vasculature.

Disclosures: **C. Li:** None. **N. Del Mar:** None. **M.S. LeDoux:** None. **S. Cuthbertson:** None. **S. Gong:** None. **P. Ryan:** None. **A. Reiner:** None. **M.E.C. Fitzgerald:** None.

Poster

077. Neural Control of Cardiovascular Function I

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 77.14/JJ26

Topic: E.04. Autonomic Regulation

Support: NIH Grant HL 49965 to DM

NIH Grant HL 59895 to DM

NIH Grant HL 72006 to DM

Title: Direct visualization of oxytocin release in the brainstem upon photoactivation of fibers originating from parvocellular neurons in the paraventricular nucleus of the hypothalamus

Authors: *H. JAMESON, R. PINOL, D. MENDELOWITZ;
George Washington Univ., WASHINGTON, DC

Abstract: In addition to the classic effects of the hormone oxytocin (OXT) on uterine contraction and milk ejection, recent work has suggested OXT can act as a neuromodulator upon synaptic release from parvocellular neurons originating in the paraventricular nucleus of the hypothalamus (PVN). One such target of PVN neurons is the parasympathetic neurons in the

brainstem that generate parasympathetic activity to the heart. Recent studies using optogenetic stimulation of the PVN neurons that express channelrhodopsin (ChR2) revealed a direct pathway from these PVN neurons to brainstem parasympathetic cardiac vagal neurons (CVNs). Photoactivation of ChR2 containing PVN fibers elicited paired-pulse facilitation of glutamatergic neurotransmission to CVNs and this enhancement was diminished after application of the OXT receptor antagonist OTA suggesting this excitatory pathway is facilitated by endogenous OXT release from these synaptic terminals. In order to further test this hypothesis and additionally elucidate the conditions required for OXT release from PVN fibers, we dispersed within the brainstem sniffer CHO cells highly sensitive to oxytocin. These sniffer CHO cells were stably transfected to express the human recombinant OXT receptor, and calcium changes within these cells could be visualized as these cells also express the red fluorescent calcium indicator, R-GECO1. Our data shows optogenetic stimulation of PVN fibers evoked large, reproducible, and transient increases in Ca2+ within the sniffer CHO cells. The photostimulation-elicited increase in Ca2+ in the sniffer CHO cells upon PVN fiber activation was abolished by application of the oxytocin receptor antagonist OTA. This work supports and extends the hypothesis that excitation of parvocellular PVN fibers releases OXT at their brainstem CVN targets. Ongoing experiments will examine whether OXT release is blunted or absent in animals exposed to chronic intermittent hypoxia/hypercapnia, an animal model of the cardiovascular disease obstructive sleep apnea.

Disclosures: **H. Jameson:** None. **R. Pinol:** None. **D. Mendelowitz:** None.

Poster

077. Neural Control of Cardiovascular Function I

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 77.15/JJ27

Topic: E.04. Autonomic Regulation

Support: Smoking Research Foundation

Title: Proton modulates axo-axonal transmission of perivascular nerves in the rat mesenteric artery

Authors: ***H. KAWASAKI**[1,2], S. TAKATORI[2], S. OZAKI[3], P. TANGSUCHARIT[4]; [1]Departmen of Clin. Pharm., Matsuyama, Ehime, Japan; [2]Dept. of Clin. Pharm., Col. of Pharmaceut. Sciences, Matsuyama Univ., matsuyama city, Ehime, Japan; [3]Dept. of Clin. Pharmaceut. Science,, Grad. Sch. of Medicine, Dent. and Pharmaceut. Sciences, Okayama Univ.,

Okayama city, Okayama, Japan; [4]Dept. of Pharmacol., Fac. of Medicine, Khon Kaen Univ., Khon Kaen, Thailand

Abstract: Previous studies demonstrated that nicotine released protons from adrenergic nerves via stimulation of nicotinic acetylcholine receptors (nAChR) and activated transient receptor potential vanilloid-1 (TRPV1) receptors located on calcitonin gene-related peptide (CGRP)-containing vasodilator (CGRPergic) nerves, resulting in vasodilation. The present study investigated whether perivascular nerves release protons that modulate axon-axonal neurotransmission. Rat denuded small mesenteric arteries with active tone produced by methoxamine were treated with a fluorescent pH indicator (Lysosensor green DND-189) and fluorescence and the width of artery were observed. Application of nicotine, acetylcholine and capsaicin increased fluorescence outside small mesenteric arteries due to perivascular pH-lowering and caused increase in width of arteries due to vasodilation. Nicotine-induced increase in fluorescence (pH-lowering) was markedly inhibited by guanethidine (82%) and Ca2+-free medium, while capsaicin pretreatment at high concentration caused significant inhibition (50%) of the nicotine-induced pH-lowering. Guanethidine, capsaicin pretreatment (high concentration) and Ca2+-free medium inhibited nicotine-induced vasodilation. Acetylcholine-induced increase in fluorescence was almost abolished by capsaicin pretreatment (high concentration) and Ca2+-free medium. Guanethidine resulted in 20% inhibition in pH-lowering induced by acetylcholine. Additionally, guanethidine, capsaicin pretreatment (high concentration) and Ca2+-free medium inhibited acetylcholine-induced vasodilation. Capsaicin (low concentration)-induced pH-lowering (increase in fluorescence) was markedly inhibited by capsazepine, capsaicin pretreatment (high concentration) and the Ca2+-free medium. Capsazepine, capsaicin pretreatment (high concentration) and the Ca2+-free medium also inhibited capsaicin-induced vasodilation. These results suggest that protons are released from perivascular adrenergic and CGRPergic nerves upon nerve excitement and that released protons modulate axo-axonal neurotransmission in perivascular adrenergic and CGRPergic nerves.

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Poster

077. Neural Control of Cardiovascular Function I

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Program#/Poster#: 77.16/JJ28

Topic: E.04. Autonomic Regulation

Support: NIH grant HL 59895 to D.M.

NIH grant HL 49965 to D.M.

NIH grant HL 72006 to D.M.

Title: The optogenetic photoactivation of the pathway from the hypothalamic paraventricular nucleus to parasympathetic cardiac neurons in the brainstem is blunted following chronic intermittent hypoxia and hypercapnia (an animal model of obstructive sleep apnea)

Authors: *O. Y. DERGACHEVA, D. MENDELOWITZ;
Pharmacol. & Physiol., GW Univ., Washington, DC

Abstract: Introduction: Patients with obstructive sleep apnea (OSA) have an increased risk of cardiovascular diseases. The mechanisms of these cardiovascular diseases likely include diminishing cardioprotective parasympathetic activity to the heart due to inhibition of excitatory inputs to cardiac vagal neurons (CVN) in the brainstem. One important excitatory pathway to CVNs originates from the hypothalamic paraventricular nucleus (PVN). We hypothesized that chronic intermittent hypoxia and hypercapnia (CIHH) inhibits the glutamatergic neurotransmission from the PVN to CVNs. **Methods**: The fluorescent tracer rhodamine was injected into the pericardial sac of rats to label CVNs. A lentivirus vector that drives expression of channelrhodopsin (ChR2) was injected into the PVN to express ChR2 in hypothalamic PVN neurons and fibers that project to CVNs. Synaptic events in CVNs were studied using whole cell patch-clamp techniques. A pulse of laser light photoactivated ChR2 PVN fibers and evoked glutamatergic monosynaptic transmission to CVNs. **Results**: Glutamatergic synaptic events, and paired pulse facilitation of this neurotransmission in CVNs evoked by photostimulation of PVN fibers were diminished following 4-weeks CIHH. Acute hypoxia and hypercapnia (H/H) did not significantly alter glutamatergic neurotransmission to CVNs in control animals; however, H/H inhibited synaptic currents in CIHH animals. **Conclusion**: The PVN excitatory pathway to CVNs is diminished in CIHH animals. This would elicit a reduced parasympathetic activity to the heart and an enhanced risk of adverse cardiovascular events in OSA.

Disclosures: **O.Y. Dergacheva:** None. **D. Mendelowitz:** None.

Poster

077. Neural Control of Cardiovascular Function I

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 77.17/JJ29

Topic: E.04. Autonomic Regulation

Title: A novel approach for the analysis of respiratory sinus arrhythmia using breath phase information

Authors: *S. BARREDA, J. R. VRANISH, E. BAILEY;
Univ. of Arizona, Tucson, AZ

Abstract: Respiratory Sinus Arrhythmia (RSA) refers to periodic oscillations in heart rate in synchrony with respiration. RSA is considered a healthy form of heart rate variability and is thought to facilitate pulmonary gas exchange and to reflect the level of cardiac vagal tone. As a result, the measurement and quantification of RSA provides researchers and clinicians with a useful, fast, and non-invasive methodology to investigate the relationship between the cardiovascular and respiratory systems, and to assess autonomic system function. Unfortunately, the most commonly used analysis methods do not typically take respiratory information into account, and seek to quantify and describe RSA based solely on fluctuations in heart rate. Here, we propose a simple method to assess RSA that accounts for respiratory information, and places changes in heart rate in the context of the respiratory cycle. In this way, heart rate variability associated with RSA may be separated from variability arising from other sources. We collected heart rate and respiration information from five healthy, young-adult subjects using a pulse oximeter and respiratory inductance plethysmography. Data was collected during two-minute intervals of eupneic breathing, followed by two minutes of volitionally controlled breathing at several different rates (6, 7.5, 10, and 15 breaths/minute). Each record was then analyzed by treating the respiratory cycle as an almost-sinusoidal oscillation, and associating each heartbeat with the phase of the respiratory cycle during which it occurred. The results of this analysis indicate that, in healthy individuals at rest, typically around 70% of the variability in heart rate may be explained by the location of a beat within the respiratory cycle. Importantly, in many cases, there are substantive differences between RSA as measured using more traditional methods which ignore respiration, and the method outlined here. Furthermore, increases in respiratory rate lead to predictable changes in the phase relationship between RSA and the respiratory cycle which are easily quantified using this methodology. We suggest that the method outlined here is a useful tool for the investigation of RSA and cardio-respiratory coupling that offers several advantages over more common time- and frequency-domain analysis methods.

Disclosures: **S. Barreda:** None. **J.R. Vranish:** None. **E. Bailey:** None.

Poster

077. Neural Control of Cardiovascular Function I

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 77.18/JJ30

Topic: E.04. Autonomic Regulation

Support: CONACyT Grant Number 281096

Title: Pharmacological analysis of the α1- and α2-adrenoceptors subtypes on the vasopressor responses induced by dihydroergotamine in pithed rats

Authors: *E. RIVERA, G. MANRIQUE-MALDONADO, A. H. ALTAMIRANO-ESPINOZA, I. RUIZ-SALINAS, B. VILLANUEVA-CASTILLO, C. M. VILLALÓN;
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Abstract: Dihydroergotamine (DHE) is a semisynthetic ergot alkaloid used in acute treatment of migraine that displays affinity for a wide variety of receptors including dopamine D_2-like, serotonergic 5-$HT_{1/2}$ and $\alpha_{1/2}$-adrenoceptors. Indeed, DHE produces: (i) vasoconstriction in the canine external carotid artery bed via 5-HT_{1B} and $\alpha_{2A/2C}$-adrenoceptors; and (ii) increases in blood pressure (systemic vasoconstriction) via 5-HT_{2A} receptors and yohimbine-sensitive α_2-adrenoceptors in pithed rats. Nevertheless, no study has yet reported the α_1- and α_2-adrenoceptor subtypes involved in DHE-induced vasopressor responses. Hence, the present study was designed to identify, using selective antagonists, the specific α_1- and α_2-adrenoceptor subtypes involved in the vasopressor responses induced by DHE in pithed rats. For this purpose, male Wistar rats were anesthetized, pithed, artificially ventilated with room air (56 strokes/min and stroke volume of 20 ml/kg) and systematically pretreated with an intravenous (i.v.) bolus injection of 100 µg/kg ritanserin (to exclude the involvement of 5-HT_2 receptors). Ten min later, i.v. bolus injections of DHE or equivalent volumes of vehicle (20% propylene glycol; 1 ml/kg given 8 times) were administered in cumulative doses (1-3100 µg/kg), and the resulting effects on diastolic blood pressure were noted. DHE, but not the corresponding vehicle, produced dose-dependent increases in diastolic blood pressure. These vasopressor responses to DHE: (i) were similar ($P>0.05$) in control animals and in animals pretreated (i.v.) with the vehicles 1% ascorbic acid or physiological saline; (ii) slightly attenuated in animals pretreated (i.v.) with the α-adrenoceptor antagonists prazosin (α_1, 10 and 30 µg/kg) or rauwolscine (α_2, 100 and 300 µg/kg); and (iii) markedly blocked with the combination of 30 µg/kg prazosin plus 300 µg/kg rauwolscine. These findings clearly support the role of α_1- and α_2-adrenoceptors in the vasopressor responses to DHE. Interestingly, when pretreating (i.v.) the animals with subtype-selective antagonists in doses high enough to block their respective receptors, the vasopressor responses to DHE were significantly attenuated after 5-methylurapidil (α_{1A}, 30 and 100 µg/kg),

L-763,314 (α_{1B}, 30 and 100 µg/kg), BMY 7378 (α_{1D}, 30 and 100 µg/kg), BRL44408 (α_{2A}, 100 and 300 µg/kg), imiloxan (α_{2B}, 1000 and 3000 µg/kg) or JP-1302 (α_{2C}, 1000 µg/kg). In conclusion, these results suggest that the vasopressor responses induced by DHE are mediated by activation of the α_{1A}, α_{1B}, α_{1D}, α_{2A}, α_{2B} y α_{2C}-adrenoceptor subtypes.

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

Poster

077. Neural Control of Cardiovascular Function I

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 77.19/JJ31

Topic: E.04. Autonomic Regulation

Support: grant (102-2314-B-010 -033 -MY3-) from the Ministry of Science and Technology (Taiwan)

grant (10201-62-004) from Taipei City Hospital

Title: Effects of cold exposure on autonomic function during sleep and early-morning blood pressure surge in prehypertensives

Authors: *C.-H. HONG, T. KUO, C. YANG;
Inst. of Brain Sci., Natl. Yang-Ming Univ., Taipei City, Taiwan

Abstract: Cardiovascular events occur more frequently in the morning, during which there is an acute change in blood pressure (BP), called morning blood pressure surge (MBPS). Prehypertension is related to a high risk of cardiovascular events than normotension. Our previous study reported that cold exposure elevates the amplitude of MBPS and is associated with late sleep stage transition sympathetic activation, which might be important implications for cold-related cardiovascular events in normotensives. However, few studies have been done on the effects of cold exposure on autonomic function during sleep stage transitions and changes of sleep architecture of prehypertensives. Therefore, we carried out an experiment aiming to test the hypothesis that the effects of cold exposure on changes of autonomic function during sleep and MBPS in young prehypertensives were more exaggerate than young nonmotensives. Fourteen normotensive and prehypertensive male adults with a mean age 24.36±0.77 and 27.00±1.38, respectively. Subjects underwent cold (16 ℃) and warm (23 ℃) conditions randomly. BP was

measured every 30 minutes by an autonomic BP monitor. The electroencephalograms (EEGs), electrooculograms (EOGs), electromyograms (EMGs), electrocardiograms (ECGs) and near body temperature were recorded by miniature polysomnography. Under cold exposure, (1) a significant higher amplitude of MBPS than in warm conditions among normotensives ($p < .05$), however, such changes were more exaggerate in prehypertensives. (2) a significant decrease on HF during the last sleep stage transition from NREM to REM in prehypertensives, whereas no such change was found in normotensives; (3) a higher early-morning (2 h before waking) surge on BP and on sympathetic activity than that during the first 2 h of sleep period ($p < .05$) in prehypertensives than normotensives; (4) a significant decrease in number of REM periods ($p < .05$) was observed under cold exposure in normotensives, whereas no such changes was found in prehypertensives. Our study reinforces cold exposure might increase the risk of sleep-related cardiovascular events for prehypertensives.

Disclosures: **C. Hong:** None. **T. Kuo:** None. **C. Yang:** None.

Poster

077. Neural Control of Cardiovascular Function I

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Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 77.20/JJ32

Topic: E.04. Autonomic Regulation

Support: Veterans Affairs A NURA-009-13S (QH, CD)

I01 BX000481 (JS)

Research and Education Component of the Advancing a Healthier Wisconsin Endowment at MCW (CH)

Title: Altered endocannabinoid signaling influences baseline heart rate

Authors: *C. DEAN-BERNHOFT[1], C. J. HILLARD[2], J. L. SEAGARD[1], F. A. HOPP[1], Q. H. HOGAN[1];
[1]Anesthesiol., Med. Col. of Wisconsin, Zablocki VA Med. Ct, Milwaukee, WI; [2]Pharmacol., Med. Col. of Wisconsin, Milwaukee, WI

Abstract: There is growing recognition of descending endocannabinoid modulation of sensory pathways from the midbrain dorsal periaqueductal gray (DPAG) contributing to the relief of neuropathic pain. Endocannabinoids also activate the sympathetic nervous system through

actions at cannabinoid 1 receptors (CB1Rs) in the stress networks of the dPAG. We have established a link between pain susceptibility and autonomic activation by showing that rats with elevated initial heart rate (HR) and sympathetic tone do not develop neuropathic hyperalgesia when subjected to spinal nerve ligation. These studies were designed to determine if baseline HR is correlated to altered components of the endocannabinoid system in the dPAG. Male Sprague-Dawley rats (320-340 gm) were implanted with telemetric units to monitor blood pressure. HR and HR variability were derived from the blood pressure measurements on days 5-7 following implantation surgery. Brains were removed on day 7, and the dPAG region was excised and quick-frozen in liquid nitrogen. Real time RT-PCR was performed to analyze the tissue samples for mRNA expression of the endocannabinoid metabolic enzymes fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL) and for CB1Rs. Transcript levels were plotted against HR and HR variability for regression analysis to determine correlations between components of the endocannabinoid system in the dPAG and baseline HR and sympathetic tone. We found a significant, inverse correlation between HR and amounts of FAAH and MAGL mRNA. There was a similar tendency for the low frequency/high frequency ratio of HR variability, an indicator of sympatho-vagal balance. Transcript levels for CB1R did not change with HR or HR variability. Downregulation of FAAH and MAGL expression at high baseline HR would be expected to result in increased concentrations of the endocannabinoids, anandamide and 2-arachidonoylglcyerol, respectively. These data suggest a link between altered endocannabinoid signaling in the dPAG, sympathetic tone and predisposition to neuropathic pain. Supported by Veterans Affairs A NURA-009-13S (QHH, CD), I01 BX000481 (JLS) and the Research and Education Component of the Advancing a Healthier Wisconsin Endowment at MCW (CJH).

Disclosures: **C. Dean-Bernhoft:** None. **C.J. Hillard:** None. **J.L. Seagard:** None. **F.A. Hopp:** None. **Q.H. Hogan:** None.

Poster

078. Stress: Corticotropin-Releasing Factor

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 78.01/JJ33

Topic: E.05. Stress and the Brain

Support: NIH Grant NS28912

NIH Grant NS45260

NIH Grant MH73136

Title: The stressed synapse: Effects of corticosterone (CORT) and corticotropin-releasing hormone (CRH) on hippocampal dendritic spines

Authors: *Y. CHEN[1], P. SEE[1], J. MOLET[2], X. ZHUO[1], T. BARAM[3];
[1]Pediatrics, [2]Anatomy/Neurobiology, [3]Pediatrics & Anatomy/Neurobiology, Univ. Cal Irvine, IRVINE, CA

Abstract: Rationale: Multiple molecules are released in response to stress, and both steroids and the stress hormone CRH bathe hippocampal synapses during stress. Both CORT and CRH contribute to stress-induced deficits in hippocampus-dependent memory, and the mechanisms seem to involve the integrity and function of hippocampal synapses located on dendritic spines. Much work has focused on the individual effects of these stress-related molecules on dendritic spines: CORT has complex effects on spine structure and turnover, and we found that CRH application results in selective loss of thin spines already within hours. Here we describe the individual and combined effects of CORT and CRH on spines of CA1 pyramidal neurons. Methods: Dorsal hippocampal slices from 3-month-old Thy1-YFP mice or C57BL/6 were assigned to one of four experimental groups: vehicle, CORT, CRH, and CORT plus CRH. CORT (100 nM) and CRH (50-100 nM) alone or together were infused for 40-120 min. Spine numbers and types were assessed in YFP-expressing mice on specific dendritic segments of CA1 pyramidal neurons. Hippocampal synaptic structure was assessed using quantitative immunohistochemistry for postsynaptic density protein (PSD)-95 followed by deconvolution analysis of wide-field 3D images. In addition, the fate of individual spines was assessed dynamically, using live imaging with two-photon microscopy. Results: Upon a two hour exposure, CORT alone had little effect on thin spines and enhanced the size of mushroom spines, as reflected also from a shift in the size distribution of synapses. CRH alone resulted in reduction of thin spine numbers, with little effect on mushroom-type spines, leading to augmented relative abundance of mushroom-type spines. The combined application of CORT and CRH led to a significant shift of synapse size distribution as represented by an increased number of large PSD-95-immunoreactive puncta (between 0.3-0.8 μm3) compared with vehicle, CRH, or CORT alone. The underlying mechanisms and the relevance to memory processes are under current examination. Conclusions: These preliminary data suggest that the effects of combined CORT and CRH are not simply additive, and have implications for understanding the effects of stress on hippocampal synaptic structure and memory functions.

Disclosures: **Y. Chen:** None. **P. See:** None. **J. Molet:** None. **X. Zhuo:** None. **T. Baram:** None.

Poster

078. Stress: Corticotropin-Releasing Factor

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 78.02/JJ34

Topic: E.05. Stress and the Brain

Support: CIHR & NSERC Grants to MNH.

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JMG is supported by a Postdoctoral Fellowship from Alberta Innovates Health Solutions.

DJH is supported by NIH predoctoral Fellowship DA031572.

Title: Corticotropin-releasing hormone drives anandamide hydrolysis in the amygdala to promote anxiety

Authors: *J. GRAY[1,2,3], H. A. VECCHIARELLI[1,2,4], T. T. Y. LEE[5], M. MORENA[6], D. J. HERMANSON[7], A. KIM[1], R. J. MCLAUGHLIN[8], K. HASSAN[1], C. KUHNE[9], C. T. WOTJAK[9], J. M. DEUSSING[9], S. PATEL[10], M. N. HILL[1,2,3];
[1]Hotchkiss Brain Institute, Univ. of Calgary, Calgary, AB, Canada; [2]Mathison Ctr. for Mental Hlth. Res. & Educ., Calgary, AB, Canada; [3]Depts of Cell Biol. & Anatomy, and Psychiatry, Univ. of Calgary, Calgary, AB, Canada; [4]Neurosci. Dept, Univ. of Calgary, Calgary, AB, Canada; [5]Dept of Psychology, Univ. of British Columbia, Vancouver, BC, Canada; [6]Sapienza Univ., Rome, Italy; [7]Dept of Chemistry, Vanderbilt Univ., Nashville, TN; [8]Douglas Hospital, McGill Univ., Montreal, QC, Canada; [9]Max Planck Inst. of Psychiatry, Munich, Germany; [10]Dept of Psychiatry and Mol. Physiol. & Biophysics, Vanderbilt Univ., Nashville, TN

Abstract: Objective: Corticotropin-releasing hormone (CRH) activation of the R1 receptor acts throughout the brain and pituitary to facilitate endocrine and behavioral stress responses, while the endocannabinoid (eCB) CB1 receptor acts in an opposite direction. Although these signaling systems have overlapping distribution, few have investigated if these systems interact. Methods & Results: Using adult male Sprague Dawley rats, we found that icv CRH decreases eCB levels (anandamide) and increases FAAH activity in the amygdala, but not the PFC, at both 10 and 30 min post-CRH administration, and that these effects are mediated by CRH-R1 activation and not CRH-R2. Consistent with these findings, CRH-R1 antagonism attenuated stress-induced increases in FAAH activity and reduced amygdalar AEA content. Mice lacking CRH-R1 exclusively on glutamatergic forebrain neurons similarly showed a lack of stress-induced changes in amygdalar FAAH activity and AEA tone, suggesting CRH-R1 activation removes the 'tonic brake' normally exerted by anandamide within the basolateral amygdala, by rapidly increasing anandamide hydrolysis via FAAH activation. We also revealed that CRH-R1

glutamatergic cells co-express both FAAH protein and mRNA, suggesting this cascade appears specialized to excitatory pyramidal cells. Lastly, we have also found that CRH-R1 activation of FAAH increases anxiety-related behavior, and that this effect is attenuated by pretreatment with FAAH inhibitor. Conclusions: This CRH-driven anandamide decline seems to disinhibit the amygdala and contribute to the generation of behavioral and endocrine stress responses. Interestingly, this model likely accounts for why FAAH inhibitors only exert anxiolytic actions in aversive environments as they simply block the effects of CRH signaling.

Disclosures: **J. Gray:** None. **H.A. Vecchiarelli:** None. **T.T.Y. Lee:** None. **M. Morena:** None. **D.J. Hermanson:** None. **A. Kim:** None. **R.J. McLaughlin:** None. **K. Hassan:** None. **C. Kuhne:** None. **C.T. Wotjak:** None. **J.M. Deussing:** None. **S. Patel:** None. **M.N. Hill:** F. Consulting Fees (e.g., advisory boards); MN Hill is a scientific consultant for Pfizer..

Poster

078. Stress: Corticotropin-Releasing Factor

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 78.03/JJ35

Topic: E.05. Stress and the Brain

Support: NIH Grant DA020129

Title: Cannabinoid type 1 receptor co-localizes with corticotropin-releasing factor in the noradrenergic nucleus locus coeruleus

Authors: *R. WYROFSKY, B. A. S. REYES, E. J. VAN BOCKSTAELE;
Pharmacol. & Physiol., Drexel Univ. Col. of Med., Philadelphia, PA

Abstract: The noradrenergic system has been shown to play a key role in the regulation of stress responses, arousal and mood. The locus coeruleus (LC) provides the sole source of norepinephrine (NE) to the frontal cortex and dysregulation of cortical NE has been implicated in the pathophysiology of stress-related psychiatric disorders. Via corticotropin-releasing factor (CRF) neurotransmission, stress exposure activates LC neurons resulting in an increase in LC firing rate and increased release of NE. The endocannabinoid (EC) system has been shown to modulate NE through modulation of cannabinoid type 1 receptors (CB1R). While it is known that both CRF and CB1R regulate noradrenergic neurons in the LC, whether CB1R are co-localized to CRF-containing axon terminals in the LC has not yet been examined. To further investigate the cellular sites for interactions between CB1R and CRF, coronal sections through

the LC were processed for immunocytochemical detection of CB1R and CRF in male rat LC. Using immunofluorescence microscopy, we observed that CB1R and CRF are frequently co-localized within varicose processes in the LC. High-resolution immunoelectron microscopy using gold-silver labeling for CB1R and immunoperoxidase labeling for CRF confirmed that single axon terminals exhibited both CB1R and CRF immunoreactivities in the LC. Semi-quantitative preliminary analysis revealed that of 228 dual-labeled CB1R and CRF-containing axon terminals, 25% (56/228) formed symmetric-type synapses while 17% (39/228) formed asymmetric-type synapses with dendritic processes in the LC. The remainder of the dually labeled axon terminals analyzed (58%, 133/228) could not be unequivocally classified into either category based on the plane of section analyzed and were classified as undefined. These results reveal an anatomical basis for modulation of CRF neurotransmission by CB1R, indicating that the endocannabinoid system is positioned to modulate stress-responses in the LC. Grant **Support:** DA020129

Disclosures: **R. Wyrofsky:** None. **B.A.S. Reyes:** None. **E.J. Van Bockstaele:** None.

Poster

078. Stress: Corticotropin-Releasing Factor

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 78.04/JJ36

Topic: E.05. Stress and the Brain

Title: Noradrenergic activation of CRH neurons via a novel retrograde trans-neuronal-glial circuit

Authors: *C. CHEN, Z. JIANG, J. G. TASKER;
Cell & Mol. Biol., Tulane Univ., New Orleans, LA

Abstract: Corticotropin releasing hormone (CRH) neurons of the hypothalamic paraventricular nucleus (PVN) release CRH as an initial signal in the hypothalamic-pituitary-adrenal (HPA) response to stress. CRH neurons in the PVN are activated by ascending noradrenergic projections from the A2 cell group in the caudal nucleus. The noradrenergic afferents provide a major excitatory drive to the HPA axis. Using whole-cell patch clamp recordings of identified CRH neurons in hypothalamic brain slices from CRH-eGFP mice, we found that norepinephrine (NE) activated local glutamate and GABA synaptic circuits via a postsynaptic α1-adrenoceptor-mediated retrograde nitric oxide release and activation of glial signaling. Glial calcium signaling resulted in ATP release and P2X purinergic receptor activation of presynaptic glutamate and

GABA neurons, which resulted in a net excitation of the CRH neurons. This represents a novel mechanism of stimulation of the CRH neurons by NE via a neuronal-glial retrograde trans-synaptic circuit.

Disclosures: **C. Chen:** None. **J.G. Tasker:** None. **Z. Jiang:** None.

Poster

078. Stress: Corticotropin-Releasing Factor

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 78.05/KK1

Topic: E.05. Stress and the Brain

Support: DA 09082

DA 018326

Title: Cellular substrates for interactions between delta opioid receptors and corticotropin-releasing factor in the basolateral nucleus of the amygdala

Authors: ***N. A. HELDT**[1], B. A. S. REYES[1], J. STERLING[1], E. M. UNTERWALD[2], E. J. VAN BOCKSTAELE[1];
[1]Dept. of Pharmacol. & Physiol., Drexel Univ. Col. of Med., Philadelphia, PA; [2]Ctr. for Substance Abuse Res. & Dept. of Pharmacol., Temple Univ. Sch. of Med., Philadelphia, PA

Abstract: The delta opioid receptor (DOR) plays an important role in mood, learning and memory. Dysregulation of the DOR system may contribute to heightened levels of anxiety, particularly during withdrawal from chronic cocaine administration. Anatomical studies show that DOR levels are high in the basolateral nucleus of the amygdala (BLA), a region involved in orchestrating emotional and behavioral responses to drug withdrawal. Withdrawal from drugs of abuse is known to engage the corticotropin-releasing factor (CRF) system, and CRF antagonism can block anxiety-like behaviors. The present study sought to elucidate the distribution of DOR with respect to CRF and the norepinephrine transporter (NET) using light, immunofluorescence and immunoelectron microscopy in the BLA. Forty-micron thick tissue sections of male Sprague Dawley were collected and processed for immunocytochemical detection of DOR, CRF, and NET. Light microscopy showed robust and a very dense distribution of DOR in the BLA. Using immunofluorescence, DOR exhibited a topographic distribution within the BLA, and frequently co-localized with CRF-immunoreactive neurons. NET-containing fibers were localized in close

proximity with DOR and CRF-containing amygdalar neurons. Using immunoelectron microscopy, rat brain sections were processed for the detection of DOR or DOR and CRF. Immunoperoxidase labeling for DOR was localized to both dendritic processes and axon terminals. Dual immunoelectron microscopy using gold-silver labeling for DOR and immunoperoxidase labeling for CRF, confirmed that dendritic processes exhibited both DOR and CRF immunoreactivities in the BLA. In addition, DOR was localized to axon terminals and formed synapses with CRF-containing neurons in the BLA. These results provide anatomical evidence for the interaction of the DOR and CRF systems. The present results also suggest a role for NE in modulating DOR and CRF-containing neurons that may be critical for the regulation of anxiety-like behaviors. Grant **Support:** R01 DA 09082 (EVB) and R01 DA018326 (EMU).

Disclosures: **N.A. Heldt:** None. **B.A.S. Reyes:** None. **J. Sterling:** None. **E.M. Unterwald:** None. **E.J. Van Bockstaele:** None.

Poster

078. Stress: Corticotropin-Releasing Factor

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 78.06/KK2

Topic: E.05. Stress and the Brain

Support: Center for Biological Control and Analysis by Applied Photonics

Title: Cross-talk between orexin/hypocretin and corticotropin releasing factor systems

Authors: J. K. ACHUA[1,2], L. B. CALLAHAN[3], J. J. BRUDVIG[1], C. H. SUMMERS[1], *P. J. RONAN[3];
[1]USD Neurosci. Group, USD, SD; [2]Res. Service, Sioux Falls VA Healthcare Syst., Sioux Falls, SD; [3]Neurosci. Group, Avera Res. Institute/USD Med./VA Res., SIOUX FALLS, SD

Abstract: Corticotropin releasing factor (CRF) and orexin/hypocretin play key, sometimes parallel, roles in a host of arousal/stress responses. They have been implicated in a range of stress-induced psychiatric disorders including addiction, depression and sleep disorders. There is evidence that these systems interact and regulate each other - perhaps providing a feed forward mechanism for enhanced stress responses. Numerous lines of evidence have shown that these neuropeptide systems mutually excite each other. We and others have provided clear anatomical evidence of CRF receptors on ORX neurons and the reciprocal; ORX innervation and ORX receptors on CRF neurons. Finally, Scammell and Yanigasawa's groups have done excellent

work elucidating general afferent pathways of orexin neurons. Orexin neurons receive inputs from many CRF-rich neuronal fields including the paraventricular nucleus of the hypopthalamus (PVN), bed nucleus of the stria terminalis (bnst) and central nucleus of the amygdala (CeA). Using a combination of neuronal tracing methods and immunohistochemistry we sought to build on this earlier work and clarify if these afferent pathways include CRF neurons. This is the first study to identify CRF neurons in these regions specifically projecting to ORX neuron fields. There appears to be specific topographic distribution of these CRF inputs to different regions of the LH that supports the hypothesis that perifornical ORX neurons preferentially contribute to stress and anxiety responses.

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Poster

078. Stress: Corticotropin-Releasing Factor

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 78.07/KK3

Topic: E.05. Stress and the Brain

Support: NIH Grant DK-26741

Title: Localization of crf receptors using a newly-elucidated radioligand, pd-sauvagine

Authors: *L. A. TAN[1], M. H. PERRIN[2], J. M. VAUGHAN[2], K. A. LEWIS[2], C. J. DONALDSON[2], J. E. RIVIER[2], P. E. SAWCHENKO[1];
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Abstract: The corticotropin-releasing factor (CRF) family of peptides includes CRF and the three urocortins, which signal through two distinct G-protein coupled receptors, CRFR1 and CRFR2. These receptors have been intensely studied since their discovery in the mid-90s for their role in mediating/integrating the neuroendocrine, autonomic, and behavioral responses to diverse stressors. Although the CRFR distribution has been well characterized at the mRNA level, the mapping of CRFR proteins have been limited by the absence of reliable methods for immunolocalization of either receptor. Binding of radiolabeled sauvagine, a CRF-related peptide from the skin of the frog, Phyllomedusa sauvagei, has provided perhaps the best available means of localizing CRF binding sites in the brain. Recently, a similar ligand was isolated from another

frog species, Pachymedusa dacnicolor, and called PD-sauvagine. In binding studies on membranes from COS cells transfected with cloned receptors, we confirmed that PD-sauvagine is a high-affinity agonist for both CRFRs. The radiolabeled analog, [125I-Tyr0,Glu1]-PD-sauvagine, reveals significantly more CRFR binding sites than the traditional radioligand, [125I-Tyr0,Glu1,Nle17]-sauvagine, owing likely to a slower off rate. When applied to fresh frozen sections of mouse brain and other tissues, the PD-sauvagine radioligand binds more robustly than the traditional sauvagine radioligand, and in a manner more consistent with the (cellular) CRFR mRNA distribution. Binding is competed in a dose-related and region-specific manner by subtype-specific antagonists, and is absent in double CRFR knockout mice. *In situ* binding of radiolabeled PD-sauvagine represents a sensitive and accurate means for localizing CRFR proteins in future studies.

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Poster

078. Stress: Corticotropin-Releasing Factor

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 78.08/KK4

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: NIH Grant AA019455

NIH Grant AA021623

Title: Ketamine reduces BNST CRF levels and ethanol withdrawal-induced depressive behaviors

Authors: *K. LOUDERBACK[1,2,3,4], T. L. FETTERLY[1,2,3], H. H. WILSON[2], D. G. WINDER[1,2,3,4];
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Abstract: Low-dose ketamine induces rapid and long-lasting antidepressant effects in humans, and this effect has been replicated in rodent models of depression. Ketamine acts as a noncompetitive N-methyl D-aspartate receptor (NMDAR) antagonist, but the mechanisms underlying its antidepressant effect are not fully understood. Studies, including our own, have

demonstrated that the ifenprodil derivative Ro 25-6981 (Ro), which inhibits GluN2B-containing NMDARs, is also capable of reducing depression-like behaviors in rodents. We have demonstrated that targeted knockdown of GluN2B within the bed nucleus of the stria terminalis (BNST) produces reductions in depression-like behavior in the novelty-induced hypophagia (NIH) test similar to effects observed with systemic ketamine or Ro administration. We sought to understand how GluN2B containing NMDARs in the BNST may be involved in modulation of affective behavior. It has been demonstrated that corticotropin releasing factor (CRF) within the BNST also plays a role in depression-like behaviors. CRF enhances excitatory drive onto VTA-projecting BNST neurons, and overexpression of CRF within the BNST results in increased immobility in the FST. We hypothesized that ketamine may act to inhibit activity-dependent CRF gene expression in the BNST through its blockade of NMDARs. Male C57Bl/6J mice were treated with 3mg/kg ketamine 4 hours, 24 hours, or one week prior to collection of punches containing the BNST from coronal sections. Using qRT-PCR, we observed a significant decrease in CRF transcript levels at the 24 hour time point, compared to vehicle treated controls, which returned to control levels by the one week time point. Finally, our lab has previously identified GluN2B as a major contributor to BNST NMDA receptor acute and chronic ethanol sensitivity. Long-term withdrawal from chronic voluntary ethanol administration has been demonstrated to induce depression-like behaviors in forced swim (FST) and novelty-suppressed feeding (NSFT) tests in female C57Bl/6J mice that are individually-housed. We found that these behaviors are ameliorated with systemic administration of ketamine (3mg/kg) in withdrawn mice.

Disclosures: **K. Louderback:** None. **T.L. Fetterly:** None. **H.H. Wilson:** None. **D.G. Winder:** None.

Poster

078. Stress: Corticotropin-Releasing Factor

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 78.09/KK5

Topic: E.05. Stress and the Brain

Support: NIH Grant MH090297

Title: Basolateral Amygdala circuitry underlying modulation of stress-related behavior

Authors: ***M. BOMPOLAKI**[1], T. UNHAVANE[1], W. F. COLMERS[2], J. H. URBAN[1];
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Abstract: The amygdala is traditionally regarded as the emotional center of the brain, and as such it functions as a central hub interconnecting a variety of brain regions. The basolateral nucleus of the amygdala (BLA) plays an important role in the balance between anxiolysis or stress resilience (seen with intra-BLA effects of neuropeptide Y-NPY) and anxiogenesis or stress vulnerability (seen with intra-BLA effects of corticotropin releasing factor-CRF). The effects of these endogenous neuropeptides are mediated by inhibition or potentiation, respectively, of the hyperpolarization activated current (Ih). These actions result in inhibition or potentiation of BLA output, which is then consistent with the behavioral outcomes observed. However, the neuroanatomical circuit responsible for the manifestation of these behaviors in response to NPY and CRF is currently not well understood. The goal of these studies was to examine the neurochemical phenotype of the BLA projections to the central nucleus of the amygdala (ceA) and to the bed nucleus of the stria terminalis (BST). We used a combination of retrograde and anterograde tracers combined with immunohistochemistry to characterize BLA projections and their downstream targets. The anterograde tracer Phaseolus vulgaris-leucoagglutinin (PHAL) was injected into the BLA. Examination of the tissue 7 days later demonstrated strong projections to the ceA, the lateral division of BST and the oval BST. Furthermore, the retrograde tracer Fluorogold (FG) was injected into either the ceA or BSTL resulting in retrograde identification of BLA neurons. Multiple-label immunohistochemistry was used to characterize whether these cells expressed hyperpolarization-activated, cyclic nucleotide-gated channel subunit 1 (HCN1) immunoreactivity. A number of FG-filled cells expressing HCN1 immunoreactivity were identified within localized regions of the BLA. These data indicate that alterations in BLA output mediated by the Ih current are poised to manipulate the activity of ceA and BSTL. This knowledge will aid our understanding of the functional neuroanatomy of NPY- and CRF-related circuits in the BLA and how their projections coordinate stress or anxiety related behaviors.

Disclosures: **M. Bompolaki:** None. **W.F. Colmers:** None. **J.H. Urban:** None. **T. Unhavane:** None.

Poster

078. Stress: Corticotropin-Releasing Factor

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Topic: E.05. Stress and the Brain

Support: NIH Grant K99MH-096746

NIH Grant RO1MH-072908

National Center for Research Resources P51RR169

Title: Mapping CRF projections from the bed nucleus of the stria terminalis (BNST) using adeno-associated viral vectors in mouse and rat

Authors: *J. A. DABROWSKA, D. G. RAINNIE;
Emory Univ., ATLANTA, GA

Abstract: The bed nucleus of the stria terminalis (BNST) is known to play a critical role in mediating the behavioral and autonomic responses to a variety of stressors. The oval and fusiform nuclei of the anterolateral cell group of the BNST (BNSTALG) contain cell bodies that synthesize the stress hormone, corticotropin releasing factor (CRF). Although afferent fibers originating from the BNSTALG have been shown to innervate several key structures of the neuroendocrine and central autonomic system, the question remains as to whether, some of these fibers are CRF-positive. To directly address this question, we injected a "floxed" anterograde tracer (rAAV5/EF1a-DIO-mCherry) into the oval nucleus of the BNSTALG of CRFp3.0CreGFP transgenic mice, which express a green fluorescent protein (GFP) under the control of the CRF promoter. Two weeks after the AAV injection, serial sections were cut through the entire brain and analyzed for the presence of dual-labeled fibers (GFP-mCherry) in potential projection sites using confocal microscopy. Our results show high GFP-mCherry co-expression in cell bodies of the oval nucleus of the BNSTALG and in several terminal fields. Notably, dual-labeled fibers were observed in the dorsal raphe nucleus (DRN), the vetral tegmental area (VTA), as well as in the paraventricular nucleus of the hypothalamus (PVN). To further explore whether CRF neurons in the rat BNSTALG send analogous projections, we injected the oval nucleus with an AAV in which the human synapsin promoter-5 (hSyn) drives GFP expression. Six weeks later serial sections were cut from the entire brain, stained with an anti-CRF antibody, and visualized with an Alexa 586 secondary antibody. Sections were analyzed for the presence of dual-labeled fibers (GFP-Alexa 586). Injection of rAAV5-hSyn-GFP showed robust somatodendritic GFP expression in the oval nucleus of the BNSTALG. Dual-immunofluorescence studies revealed that the subpopulation of GFP-positive neurons in the BNST co-expressed CRF. AAV5-hSyn-GFP also resulted in robust GFP expression in fibers and terminal fields. Analysis of dual-labeled fibers confirmed the presence of CRF-GFP fibers in several divisions of the PVN: namely the anterior (PaAP), and the medial parvicellular (PaMP) divisions, the ventral (PaV), the posterior (PaPO), and PaLM-lateral magnocellular division. CRF-GFP terminals were also seen in the VTA and the central nucleus of the amygdala. The results of our study indicate that CRF neurons in oval nucleus of the BNSTALG send projections not only to the regions critically involved in the neuroendocrine and autonomic regulation, but also to centers modulating reward and affective behavior.

Disclosures: **J.A. Dabrowska:** None. **D.G. Rainnie:** None.

Poster

078. Stress: Corticotropin-Releasing Factor

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Topic: E.05. Stress and the Brain

Support: NIH Grant 1R15MH85266-1A1

Title: Behavioral effects of chronically elevated corticosterone in the bed nuclei of stria terminalis: Role of corticotropin releasing factor

Authors: *J. D. SHEPARD, K. KINSLEY, E. TYLER, E. GLOTFELTY;
Towson Univ., Towson, MD

Abstract: Glucocorticoids secreted during adversity play an important role in sustaining fear and anxious behaviors during a protracted threat and may make the organism more reactive to novel threats in the future. The bed nuclei of stria terminalis (BNST) expresses glucocorticoid receptors and is a key limbic structure involved in fear and anxiety. We previously discovered that local administration of corticosterone (CORT) into the lateral BNST increases anxiety-like behavior in rats. The present study extends these findings by examining the effects of glucocorticoids in the ventromedial BNST (Exp. 1); effects of CRF in mediating the anxiogenic effects of CORT in the BNST (Exp. 2); and the potential role of CRF within the BNST in mediating anxiety (Exp. 3). We used the elevated plus maze to assess anxiety 7 days post treatment in all 3 experiments. For Exp 1, micropellets containing 30 μg of CORT or cholesterol (CHOL; control) were stereotaxically implanted in the vmBNST of Wistar rats. In contrast to the anxiogenic effects of CORT in the dlBNST, there was no effect on open arm exploration with elevated CORT in the vmBNST (ps > 0.40). Taken with earlier findings, this result supports regional specificity for the effects of CORT in the BNST. Next, we examined the effects of a CRF1 receptor antagonist (Antalarmin) on the anxiogenic effects of elevated CORT in the dlBNST (Exp. 2). One week after implanting CORT in the dlBNST rats received the plus-maze test preceded with a systemic injection of Antalarmin. Chronically elevated CORT in the dlBNST increased indices of anxiety (ps < 0.04) and this effect was abolished with Antalarmin treatment (ps < 0.05). We then instrumented rats with guide cannulas in the dlBNST and elevated systemic CORT using subcutaneous implants. Preliminary data indicate that elevated CORT increased anxiety (ps < 0.05) with a trend toward Antalarmin blocking the anxiogenic effect (ps < 0.14). Conclusions: The behavioral effects of chronically elevated CORT can be

localized to specific regions of the BNST. Further, preliminary data suggest CRF1 receptors in the dlBNST may play an important role in mediating the anxiogenic effects of chronically elevated CORT.

Disclosures: **J.D. Shepard:** None. **K. Kinsley:** None. **E. Tyler:** None. **E. Glotfelty:** None.

Poster

079. Stress: Genes and Epigenetics

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Program#/Poster#: 79.01/KK8

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: The Hope for Depression Research Foundation

The American Foundation for Suicide Prevention

Title: Epigenetics of stress effects in hippocampus: The ehRatio on H3K27

Authors: *B. S. MCEWEN[1], B. BIGIO[2], D. ZELLI[1], C. NASCA[1];
[1]Lab. of Neuroendocrinology, [2]Ctr. for Clin. & Translational Sci., The Rockefeller Univ., NEW YORK, NY

Abstract: A wealth of studies has shown the involvement of histone modifications in several behavioural and neurobiological phenomena. However, in the investigation of epigenetic control of gene transcription via histone modifications, one of the challenges is yet to understand potential interplays between different processes of histone modifications and how the large numbers of histone modifications interact with each other. The evidence that H3K27ac is implicated in the mechanism of action of a novel antidepressant candidate, acetyl-L-carnitine (LAC), in either stressed or genetically vulnerable animals led us to investigate BDNFVal/Met SNP effects as well as acute stress (ARS) effects in BDNFVal/Val mice on a potential interplay between histone modification processes, such as acetylation and methylation and whether oral LAC treatment, which directly resets the chromatin assembly, may prevent the stress molecular and behavioral effects and restore vulnerable BDNFVal/Met phenotype. Thus, we provide evidence for an underlying molecular mechanism by which a dynamic interplay among two histone modifications on histone H3 controls the brain responses to environmental stressors regulating the expression of a gene, mGlu2, in hippocampus that is a marker of anxiety- and mood-related behaviors. In particular, ARS-BDNFVal/Val mice show reduced mGlu2 and

mGlu3 transcripts in hippocampus but not in prefrontal cortex (PFC), whereas BDNFVal/Met mice show a selective mGlu2 reduction in hippocampus. mGlu2 reduction correlates with a dynamic interplay between H3K27acetylation and H3K27trimethylation and chromatin immunoprecipitation (ChIP) assays link H3K27acetyl with mGlu2, but not mGlu3 reduction, in hippocampus. Both mGlu2 and histone mark changes are prevented by oral LAC treatment. ARS-induced elevated anxiety-like behavior in a light-dark test and the associated anxiety behaviour of SSRI-resistant BDNFVal/Met mice are rescued by one day of LAC oral treatment. Furthermore, BDNFVal/Met mice and ARS on BDNFVal/Val mice show reduced expression of the transcriptional coactivator with intrinsic histone acetyltransferase activity (HAT), P300, which is prevented by LAC, while the methylating enzyme, Ezh-2 is not affected. We propose an Equilibrium Histone Ratio (ehRatio) that underscores the importance of interplay between multiple epigenetic modifications, in the control of stress responses and related anxiety-like behaviors. Given the reversible nature of histone modifications, next-generation epigenetic drugs, such as HDACs' inhibitors and LAC, provide some hope for developing rapidly acting psychiatric drugs.

Disclosures: **B.S. McEwen:** None. **B. Bigio:** None. **D. Zelli:** None. **C. Nasca:** None.

Poster

079. Stress: Genes and Epigenetics

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Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: The Hope for Depression Research Foundation

The American Foundation for Suicide Prevention

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Title: Stress exposure induces resistance to LAC oral antidepressant treatment in a subset of vulnerable FSL rats

Authors: *A. A. MATHE[1], C. NASCA[2], V. SOUSA[1], T. L. STAN[1], P. SVENNINGSSON[1], B. S. MCEWEN[2];
[1]Clin. Neurosci., Karolinska Inst., Stockholm, Sweden; [2]Dept. of Neuroendocrinology, The Rockefeller Univ., New York, NY

Abstract: Depression, a growing problem that needs new therapeutic approaches, is often triggered by stressful experiences through a stress induced epigenetic reprogramming of gene expression. Current antidepressants require in general 2-3wks to significantly improve mood and approximately 40% of patients are considered treatment resistant. Recently, we showed that the intraperitoneal administration of acetyl-L-carnitine (LAC) induces rapid and long lasting antidepressant effects in two rodent models of depression through the epigenetic regulation of the metabotropic glutamate receptor, mGlu2, in brain regions critically involved in the pathophysiology of depression: the hippocampus and prefrontal cortex. Here we asked whether LAC, administered orally, will rescue the depressive-like phenotype of Flinders Sensitive Line (FSL) rats and whether in a context of stress Thus, FSL rats and their controls (FRL) were treated with vehicle or LAC at the low dose of 300mg/Kg dissolved in the drinking water for 7 days and subjected on day 6 to 15 min long forced swim test (FST) used as stressor and as behavioral test to assess antidepressant responses. FSL/FRL rats were also subjected to a second session of FST on day 7 to test whether the treatment worked after a stress manipulation. We found that, on the first session, Veh-FSL rats showed a higher immobility time vs Veh-FRL rats. Moreover, LAC restored the depressive-like FSL behavior in the FST. In agreement with previous findings LAC showed no effect on FRL rats, supporting the idea that LAC may supplement the lower endogenous levels of acetylcarnitine found in FSL rats. In the second FST, we again found an increased immobility time of Veh-FSL rats vs. Veh-FRL rats. Interestingly, LAC restored immobility time only in a subset of FSL rats, suggesting that the first swim session may induce a resistance in the brain responses to antidepressant treatment because of individual differences in stress responsiveness. A further molecular analysis is in progress to better understand the nature of the individual difference in brain response to potential next-generation treatments (LAC). Moreover, ChIPseq analysis will investigate differences in the chromatin structure which may account for the stress-induced resistance to LAC in a subset of animals. Comparing animals which differ in responsivity to antidepressant treatments may provide clues for identifying markers of risk and resilience to stress that can be used to promote early intervention and develop therapeutic next-generation treatments.

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Poster

079. Stress: Genes and Epigenetics

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Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: The Hope for Depression Research Foundation

The American Foundation for Suicide Prevention

Title: mGlu2 is a key mediator in the responses to next-generation antidepressant treatments: Epigenetic mechanisms of neuronal plasticity

Authors: *C. NASCA, D. ZELLI, B. S. MCEWEN;
Neuroendocrinology, The Rockefeller Univ., New York, NY

Abstract: Targeting the epigenetic control of the glutamatergic synapse has the potential for rapid treatment of stress-related actions on brain, including mood related disorders. Recently, we showed that the naturally-occurring compound Acetyl-L-carnitine (LAC) causes rapid antidepressant effects through the epigenetic induction of mGlu2 receptors in both a genetic rat model of susceptibility, FSL rats, and following chronic unpredictable stress exposure. Since there is no single animal model that is relevant to human psychiatric disorders in their multiple forms, the efficacy of a new treatment should be validated in multiple animal models. Here, we tested the efficacy of LAC in effects of chronic restraint stress (CRS) in C57bl mice, comparing LAC efficacy with the SSRI fluoxetine (FLUOX) and the acetyl-donor N-acetyl-cysteine (NAC) with the ultimate goal to determine whether acetyl-donors may correct the stress-reprogrammed transcriptional machinery and promote antidepressant-like responses. In particular, we found that exposure to chronic restraint stress alters the glutamatergic homeostasis in the hippocampus and in the prefrontal cortex, causing strong reductions in mGlu2 mRNA levels, which exert an inhibitory tone on glutamate release, and that CRS does this via a down-regulation in the histone H3K27ac, a mark of active transcription. The acetyl-donors LAC and NAC, orally administered for 3 days to CRS mice, restored mGlu2 mRNA levels in the hippocampus and prefrontal cortex, whereas 3 days of FLUOX did not block the stress effects. Moreover, the stress induced dysregulation of the glutamatergic homeostasis is associated with occurrence of a depressive-like behavior, which we show is rescued by 3 days of oral administration with LAC and NAC, whereas 14 days of FLUOX oral administration are required to restore the CRS-induced depressive behavior. A chromatin immunoprecipitation analysis (ChIP and ChIPseq) for the histone H3K27ac is in progress to test the hypothesis that next-generation epigenetic drugs exert fast antidepressant responses correcting the stress-altered chromatin remodeling. Understanding the epigenetic hypothesis of the rapid treatment of stress-related mood disorders may also facilitate development of a rapidly acting suicide deterrent.

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Poster

079. Stress: Genes and Epigenetics

Location: Halls A-C

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Program#/Poster#: 79.04/KK11

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: HDRF RGA: 13-004

Title: Young BDNFmet carrier mice show a positive effect to stress: Is such positive effect lost with aging?

Authors: *E. M. WATERS, D. A. ZELLI, S. MAZID, B. S. MCEWEN, C. NASCA;
Lab. of Neuroendocrinology, Rockefeller Univ., NEW YORK, NY

Abstract: The hippocampus, which is involved in stress responses and mood regulation as well as in memory, displays remarkable structural and functional plasticity over the life course. Our and other labs supported a key role of the glutamatergic activity in stress-related mood disorders. Recently, we showed that acute restraint stressed (ARS) BDNFVal/Val mice and naïve BDNFVal/Met mice show a selective mGlu2 reduction in hippocampus which correlates in a light-dark test with elevated anxiety-like behavior. This evidence led us to investigate whether the met allele may confer a greater or lower vulnerability to negative events, such as a single episode of ARS. Interestingly, we found that ARS in BDNFVal/Met mice results in a decreased anxiety-like behavior in the light-dark test. Moreover, the positive response to stress in BDNFVal/Met mice correlates with an increased mGlu2 mRNA levels in hippocampus and no change in the cognate presynaptic mGlu3 receptor, suggesting that the met allele, which is carried by the 33% of human population, makes individual more reactive to either a negative or, most likely, to a positive events. Since a wealth of studies suggest that age-reduced BDNF level correlate with decreased hippocampal neuronal plasticity and cognitive functions, ongoing studies are investigating whether the ARS-induced positive response is impaired with aging in BDNFmet carrier mice. These findings open up a window of opportunity for intervention whereby BNDF signaling on glutamatergic function may be a counter-regulatory advantage under negative life events.

Disclosures: **E.M. Waters:** None. **D.A. Zelli:** None. **S. Mazid:** None. **B.S. McEwen:** None. **C. Nasca:** None.

Poster

079. Stress: Genes and Epigenetics

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Program#/Poster#: 79.05/KK12

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: The Hope for Depression Research Foundation

The American Foundation for Suicide Prevention

Title: Stress elicits contrasting patterns of responses in the dorsal vs ventral hippocampus

Authors: *D. A. ZELLI[1], B. BIGIO[2], S. CHATTARJI[3], B. S. MCEWEN[1], C. NASCA[1]; [1]Neuroendocrinology, [2]Ctr. for Clin. and Translational Sci., The Rockefeller Univ., New York, NY; [3]Neurobio., Natl. Ctr. for Biol. Sci., Bangalore, India

Abstract: The hippocampal formation, which is a target of stress and stress hormones, is a functionally and structurally complex brain region that plays a key role in regulating mood as well as memory. Previously, our and other labs have shown that stress acts synergistically with excitatory amino acids (EAA) to induce complex changes in various brain regions at several points of regulatory control. In particular, we have discovered that the glutamatergic synapse is differentially responsive to acute and chronic stress since, shedding light on a more sensitive response of the hippocampus to an acute stress and its dynamic habituation induced by chronic stress in regard to the glutamatergic synapse, except for the presynaptic mGlu2 receptors. Here we asked whether the two subregions of the hippocampus, the dorsal and ventral parts, show a different vulnerability to chronic restraint stress (CRS). The dorsal hippocampus, which corresponds to the human posterior hippocampus, is principally involved in memory function, whereas the ventral hippocampus, which corresponds to the anterior hippocampus in humans, modulates emotional and affective processes. Thus, we used both the Zivic brain blocks 1.0 and 0.5 ml to dissect the two brain regions from coronal sections of mice subjected to 21 days of chronic stress and their controls. We found that exposure to chronic stress in mice caused substantial habituation within the dorsal hippocampus, underlined by reductions in the cystine/glutamate exchanger xCT and up-regulations in the mGlu2, mGlu3, NMDA receptors as well as in the glial glutamate transporter Glt-1. Interestingly, we found that the ventral portion appears to be selectively vulnerable to chronic stress, showing a deregulated glutamate homeostasis, as underlined by decreased mGlu2 and mGlu3 receptors and no changes in the NMDA receptor and xCT and Glt-1 transporters. A further RNA-seq analysis will investigate the

different patterns elicited by CRS in the two hippocampal subregions. In parallel, presynaptic glutamate release will be studied to investigate more in depth the opposite changes induced by CRS in the two hippocampal subregions. These results underscore the importance of better understanding how stress induces transcriptional changes in hippocampal subregions that are known to play different roles in brain functions and, by extension, structural and functional changes in higher level cognition brain regions.

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Poster

079. Stress: Genes and Epigenetics

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Program#/Poster#: 79.06/KK13

Topic: E.05. Stress and the Brain

Support: Defense Advanced Research Projects Agency (DARPA) and the U. S. Army Research Office under grant number W911NF101009

Title: MicroRNA profiles in medial prefrontal cortex and amygdala in rats resilient or vulnerable to chronic stress

Authors: ***R. CHEN**[1], S. BELTRAMI[1], G. KELLY[1], A. SENGUPTA[1], B. NICHOLAS[1], S. LUZ[1], W. HEYDENDAEL[1], S. BHATNAGAR[1,2];
[1]Children's Hosp. of Philadelphia, Philadelphia, PA; [2]Univ. of Pennsylvania Sch. of Med., Philadelphia, PA

Abstract: MicroRNAs are small endogenously expressed non-coding RNA molecules that are important post-transcriptional regulators of gene expression. Changes in expression of some microRNAs have been observed in neuropsychiatric and neurodegenerative disorders. However, little is known about changes in brain microRNAs as a result of stress, which is important in the etiology of many psychiatric disorders. In the present studies, we assessed profiles of all known mature microRNAs in two brain regions in rats that regulate behaviors relevant to psychiatric disease- the medial prefrontal cortex (mPFC) and basolateral amygdala (BLA). We exposed rats to chronic social defeat stress in a 7-day resident-intruder paradigm, in which we have previously shown the emergence of two subpopulations: a vulnerable subpopulation that exhibits short latencies (SL) to defeat associated with increased anxiety- and depressive-type behavior and

neuroendocrine dysfunction and a resilient subpopulation that resists defeat (long latencies, or LL) and that does not exhibit behavioral or neuroendocrine dysfunction. We analyzed microRNAs in the mPFC and in the BLA 24 hours after the end of the 7 days of stress. MicroRNAs were isolated and their content was analyzed on Affymetrix GeneChip microRNA 3.0 Arrays, which analyze expression of known pre- and mature microRNAs in the miRBase database (v17). In the mPFC, we observed elevated expression of miR-126 in SL vulnerable rats compared with LL resilient rats and elevated expression of miR-708 in SL rats compared with LL rats and controls. 30 total gene targets were implicated by the 2 differentially expressed microRNAs and current work is aimed at identifying pathways enriched with these gene targets. In the BLA, the expression of a total of 77 microRNAs was significantly different between SL rats and control rats, and a total of 42 microRNAs was significantly different between LL rats and control rats. All but one of the microRNAs (miR-27a) that were significantly different between LL and control rats were also significantly different between SL and control animals. For the remaining microRNAs that were significantly different between SL and controls, the general trend was one of similar effects in LL rats compared to controls as well. These results suggest 1) a potentially important role for microRNAs in the mPFC in mediating stress vulnerability but 2) that microRNAs in the BLA are not important in differentiating resilient from vulnerable phenotypes. Together, the results indicate that microRNAs are potential novel regulators of adaptations to stress.

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Poster

079. Stress: Genes and Epigenetics

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Topic: E.05. Stress and the Brain

Support: DARPA ARO grant W911NF1010093

Title: Circulating blood microRNAs are biomarkers for resilience or vulnerability to the effects of chronic social stress in rats

Authors: ***S. BELTRAMI**[1], R. CHEN[1], G. KELLY[1], A. SENGUPTA[1], W. HEYDENDAEL[1], B. NICHOLAS[2], S. LUZ[1], S. BHATNAGAR[1,3];

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Abstract: Small, noncoding RNAs, referred to as microRNAs (miR), regulate expression of a significant number of protein-encoding genes, generally repressing their expression. Circulating microRNAs have become well-validated biomarkers of immune and cardiovascular diseases. More recently miRNAs been identified as non-invasive biomarkers for several diseases of the central nervous system, including Multiple Sclerosis and Alzheimer's Disease. However, much less is known about circulating miRNAs and psychiatric illnesses. Symptoms of psychiatric illnesses such as anxiety and depression can be precipitated or exacerbated by stressful life events. Some individuals are more vulnerable to these effects of stress while others are more resilient. Identifying non-invasive biomarkers of resilience or vulnerability to the effects of stress would provide valuable information for the prevention and/or treatment of stress-related diseases. To investigate the utility of microRNAs as biomarkers of resilience or vulnerability to stress, adult male Sprague Dawley rats were subjected to chronic social defeat using the resident-intruder paradigm with exposure to an aggressive resident rat for 30min per day for 7 days. In previous work (Wood et al., 2010), we showed that rats vulnerable to chronic stress are rapidly defeated by the resident rat and exhibit increased anxiety- and depressive-type behaviors and neuroendocrine dysfunction. In contrast, rats that are resilient to chronic stress resist being defeated and do not exhibit behavioral or neuroendocrine dysfunctions. Circulating blood microRNAs were isolated from these rats and a group of control rats prior to the start of 7 days of defeat and at 24h after the last defeat and were analyzed on Affymetrix GeneChip microRNA 3.0 arrays, encompassing all known rat microRNAs. Prior to onset of social defeat, expression of miR-24-2-star, miR-27a, miR-30e, miR-362-star, miR-3590-3p, miR-532-5p was reduced in rats that later became vulnerable to stress compared to control rats. In contrast, after 7 days of defeat, resilient rats displayed reduced expression of miR-139-5p, miR-28-star, miR-326, and miR-99b compared to controls. These results provide proof-of-principle that assessment of circulating microRNAs may be useful in identifying individuals that are vulnerable to future stress or individuals that have become resilient to ongoing stress.

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Poster

079. Stress: Genes and Epigenetics

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Dielmann Genetic and Environmental Risk Endowment

Title: Identifying alterations in gene regulatory networks in Post-Traumatic Stress Disorder: Evidence from preclinical models

Authors: *K. SMITH[1], D. A. CRUZ[2], B. C. BINGHAM[3], D. A. MORILAK[3], D. E. WILLIAMSON[2];
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Abstract: Ongoing research by our group involves interrogating the genetic mechanisms underlying post-traumatic stress disorder (PTSD). Using a whole-genome microarray approach, we previously examined mRNA in the medial orbital frontal cortex (OFC) of human postmortem brain samples and observed 51 genes differentially expressed in PTSD subjects compared to controls. This study further examined these genes in a rat preclinical model of PTSD, the Chronic plus Acute Prolonged Stress (CAPS) model that has been shown to induce PTSD-like behavioral changes. In addition, we subjected rats to a session of fear extinction training following CAPS treatment to model prolonged exposure therapy, an effective form of cognitive behavioral therapy for PTSD in humans. Of the top 45 genes that showed differential expression in the human postmortem OFC, only mitochondrial ribosomal protein S9 (Mrps9) was differentially expressed in the OFC of CAPS-stressed rats compared to controls following treatment. Further, while human MRPS9 showed increased expression in the OFC of PTSD subjects, we found that the expression of this gene was reduced in the OFC of rats that had undergone fear extinction training following CAPS treatment. These preliminary results suggest that cognitive treatment indexed by fear extinction may reverse changes in the expression of gene networks observed in human PTSD. Future studies will investigate the effects of CAPS treatment alone (i.e., without fear extinction) on gene expression compared to unstressed controls. Additionally, work is currently underway to examine whole-genome changes in mRNA in the rat model of PTSD to identify other changes in gene expression immediately following exposure to the traumatic stress procedure. Understanding how changes in gene expression converge between human clinical PTSD and preclinical models of PTSD will ultimately aid in uncovering the root causes of this debilitating condition, and may suggest potentially novel therapeutic targets.

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Poster

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Title: Heteromerization of wild-type P2X7R with the mood disorder-associated Gln460Arg variant alters receptor function and conveys enhanced stress vulnerability

Authors: *J. M. DEUSSING[1], F. APRILE-GARCIA[3], M. W. METZGER[1], N. DEDIC[1], S. M. WALSER[1], V. JAKUBCAKOVA[2], D. CZAMARA[2], M. MITKOVSKI[4], D. REFOJO[1], B. MÜLLER-MYHSOK[2], M. KIMURA[2], W. WURST[5], W. STÜHMER[4], F. HOLSBOER[2], E. ARZT[3];
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Abstract: A single-nucleotide polymorphism (rs2230912) in the purinergic P2X7 receptor (P2X7R) gene leading to a glutamine (Gln) by arginine (Arg) substitution at codon 460 (Gln460Arg) has been associated with mood disorders in many but not all human genetic studies. Therefore, we conducted a meta-analysis taking into account all published studies that consider association between case-control status and the P2X7R-Gln460Arg polymorphism. We found a significant effect of rs2230912 on case-control status, which can be explained by a dominant or a heterozygote disadvantage model. This finding supports a contribution of the P2X7R Gln460Arg polymorphism to increased susceptibility for the development of mood disorders. In contrast to the many loss- and gain-of-function polymorphisms that have been identified, seemingly the mood disorder associated P2X7R-Gln460Arg variant is not compromised in its activity. However, we could demonstrate at molecular and cellular level that hetero-oligomerization between human wild-type P2X7R (hP2X7R-WT) and hP2X7R-Gln460Arg impairs normal receptor function, which depends on direct physical interaction of the two P2X7R isoforms. To

further investigate the functional significance *in vivo*, we generated mice expressing hP2X7R-WT and hP2X7R-Gln460Arg, respectively. Under basal conditions humanized mice did not show any alterations in a battery of test assessing endophenotypes related to mood disorders. But interestingly, mice heterozygous for both variants showed signs of a pre-symptomatic disease stage as reflected by a decreased startle response and alterations in sleep quality and architecture. Along these lines heterozygote hP2X7R mice revealed an increased vulnerability to develop mood disorder-related endophenotypes in response to chronic stress. These results suggest that heterozygosity of wild-type P2X7R with P2X7R-Gln460Arg alters receptor function and thereby impacts the stress vulnerability. Taken together, these humanized mouse lines provide functional evidence supporting P2RX7 as a susceptibility gene of mood disorders.

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Poster

079. Stress: Genes and Epigenetics

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Program#/Poster#: 79.10/KK17

Topic: E.05. Stress and the Brain

Support: NWO / VIDI

Title: The 5-HTT genotype dependent effects of stressor controllability on dorsal raphe nucleus activation

Authors: *P. SCHIPPER, R. REINTJES, D. LOPRESTO, J. HOMBERG;
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Abstract: The 5-HTTLPR short allele, which affects serotonin homeostasis by reducing expression and function of the serotonin transporter (5-HTT), is well known for modulating the effects of stress on the pathogenesis of psychiatric disorders. The severity of a stressor's impact is strongly influenced by an individual's controllability of that stressor, with uncontrollable stressors having a much more detrimental effect on subsequent emotional behavior; these effects are known to be mediated by a serotonergic mechanism. Here, we subjected 5-HTT deficient rats and wild-type controls to a signaled active stressor avoidance behavioral paradigm, as well as a

yoked control experiment wherein the same stressors were given but the animals had no control over the stressor. Subsequently, the animals were sacrificed and serotonergic neuronal activation in the dorsal raphe nucleus (DRN) was assessed, as well as neural activation in the prefrontal cortex. We found that 5-HTT deficient rats acquired the task quicker than wild-types, resulting in lower response latency for this genotype. Whereas several effects of stressor controllability on serotonergic activation are seen throughout different regions of the DRN in the wild-type animals, few effects are seen in the 5-HTT knockout animals. Furthermore, escape latency correlated with DRN serotonergic activation in wild-types, but not in 5-HTT knockout animals. These findings indicate that the mechanisms directing the effects of stressor controllability on subsequent behavior are 5-HTT genotype dependent.

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Poster

079. Stress: Genes and Epigenetics

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Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: NIH Grant MH091633

Virginia Commonwealth University

Title: Elevated anxiety in serotonin transporter knock in mice carrying I425V coding variant associated with obsessive compulsive disorder and tourette disorder

Authors: *S. RAMAMOORTHY[1], J. CROWELY[1], Z. DANEVA[1], P. MANNANGATTI[1], J. RAJAMANICKAM[1], D. L. MURPHY[2], L. D. JAYANTHI[1];
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Abstract: Obsessive-Compulsive Disorder (OCD) is a complex neuropsychiatric disorder with an estimated lifetime prevalence of 2-3% worldwide. Neuroimaging studies have indicated that OCD individuals have decreased serotonin transporter (SERT) expression in the midbrain and brainstem. Moreover, Serotonin Reuptake Inhibitors (SRIs) are currently the only clinically effective medications for the treatment of OCD. The neurobiological substrates and cellular mechanisms by with SRIs produce therapeutic efficacy in OCD remain unclear. A rare single

nucleotide polymorphism, isoleucine to valine at amino acid 425 (I425V) in SERT is implicated in the pathogenesis of OCD, Tourette Disorder, alcohol abuse/dependence, anorexia nervosa and pervasive developmental disorders. However, the physiological effects of V425-SERT in serotonergic signaling and whether V425-linked dysregulation of SERT imposes behaviors contributing to OCD and possibly to other psychiatric disorders are unknown. To investigate this in a physiological context, we replaced isoleucine to valine at position 425 of exon 9 in the SERT gene (Slc6a4) and generated V425-SERT knock-in mice. The presence of the V425 mutation in SERT is confirmed by sequencing. V425-SERT mice have a birth rate consistent with Mendelian inheritance ratios, indicating that the V425 mutation in SERT did not create a trans-dominant lethal phenotype. Both male and female homozygous mice are viable, displayed no major developmental abnormalities and exhibit normal growth and size. As reported in human subjects, V425-SERT mice have reduced SERT expression and function in the midbrain and hippocampus but not in the cortex. Homozygous V425-SERT mice display elevated anxiety levels in open-field, elevated-zero maze and light-dark box tests. These results indicate that introducing naturally occurring OCD-associated human SERT coding variant leads to perturbations in normal SERT functional expression and subsequently altering serotonergic regulation of anxiety-related behaviors. Given the value of SERT offering a pharmacological target in the treatment of OCD as well as I425V association with OCD and TD, V425-SERT knock-in model provides a novel tool to further our understanding of the role of SERT in neuropsychiatric and developmental disorders. The knowledge we gain from such studies may aid in developing potential pharmaco-therapeutic strategies for the treatment of these disorders.
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Poster

080. Adolescent Stress

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Topic: E.05. Stress and the Brain

Support: RO1 MH083324

P30 NS047243

Title: Adolescent and female specific roles of RasGRF1 in the stress response

Authors: G. UZTURK[1], *L. A. FEIG[2];
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Abstract: Females and adolescents are both known to be particularly sensitive to stress. Studies have shown that this may be due, at least in part, to enhanced sensitivities of their HPA axis to stress, yet the molecular mechanisms underlying these differences are poorly understood. RasGRF1 (GRF1) and RasGRF2 (GRF2) form a family of calcium-activated guanine nucleotide exchange factors (GEFs) that activates Ras and Rac GTPases and regulates multiple forms of synaptic plasticity in the CA1 of hippocampus. GRF1 contributes to LTP mediated by calcium-permeable AMPA receptors and LTD mediated by NMDA receptors. Because the hippocampus is known to modulate the HPA axis that regulates the stress response, we tested the involvement of GRF1 in this process by comparing the response of GRF1 knockout (GRF1(-/-)) mice and control mice after exposure to short-term and chronic restraint stress. We found that enhanced 'risk taking' behavior in the elevated plus maze and open-field tests, as well as elevated corticosterone (CORT) levels that normally occur after chronic restraint stress (30 min/day for 7 days) are blocked in GRF1(-/-) mice in an age and sex dependent manner. In particular, these responses to chronic stress are blocked in adolescent female GRF1(-/-) mice, but normal in both their adolescent male and adult female GRF1(-/-) counterparts. Strikingly, the opposite phenotype is found after acute (a single 30 min restraint) and short-term (30 min/day for 3 days) stress, such that adolescent female, but not adolescent male or adult female GRF1(-/-) mice display enhanced CORT compared to their control counterparts. These results show that GRF1 plays an adolescent and female specific roles in the regulation of HPA axis that differs after exposure to short-term and chronic restraint stress. These observations may yield new insights into the mechanisms underlying stress induced activation of HPA axis that occurs in an age and sex dependent manner.

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Poster

080. Adolescent Stress

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Topic: E.05. Stress and the Brain

Support: NIDA Grant 1T32DA031111-01

NIDA Grant DA027679

Title: Early life experience modulates the effects of unpredictable chronic mild stress during adolescence

Authors: *E. K. KIRSCHMANN[1,2,3], J. C. MAUNA[1,2], C. O'CONNOR[1], J. LU[1], E. C. DONNY[3,4], A. F. SVED[2,4], E. THIELS[1,2,3];
[1]Neurobio., Univ. of Pittsburgh, Pittsburgh, PA; [2]Ctr. for Neurosci., [3]Ctr. for the Neural Basis of Cognition, [4]Psychology, Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Adolescence is a period of many neural, physiological, and behavioral changes, and may be a period of vulnerability to psychiatric disorders. Unpredictable chronic mild stress (UCMS) is a widely-used model for inducing depressive- and anxiety-like behaviors in adult rodents. We examined the effect of UCMS during adolescence on behavior, hypothalamic-pituitary-adrenal (HPA) axis function, and the brain of adolescent rats, and also tested whether early life experience (e.g., shipping conditions) influences experimental outcome. We ran 2 studies in parallel: 1) male rats weaned and shipped from the supplier on postnatal day 21 (p21; w/s condition); and 2) male rats shipped with a foster dam on p18 and weaned in-house on p21 (d/s condition). Starting at age p28-31, rats were exposed to 5 weeks of UCMS (n = 10 w/s, 9 d/s) or control conditions (Con; n = 10 w/s, 9 d/s), and we assessed effects on body weight, sucrose preference, activity in a 2hr open-field test (OF), and performance on the elevated plus maze (EPM). Rats were sacrificed at p62-65 for collection of trunk blood and brains, to examine levels of stress hormones in plasma and stress-relevant proteins in select brain regions. Prior to sacrifice, some rats were treated with saline/dexamethasone and exposed to an acute 5min swim stress, to assess HPA axis function. In the w/s condition, UCMS attenuated growth and resulted in a phenotype characterized by hyperactivity and reduced anxiety; total weight gain was significantly lower, whereas activity and time in the center of the OF arena and exploration of the open arms of the EPM were significantly higher in UCMS- compared to Con rats. Sucrose preference was significantly reduced after 1 week of UCMS, but rebounded by week 2. Plasma corticosterone levels were significantly blunted at baseline and after acute stress, and glucocorticoid receptor (GR) levels in the paraventricular nucleus were lower in UCMS- compared to Con rats. GR levels in the basolateral amygdala tended to be higher in UCMS rats. Results were very different for rats in the d/s condition. For all measures except OF locomotor activity, no differences emerged between UCMS and Con. This was not because the UCMS effect was blunted, but because the results of d/s Con and UCMS rats resembled those of w/s UCMS rats. The w/s controls, but not the d/s controls, had a phenotype similar to controls born in-house. Taken together, our findings suggest that: 1) UCMS during adolescence causes a hyperactive/risk-seeking phenotype, which is accompanied by decreased HPA axis function and changes in brain stress systems; and 2) shipping with a foster dam alters control phenotype, whereas rats weaned and shipped resemble in-house rats.

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Poster

080. Adolescent Stress

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Topic: E.05. Stress and the Brain

Support: USAMRMC award (10071009)

German Israeli Project Cooperation (DIP) RI 1922/1-1 HE 1128/16-1

Title: Pre-pubertal ('juvenile') stress-induced susceptibility to PTSD, which is associated with selective alterations in GABAAR alpha1 subunit in the dentate and amygdala, is rescued by juvenile but not adulthood exposure to 'Enriched Environment'

Authors: *G. RICHTER-LEVIN[1], Z. ARDI[2], A. ALBRECHT[3], A. RICHTER-LEVIN[3]; [1]Univ. Haifa, Haifa, Israel; [2]Sagol Dept. of Neurobio., [3]Univ. of Haifa, Haifa, Israel

Abstract: Despite the intuitive association of Post-Traumatic Stress Disorder (PTSD) with an exposure to a trauma, most individuals are able to cope with it, and only a minority will exhibit prolonged maladaptive responses to the traumatic experience. Thus, the exposure to the trauma may be a necessary, but not a sufficient condition to induce PTSD. There must be additional factors that determine the outcome of the exposure to the trauma. Understanding the neurobiology of those risk factors holds great importance for understanding PTSD. We have developed an animal model of PTSD, which takes into consideration the exposure of rats to pre-pubertal (juvenile) stress, as a risk factor, and measuring behavioral and neurbiological outcomes one month after the adulthood trauma, to ensure the relevance of our findings to PTSD. We have demonstrated that exposure to juvenile stress is a significant risk factor for the development of PTSD (Horovitz et al, 2012; 2014). 'Enriched Environment' (EE) has been suggested to have the ability to rescue abnormal anxiety behaviors induced by stress in adulthood. In the current study we examined the ability of one month exposure to EE from juvenility to adulthood to prevent the effects of pre-exposure to 'Juvenile stress', and compared that to the ability of one month exposure to EE from after the exposure to the adulthood trauma to rescue PTSD-like symptoms following the exposure to the combination of 'Juvenile + adulthood stress'. Furthermore, we have analyzed hippocampal and amygdala tissue for correlated alterations in expression of proteins

associated with gultamatergic and GABAergic neurotransmission. Results indicate that exposure to EE from juvenility to adulthood could prevent the effects of pre-exposure to 'Juvenile stress'. In contrast, exposure to EE from after the exposure to the adulthood trauma failed to rescue PTSD-like symptoms following the exposure to the combination of 'Juvenile + adulthood stress'. In addition, we identified a selective increase in expression of GABAAR alpha1 subunit only in the basolateral amygdala and the ventral dentate gyrus, which was correlated with the PTSD symptoms. This increased expression of GABAAR alpha1 subunit was also prevented by EE from juvenility to adulthood. The results demonstrate the effectiveness of the 'Juvenile stress' model of PTSD as a platform for identifying target genes associated with PTSD psychopathology, but also with environmental risk factors for developing PTSD. In addition, the results point to selective alterations in GABAR subunit that may be part of the neurobiology of PTSD.

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Poster

080. Adolescent Stress

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Program#/Poster#: 80.04/KK22

Topic: E.05. Stress and the Brain

Title: Repeated exposure to threat in adolescence increases defensive behaviors, but decreases avoidance in highly arousing test conditions

Authors: *M. L. JACOBSON, J. LIEBMAN, N. ODYNOCKI, D. AWALT, Jr., P. PEDULLA, B. J. ANDERSON;
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Abstract: Stressors experienced in daily life may elicit responses that are adaptive in matched environments and maladaptive in mismatched environments. To help understand adaptations in different contexts, our lab has designed a rodent living environment that allows manipulation of threat prediction and control. When presenting repeated unpredictable threats (UT) without harm, rodents develop heightened responses to aversive stimuli, including potentiated defensive burying and sensitization of acoustic startle. These effects represent hyper-reactivity to threat and hypervigilance, two features of anxiety. Both effects are in response to stimuli that increase arousal, but there were no effects on passive avoidance in the elevated plus maze (EPM), a test

sensitive to anxiolytics and typically tested in neutral test conditions. To understand whether group differences emerge only in high arousal conditions or to focused threat, we retested avoidance of open arms in the EPM in highly arousing conditions. Rats were housed with resources (food and water) separated by a tunnel for three weeks. Simultaneous presentations of predator odor, abrupt lights and sound were presented randomly (p=.25) in the center of the tunnel during crossings in the UT condition, whereas control (CT) rats crossed without risk. After the condition, animals were tested in the EPM with wind and variable room noise. UT rats explored the open arms of the maze more than CT during the first five minutes of maze exposure, and the effect was not explained by differences in locomotion. Regardless of testing conditions, ambiguous or aversive, repeated threats failed to increase passive avoidance. In contrast, when tested in the Barnes maze, previous threat exposure impaired memory in tests of low arousal test conditions, but improved memory in highly arousing test conditions. The increase in entries into open arms of the EPM in aversive test conditions suggest that improved memory in identical aversive test conditions is not accounted for by motivation to avoid open spaces. In summary, exposure to repeated threats increases active defensive behaviors without increasing passive avoidance; therefore repeated threats produce behaviors that favor active over passive coping forms of coping, and match the positive symptoms of anxiety, but not negative symptoms. Future studies will investigate other symptoms of anxiety, including impaired safety learning.

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Poster

080. Adolescent Stress

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Topic: E.05. Stress and the Brain

Support: NSF IOS 1257679 (MJW)

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Title: Changes to tyrosine hydroxylase activity in the adult medial prefrontal cortex following adolescent social defeat

Authors: *M. A. WEBER[1], J. L. SCHOLL[1], G. L. FORSTER[1], K. J. RENNER[2], M. J. WATT[3]; [1]Basic Biomedial Sciences, Univ. of South Dako, Vermillion, SD; [2]Biol., Univ. of South Dakota, Vermillion, SD; [3]Basic Biomed. Sciences, Univ. of South Dakota, Vermillion, SD

Abstract: Negative social experience in adolescence is associated with increased incidence of psychiatric disorders manifesting both acutely and later in life, which are characterized by deficits in cognitive processing. Such deficits may result from stress-induced disruption of the developing adolescent prefrontal cortex (PFC) dopamine (DA) system. We have shown that male rats exposed to repeated social defeat during adolescence exhibit decreased medial PFC (mPFC) DA activity in adulthood, along with associated behavioral changes such as impaired working memory. Here, we investigated whether mPFC DA hypofunction could result from reductions in activity of tyrosine hydroxylase (TH), the rate-limited enzyme in DA synthesis. Male rats were defeated daily for 5 days from postnatal days (P) 35-39 using a modified resident-intruder paradigm. Controls did not experience social defeat, but were exposed to a novel empty cage for the duration of each defeat trial. After maturing undisturbed to early adulthood (P56), rats received injections of either vehicle or the aromatic amino acid decarboxylase (AADC) inhibitor NSD-1015 (100 mg/kg, ip.), with accumulation of the DA precursor DOPA in the mPFC serving as a measure of *in vivo* TH activity. Subjects were decapitated 30 min after injection, and tissue content of mPFC DOPA quantified using HPLC with electrochemical detection. Inhibition of AADC resulted in greater DOPA accumulation compared to vehicle treatment. However, previously defeated rats showed significantly higher DOPA content 30 min following AADC inhibition than controls. Current studies are examining additional time points following AADC inhibition to determine if the time course of DOPA accumulation differs between defeated and control rats. Western immunoblots are also being used to measure expression of both total and phosphorylated TH as a complimentary analysis of changes to mPFC TH activity following adolescent defeat. Findings to date suggest that experience of social defeat in adolescence has long-lasting effects on mPFC TH activity, implying alterations to DA synthesis.

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Poster

080. Adolescent Stress

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Title: Adolescent experience of food restriction results in delayed enhancement of spatial learning in female rats

Authors: *T. G. CHOWDHURY[1], A. A. FENTON[2], C. AOKI[2];
[1]NYU Ctr. For Neural Sci., New York, NY; [2]Ctr. for Neural Sci., New York Univ., New York, NY

Abstract: Adolescence is a period of vulnerability to onset of psychiatric illnesses such as depression, schizophrenia, addictions, and eating disorders. We have shown that adolescence is a critical period for experience-dependent plasticity in the ventral hippocampus, a region important for regulation of anxiety. In the ventral hippocampus of female rats, we observed a transient increase in the number of apical dendritic branches and a steadily increased proportion of mature spines on CA1 pyramidal cells during the period between puberty and adulthood. These increases were followed by pruning of branches by adulthood with no change in the proportion of mature spines. These anatomical changes occurred precociously in animals that experienced activity-based anorexia (ABA), a rodent model of anorexia nervosa that combines the experience of food restriction and voluntary wheel-running exercise (Chowdhury et al., 2014). To study the effects of exercise and food-restriction separately, and jointly as ABA, on hippocampus-dependent behaviors, we tested spatial learning, anxiety, and cognitive flexibility. Four experimental groups of females were evaluated: CON (control rats with no running wheel experience or food restriction), ABA (housed with a running wheel from P36-44 and food restricted from P40-44), EX (housed with a running wheel from P36-44), and FR (food restricted from P40-44). Behavioral outcomes were measured at two ages: P47 and P53. We trained rats across two days in an active place avoidance task that requires persistent hippocampal synaptic plasticity (Pastalkova et al, Science, 2006). Day 1 included an open field test on the rotating circular arena in dim light and without shock, followed by three 10-min training sessions to actively avoid the zone of a 500 ms, 0.3 μA shock that stays stationary relative to room-based spatial cues. The day 2 retention test was followed by three conflict sessions with the shock zone in the opposite location to test cognitive flexibility. At P47, we found that EX and FR groups

entered the shock zone during the 2nd training session less than CON and ABA groups. EX and FR groups also entered less during the 1st conflict trial than CON and ABA groups. At P53, animals that experienced food restriction from P40-44 (ABA and FR groups) learned to avoid more rapidly than CON and EX groups. In the first two training sessions, ABA and FR groups had half as many entries as CON and EX groups. These results suggest that the experience of exercise during adolescence transiently improves spatial learning while food restriction improves learning through young adulthood. ABA has a delayed effect of improved spatial learning in young adulthood.

Disclosures: **T.G. Chowdhury:** None. **A.A. Fenton:** None. **C. Aoki:** None.

Poster

080. Adolescent Stress

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 80.07/KK25

Topic: E.05. Stress and the Brain

Support: DA07606

Title: Acute stress to adolescent rats reduces p-CREB in adulthood

Authors: *V. M. CHIU, B. K. YAMAMOTO;
Dept. of Neurosciences, Univ. of Toledo, Toledo, OH

Abstract: Early-life stress may cause long-term effects that are evident in adulthood. Previous studies have shown that repeated stress during adolescence decreases glutamate receptor expression in the prefrontal cortex. However, it is unknown whether acute traumatic stress during adolescence could alter transcription factors that may be associated with long term effects in adulthood. The current study exposed rats to acute traumatic stress during adolescence and examined the transcription factory, CREB in adulthood. An acute, single prolonged stress (SPS) paradigm was used that included restraint for 2 hours followed by forced swim for 20 min and anesthetization with isoflurane 15 min later. This paradigm has been used to model a stressful traumatic experience. Rats were exposed to SPS or no stress as a control at PND 28 and left undisturbed until adulthood and killed on PND 60. Preliminary results show that prior exposure to SPS, decreased phosphorylated cAMP response element-binding protein (p-CREB) by 20% ($p < 0.029$) and histone deacetylase 2 (HDAC2) by 40% ($p < 0.003$) in the frontal cortex compared to the no stress group when examined at 60 days of age. However, the above changes were not

observed in the dorsal hippocampus. These results suggest that the effects of early childhood stress persist for at least a month in a brain region dependent manner that may involve epigenetic mechanisms. Further experiments are needed to further examine the mechanisms underlying the protracted effects of adolescent traumatic stress that exhibit in adulthood.

Disclosures: **V.M. Chiu:** None. **B.K. Yamamoto:** None.

Poster

080. Adolescent Stress

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 80.08/KK26

Topic: E.05. Stress and the Brain

Support: NSERC Grant 288348

Title: Social context differentially modulates neural Zif268 expression, behaviour, and endocrine function in response to acute and repeated stress in adolescent and adult male rats

Authors: *T. E. HODGES, C. M. MCCORMICK;
Psychology, Brock Univ., St. Catharines, ON, Canada

Abstract: Few studies have directly compared adolescent and adult hypothalamic-pituitary-adrenal (HPA) function in response to chronic stressors. In this experiment we manipulated stress history and social context: adolescent and adult rats underwent either acute (postnatal day 45; P45 or P85) or repeated (16 consecutive days from P30-45 or P70-85) one hr isolation (confined in a small ventilated container) after which they returned to either their original (familiar) or a new (unfamiliar) cage partner. Behaviour of rats upon return to the colony after isolation was monitored for 40 minutes. Blood samples and brains were collected either before the acute (baseline) or last repeated isolation, after isolation, or one hour after isolation after return to a familiar or unfamiliar cage partner for measurement of plasma corticosterone and testosterone using Elisa kits and for visualization of Zif268 expression by immunohistochemistry. Isolation stress increased in adolescent rats, and decreased in adult rats, affiliative behaviours irrespective of partner familiarity ($p<0.02$). In both adolescents and adults, there was no effect of stress history or partner familiarity on baseline corticosterone concentrations, and after 1 hr isolation, those that were undergoing their 16th isolation had lower corticosterone concentrations than those undergoing their first isolation (ps=0.01). After one hour of recovery, those with a familiar partner had lower corticosterone than did those with an

unfamiliar cage partner, irrespective of stress history. The only age difference in corticosterone was that adolescents had significantly higher corticosterone after a 16th return to an unfamiliar partner compared to a 1st return ($p=0.03$), whereas number of new partner pairings was not a factor for adults ($p>0.50$). Testosterone concentrations indicated that adult rat gonadal function was more responsive to isolation and social context than was adolescent gonadal function: adult testosterone increased after 1 hr isolation compared with baseline regardless of stress history and partner familiarity ($p=0.04$), and adult rats repeatedly paired with a familiar partner after isolation had lower testosterone concentrations compared with adult rats repeatedly paired with unfamiliar peers ($p=0.003$) and adult controls isolated for the first time ($p=0.002$). Further, adolescent and adult rats differed in Zif268 expression in a number of brain regions depending on stress history and partner familiarity. Differential responses to stressors between adolescents and adults may be based in age differences in sensitivity to social context rather than immaturity of the HPA axis.

Disclosures: **T.E. Hodges:** None. **C.M. McCormick:** None.

Poster

080. Adolescent Stress

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 80.09/KK27

Topic: E.05. Stress and the Brain

Support: CIHR

NSERC

CCIC

Title: Adolescent CB1 receptor antagonism alters adult stress responsivity and emotional behaviour in male rats

Authors: *T. T.-Y. LEE[1], M. N. HILL[2], B. B. GORZALKA[1];
[1]PSYCHOLOGY, Univ. of British Columbia, VANCOUVER, BC, Canada; [2]Anat. and Cell Biol., Hotchkiss Brain Institute, Univ. of Calgary, Calgary, AB, Canada

Abstract: Previous work from our laboratory has reported dynamic and temporal-specific changes in anandamide (AEA) content and fatty acid amide hydrolase (FAAH) activity within corticolimbic structures throughout the peri-adolescent period. Moreover, CB1 receptor

expression has been reported to peak during early to pre-adolescence, which then decreases to adult levels. Together, these findings suggest that normative adolescent endocannabinoid signaling within the corticolimbic stress circuit may be vulnerable to perturbations such as stress or exogenous cannabinoids. Therefore, we sought to examine the role of adolescent CB1 receptor activation in the development of HPA axis stress responsivity and emotionality behaviour in male rats. Between post-natal days (PND) 35-45, animals were administered daily IP injections of CB1 receptor antagonist, AM-251 (5 mg/kg), or vehicle. Upon reaching adulthood (PND 75), endocannabinoid content, HPA axis stress reactivity and emotional behaviour were assessed. AM-251 treated males exhibited greater anti-depressive-like behaviour in the forced swim test and greater risk assessment behaviour in the elevated plus maze, with no significant differences in general motor activity. Preliminary results suggest that adolescent AM-251 treatment had no significant effect on HPA axis stress reactivity to restraint. The relatively modest long term behavioural effects of adolescent CB1 receptor antagonism also resulted in moderate changes to the adult endocannabinoid system, with no significant changes to 2-AG content, and a small but significant increase and decrease in the amygdala and hypothalamus, respectively.

Disclosures: **T.T. Lee:** None. **M.N. Hill:** None. **B.B. Gorzalka:** None.

Poster

080. Adolescent Stress

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 80.10/KK28

Topic: E.05. Stress and the Brain

Support: NIH Grant MH099910

NIH Grant MH091258

NIH Grant MH087597

Title: Programming grit: Prepubertal stress combined with social support promotes resilience even in the face of aging

Authors: K. E. MORRISON[1], *C. N. EPPERSON[2], T. L. BALE[1];
[1]Univ. of Pennsylvania, Philadelphia, PA; [2]Psychiatry, Perelman Sch. of Med., Philadelphia, PA

Abstract: The prepubertal period represents a particularly sensitive window for adversity to precipitate adult affective disorders in women. In contrast, a supportive social environment following exposure to early life adversity is a strong predictor of long-term resiliency, or grit. A mild amount of stress during early life has been associated with improved coping skills in adulthood, suggesting that grit is a valuable characteristic aligned with future success. Affective disorders and cognitive deficits commonly emerge during aging, with many women reporting increased difficulty with emotional regulation as well as prefrontal cortex (PFC)-dependent executive functions. Risk factors for late-onset cognitive and affective disorders in women include prepubertal adversity, a time that coincides with PFC maturation. We have developed a novel mouse model to examine the interaction between prepubertal experience and age-related changes in cognition and stress regulation. Female mice were exposed to prepubertal chronic variable stress (CVS) from postnatal day 21-34 and either individually housed, to model stress susceptibility (CVS-S), or housed with social interaction, to model resiliency (CVS-R). One year following this stress, mice were examined in tasks to access their cognition and their HPA stress axis reactivity. We found that the aged females displayed significantly lower circulating estradiol than young controls, irrespective of prepubertal stress experience, indicating onset of reproductive senescence. To examine cognition, females were tested for spatial memory acquisition and reversal learning on a modified Barnes maze. Aged female controls displayed a deficit in reversal learning compared to young controls. Interestingly, aged CVS-R females displayed improved performance in reversal learning, suggesting that prepubertal stress with social support promotes resilience. As reversal learning is a PFC-dependent task, whole RNA was isolated from the PFC and gene expression was obtained via Affymetrix GeneChip microarray analysis. Statistical comparison revealed a subset of genes with expression patterns that reflect the findings in reversal learning, such that CVS-R females have differing expression compared to both control and CVS-S females. Prepubertal CVS did not alter stress responsivity in aged females, as assessed by both HPA stress axis responsivity and the acoustic startle response. These data suggest that prepubertal stress in the context of social support provides females with resilience to age-related cognitive decline and provides a novel mouse model for the examination of the mechanisms underlying this interaction.

Disclosures: **K.E. Morrison:** None. **C.N. Epperson:** None. **T.L. Bale:** None.

Poster

080. Adolescent Stress

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 80.11/KK29

Topic: E.05. Stress and the Brain

Support: NIH Grant R21MH091321-01

Title: Developmental high-fructose diet consumption increases depressive-like and anxiety-like behavior and remodels the hypothalamic transcriptome

Authors: ***C. S. HARRELL**, J. BURGADO, S. D. KELLY, Z. P. JOHNSON, G. N. NEIGH; Emory Univ., Atlanta, GA

Abstract: Fructose consumption, which promotes insulin resistance, hypertension, and dyslipidemia, has increased by over 25% since the 1970s. In addition to metabolic dysregulation, fructose ingestion stimulates the hypothalamic-pituitary-adrenal axis resulting in elevated glucocorticoid production. Adolescents are the greatest consumers of fructose, and adolescence is a critical period for maturation of the hypothalamic-pituitary-adrenal axis. Repeated consumption of high levels of fructose during adolescence has the potential to promote long-term dysregulation of the stress response. Therefore, we determined the extent to which consumption of a diet high in fructose affected behavior, serum corticosterone, and hypothalamic gene expression using a whole-transcriptomics approach. In addition, we examined the potential of a high-fructose diet to interact with exposure to chronic adolescent stress in male Wistar rats. Rats fed the peri-adolescent high-fructose diet showed increased anxiety-like behavior in the elevated plus maze and depressive-like behavior in the forced swim test in adulthood, irrespective of stress history. They also showed elevated basal corticosterone but a blunted response to forced swim. These behavioral and hormonal responses to the high-fructose diet did not occur in rats fed fructose during adulthood only. Finally, rats fed the high-fructose diet throughout development underwent marked hypothalamic transcript expression remodeling, with 966 genes (5.6%) significantly altered and a pronounced enrichment of altered transcripts in the POMC pathway. Collectively, the data presented herein indicate that diet, specifically one high in fructose, has the potential to alter behavior, HPA axis function, and the hypothalamic transcriptome.

Disclosures: **C.S. Harrell:** None. **J. Burgado:** None. **S.D. Kelly:** None. **Z.P. Johnson:** None. **G.N. Neigh:** None.

Poster

080. Adolescent Stress

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 80.12/KK30

Topic: E.05. Stress and the Brain

Support: NIH Grant MH099910

NIH Grant MH087597

NIH Grant MH091258

Title: Prepubertal adversity in females programs a blunted stress reactive maternal phenotype during pregnancy

Authors: *K. E. MORRISON, T. L. BALE;
Univ. of Pennsylvania, Philadelphia, PA

Abstract: The prepubertal period of development represents a particularly sensitive window for adversity to precipitate later adult affective disorders in women. However, a supportive social environment is a strong predictor of long-term resiliency, and appears to ameliorate the detrimental effects of the stress. Periods of great hormonal flux across the female lifespan are associated with the precipitation of affective disturbances, including puberty onset, pregnancy, and menopause. This suggests that prepubertal adversity may program central pathways involved with regulation of or interaction with hormonal state to determine affective symptomology. We have developed a novel mouse model to examine the factors that determine risk and resilience to prepubertal adversity, and the mechanisms involved in stress pathway dysregulation that intersect with hormonal state. Female mice were exposed to chronic stress from postnatal days 21-34 while being either individually housed, to model stress susceptibility, or housed with social interaction, to model resiliency. We examined endpoints in adults related to stress pathway regulation. Female stress dysregulation, a key feature of affective disorders, was assessed both by HPA responsivity to an acute restraint stress and the acoustic startle response, prior to pregnancy, during early (E7.5) or late (E17.5) pregnancy. We found that prepubertal stress significantly blunted the acoustic startle response, irrespective of pregnancy state. As the acoustic startle response is an indicator of PFC function, these data show that adversity can alter PFC programming that is ongoing during the prepubertal window, and that this programming is independent of hormonal status. HPA dysregulation, via a blunted corticosterone response in prepubertal stress groups was evident only during pregnancy. This suggests that HPA programming intersects with the hormonal changes that occur during pregnancy. To better understand the mechanisms by which prepubertal stress interacts with pregnancy to produce stress dysregulation, regions relevant to the stress response, such as the prefrontal cortex, hypothalamus, and amygdala, were examined. Together, these studies confirm prepubertal stress as a risk factor for endophenotypes of affective disorders, particularly during later periods of hormonal flux across the lifespan and provide a novel mouse model for the examination of the mechanisms underlying this interaction.

Disclosures: **K.E. Morrison:** None. **T.L. Bale:** None.

Poster

080. Adolescent Stress

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 80.13/KK31

Topic: E.05. Stress and the Brain

Support: NSERC

Title: Social instability stress in adolescence alters social behaviour in a food competition task

Authors: M. J. CUMMING[1], M. THOMPSON[1], *C. M. MCCORMICK[2];
[1]Neurosci., [2]Brock Univ., Saint Catharines, ON, Canada

Abstract: Social instability stress in adolescence (SS; daily 1 h isolation + new cage partners postnatal days 30 - 45; thereafter with original cage partner, also in the SS condition) and control (CTL) rats competed for access to a preferred food (sweetened condensed milk) in 5 test sessions. As adults, all rats were habituated individually to the test apparatus in three 10 min sessions (adapted from Matalynska et al., 2007). In the first test session, SS pairs displayed more aggressive behaviour (frontal attacks, $p = 0.02$; rear attacks, $p = 0.03$), and were less likely to relinquish access to the food voluntarily ($p = 0.03$) and tended to spend more time at the feeder than CTL pairs ($p = 0.06$). Pairs were considered to have formed dominant-submissive relationships (DSR) if one rat spent significantly more time at the feeder than the other across the five sessions. A similar number of SS and CTL pairs displayed significant DSRs (8 of 12 pairs each). Analyses were conducted to investigate change in behaviour from the 1st to 5th session in SS and CTL rats based on DSR status (DSR pair, no-DSR pair). Aggressive behaviour increased from the 1st to 5th session ($p < 0.001$), was greater in no-DSR than DSR pairs ($p = 0.04$; consistent with the proposed adaptive value of forming DSRs), and was higher in SS than CTL pairs ($p = 0.057$). Time at feeder increased ($p < 0.001$) from the 1st to 5th session, but the three-way interaction was significant ($p = 0.009$) for number of bouts at the feeder: SS pairs had more bouts than CTL pairs on the first day ($p = 0.02$), and in the 5th session, SS pairs had fewer bouts than CTL pairs ($p = 0.04$) among the no-DSR pairs, and did not differ for the DSR pairs ($p = 0.53$). The percentage of voluntary retreats from the feeder decreased from the 1st to 5th session, and SS pairs tended to have a lower percentage of voluntary retreats ($p = 0.08$) than did CTL pairs. These results add to our previous findings that social instability stress in adolescence modifies the adult social repertoire of rats and highlight the importance of adolescent social experiences in adult behaviour.

Disclosures: **M.J. Cumming:** None. **M. Thompson:** None. **C.M. McCormick:** None.

Poster

080. Adolescent Stress

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 80.14/KK32

Topic: E.05. Stress and the Brain

Support: NIH K99MH-094408 (MN)

NIH (AS)

Title: A critical period of vulnerability to adolescent stress: epigenetic mediators in mesocortical dopaminergic neurons

Authors: ***M. NIWA**, R. S. LEE, S.-I. KANO, A. SAWA;
Psychiaty and Behavioral Sci., Johns Hopkins Univ. Sch. of Med., Baltimore, MD

Abstract: Adolescence is a sensitive developmental period when environmental stressors can affect dynamic brain maturation, which in turn determines adult behaviors. The molecular basis of vulnerability to stress during the adolescent period is largely unknown. We recently reported that adolescent stress on a genetically vulnerable mouse model led to neurochemical abnormalities and behavioral deficits in adulthood via glucocorticoids (Niwa et al, Science 2013). In the present study, we identify potential molecular mediators that may play a role in stress-induced behavioral deficits. We report that three-week (five to eight weeks of age) adolescent stress in combination with *Disc1* genetic risk elicits alterations in DNA methylation of a specific set of genes, *Th*, *Bdnf*, and *Fkbp5*. The epigenetic changes in the mesocortical dopaminergic neurons are prevented when mice are treated with a glucocorticoid receptor (GR) antagonist RU38486 during the isolation period, which implicates the role for glucocorticoid signaling in this pathological event. We define the critical period of GR intervention as the first one-week period during the stress regimen, suggesting that this particular week in adolescence may be a specific period of maturation and function of mesocortical dopaminergic neurons and their sensitivity to glucocorticoids. Our study may provide insight into how and when individuals with genetic risk(s) may require early detection and prophylactic intervention to protect against risks of adolescent stressors.

Disclosures: **M. Niwa:** None. **R.S. Lee:** None. **S. Kano:** None. **A. Sawa:** None.

Poster

080. Adolescent Stress

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 80.15/LL1

Topic: E.05. Stress and the Brain

Support: The Klarman Foundation Grant Program in Eating Disorders Research to CA

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UL1 TR000038 from the National Center for the Advancement of Translational Science (NCATS) to TGC

Title: Voluntary wheel running exercise by female adolescent rats reduces GABAergic synaptic coverage of the CA1 pyramidal cell bodies and dendrites but the same exercise maintains GABAergic synaptic coverage and increases alpha4-GABARs at spines, if also exposed to and is resilient to food restriction-stress while evoking no change to alpha1-GABARs

Authors: *C. J. AOKI, K. TATEYAMA, I. YU, J.-Y. WANG, M. HSU, G. S. WABLE, T. G. CHOWDHURY;
Ctr. Neural Sci., New York Univ., NEW YORK, NY

Abstract: Physical exercise (EX) has many health benefits, including anxiolysis and the improvement of cognition and mood. These benefits are likely to be linked to cellular changes in the hippocampus, including angiogenesis, neurogenesis and the release of neurotrophins, but are also influenced by stressors that induce neurogenesis-dependent anxiety. For humans, dieting is one stressor that is often combined with EX. Another human condition in which stress frequently co-exists with EX is anorexia nervosa. We've shown that when food restriction (FR) is imposed upon rodents given access to a running wheel, many, but not all exhibit great increases in voluntary EX, causing them to lose more weight than by FR alone. Post mortem analysis

indicated that moderate exercisers (MEX) increase α4-containing GABA receptors (α4-GABARs) at dendritic spines of the hippocampus, while those that express life-threateningly high, i.e., excessive levels of EX (EEX) fail to up-regulate α4-GABARs (Aoki et al., 2014) and exhibit elevated anxiety (Wable et al, ms in prep). Here, we sought to determine (1) whether EX alone also induces α4-GABAR expression; (2) whether α1-GABAR expression is altered among the EEX; and (3) whether GABAergic innervation is altered by EX alone and differently, depending on whether the animal has become an EEX due to FR. Electron microscopy with a non-diffusible marker was used to probe for the presence and level of α4-GABARs and α1-GABARs at the plasma membrane, synapses and extra-synaptic sites and of the extent of GABAergic synapses formed on somatic and dendritic plasma membranes of the hippocampal CA1 of female adolescent rats. We compared the values among animals with EX only and also of those induced to become spontaneously EEX or MEX following 8 days of access to a running wheel plus 4 days of FR. We show that (1) the extent of voluntary running correlates negatively with α4-GABAR expression at excitatory axo-spinous synapses (R=-.65; p=.02) with or without FR, suggesting suppression of excitatory inputs within the MEX hippocampi that may underlie anxiolysis; (2) α4-GABARs at plasma membranes outside of axo-spinous synapses increase >100% among the MEX but not of those of EEX or EX alone (i.e., without FR); (3) α1-GABAR expression is unaltered by FR+EX; (4) GABAergic innervation is reduced ~25% by EX alone, through reduction of axon numbers at somata and shafts; (5) GABAergic innervation is also reduced by FR+EX, but only among those that become EEX. These findings indicate that EX exerts differential effects, depending on whether it is combined with FR and depending on individuals' response of becoming EEX and anxious or MEX and not anxious.

Disclosures: **C.J. Aoki:** None. **K. Tateyama:** None. **I. Yu:** None. **J. Wang:** None. **M. Hsu:** None. **G.S. Wable:** None. **T.G. Chowdhury:** None.

Poster

080. Adolescent Stress

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 80.16/LL2

Topic: E.05. Stress and the Brain

Support: PRIN GRANT 20107MSMA4

Title: Three weeks of maternal separation induced a long lasting changes in the plasticity of hippocampal neurons of offspring and mothers

Authors: *G. BIGGIO[1], P. P. SECCI[1], M. V. MELIS[1], M. C. MOSTALLINO[2];
[1]Dept. Life and Envrn. Sci., Univ. of Cagliari, Monserrato, Italy; [2]Inst. of Neurosci., Natl. research Council, Cagliari, Italy

Abstract: Neural plasticity is the capability of neurons to changes the structure, function and organization of neurons in response to new experiences. It specifically refers to strengthening or weakening nerve connections or adding new nerve cells based on environmental stimuli. These processes are responsible for physiological changes, learning and the formation of appropriate responses to external events. Neural plasticity is among the most important aspects of the field of modern neuroscience and its study is leading to a better understanding of brain development. In this study the expression levels of BDNF and Arc protein, the density of dendritic spines and the neurogenesis, were studied after a long-lasting stress, due to maternal separation Neuroplasticity was evaluated in controls (mother and offspring not exposed to maternal separation) and after the stress induced by maternal separation (3h at day from the 3rd to the 21st day after birth). The mothers were sacrificed 21 days after the birth and pups in three different age groups: 21, 30 and 60 days. In the hippocampus of non-stressed we found an increase in the expression levels of the protein BDNF and Arc, in the dendritic spines density and in the neurogenesis, a phenomenon still present at weaning (21 days postpartum). In contrast, opposite effect (decrease) was observed on all the neurogenesis parameters in the mothers separated of their pups for 3 h per day for 20 days. Similarly to the mothers, also in hippocampus of the pups separated from their mothers was present a reduction of BDNF, Arc, dendritic spines density and neurogenesis in all three ages studied (21, 30 and 60). These results demonstrate that stress due to separation in the postnatal period results in adverse effects on neuronal plasticity in the hippocampus of both mothers and offspring.

Disclosures: **G. Biggio:** None. **P.P. Secci:** None. **M.V. Melis:** None. **M.C. Mostallino:** None.

Poster

081. Stress Effects on Cortex and Other Brain Regions

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 81.01/LL3

Topic: E.05. Stress and the Brain

Support: PMSHE grant N N311 604938

NCS grant 2011/03/N/NZ29/05222

PMSHE grant N N519 657940

Title: Repeated social stress affects expression of hemoglobin genes in mouse prefrontal cortex

Authors: *A. M. STANKIEWICZ[1], J. GOSCIK[4], A. H. SWIERGIEL[5], A. MAJEWSKA[6], G. R. JUSZCZAK[2], P. LISOWSKI[3];
[2]Dept. of Animal Behavior, [3]Dept. of Mol. Biol., [1]Inst. of Genet. and Animal Breeding of the Polish Acad. of Sci., Magdalenka, Poland; [4]Fac. of Computer Sci., Bialystok Univ. of Technol., Bialystok, Poland; [5]Dept. of Human and Animal Physiol., Univ. of Gdansk, Gdansk, Poland; [6]Dept. of Physiological Sci., Warsaw Univ. of Life Sci., Warsaw, Poland

Abstract: We investigated gene expression in prefrontal cortex of mice subjected to acute and repeated social stress using microarrays. The most important finding was identification of hemoglobin genes (*Hbb-b1*, *Hbb-b2*, *Hba-a1*, *Hba-a2*, *Beta-S*) as potential markers of chronic social stress in mice. Expression of these genes was not altered by acute stress, but was progressively increasing in animals subjected to 8 and 13 days of repeated stress. Expression of hemoglobin genes was correlated with altered expression of *Mgp* (*Mglap*), *Fbln1*, *1500015O10Rik* (*Ecrg4*), *SLC16A10*, and *Mndal*. Chronic stress increased also expression of *Timp1* and *Ppbp* that are involved in reaction to vascular injury. Acute stress induced changes in expression of *Fam107a* (*Drr1*) and *Agxt2l1 (Etnppl*) that have been implicated in psychiatric diseases. The observed up-regulation of genes associated with vascular system and brain injury suggests that stress may affect brain function through the stress-induced dysfunctions of the vascular system.

Disclosures: **A.M. Stankiewicz:** None. **J. Goscik:** None. **P. Lisowski:** None. **A. Majewska:** None. **A.H. Swiergiel:** None. **G.R. Juszczak:** None.

Poster

081. Stress Effects on Cortex and Other Brain Regions

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 81.02/LL4

Topic: E.05. Stress and the Brain

Support: NSERC (Canada)

Norlien Foundation (Canada)

Title: Chronic stress induces epigenetic modification to the mPFC, OFC, and HPC of adult rats

Authors: *B. E. KOLB[1], A. MUHAMMAD[1], S. ILNYTSKYY[2], R. MYCHASIUK[3];
[1]Canadian Ctr. Behav Neurosci, [2]Biol. Sci., [3]Univ. of Lethbridge, Univ. Lethbridge, Lethbridge, AB, Canada

Abstract: Chronic stress is associated with a multitude of cognitive symptoms including deficits in emotional regulation, diminished self-regulatory and attentional function, abnormal learning, memory, and executive function. Many researchers have demonstrated that the medial prefrontal cortex (mPFC), orbital frontal cortex (OFC), and the hippocampus (HPC) work in conjunction to mediate responses to stressful experiences and have also shown that changes in these brain regions may precede the cognitive impairments. Anatomical studies have identified consistent alterations in the neuronal morphology of these brain regions, specifically demonstrating that chronic stress reduces dendritic arborization and spine formation in the mPFC and HPC, but increases the same parameters in the OFC. Little is known, however, about how experiential stressors actually change the neuronal structure and function of these brain regions. The current study examined the epigenetic changes that occur in response to chronic stress, with a specific focus on the mPFC, OFC, and HPC. Adult male and female Long-Evans rats (P70) were exposed to an elevated platform stressor for 15 minutes, twice a day, for 14 consecutive days, given a 14 day withdrawal period, and then sacrificed. DNA from the mPFC, OFC, and hippocampus was used for global DNA methylation analysis and RNA from the same brain regions was examined with an mRNA biosequencing platform to ascertain changes in gene expression. Following the two week withdrawal period, we found that exposure to chronic stress was associated with increased methylation in the mPFC and OFC for both male and female rats ($p < .05$). There was an opposite effect in the HPC of male rats, decreasing global methylation ($p < .05$), but no effect in the HPC of female rats. When examining the RNA from the same brain regions, chronic stress exposure was associated with changes in expression of 58 genes in male rats (HPC:9, mPFC:17, OFC:32) whereas the same exposure in female rats was associated with changes in expression of 76 genes (HPC:17, mPFC:18, OFC:41). There was little overlap in gene expression by sex or region. (A full list of genes and function will be included with poster). This study demonstrates that the persistent epigenetic changes associated with chronic stress are region specific and differ in males and female. It also shows a much larger change in OFC than in either mPFC or HPC, suggesting that the OFC may play a larger role in chronic effects of stress than generally believed.

Disclosures: **B.E. Kolb:** None. **A. Muhammad:** None. **S. Ilnytskyy:** None. **R. Mychasiuk:** None.

Poster

081. Stress Effects on Cortex and Other Brain Regions

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 81.03/LL5

Topic: E.05. Stress and the Brain

Support: R01 DK066596-07

R01 NS060114

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NSF GRFP

Selma Schottenstein Harris Lab for Research in Parkinson's

Title: Prefrontal cortex pituitary adenylate cyclase-activating peptide inhibits HPA axis responses to stress

Authors: *R. A. MAKINSON, M. SMELTZER, K. LUNDGREN, R. SAKAI, K. SEROOGY, J. HERMAN;
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Abstract: Pituitary adenylate cyclase-activating peptide (PACAP) neuropeptide is implicated in a number of cellular processes and complex behaviors, including learning and memory, synaptic plasticity, neuronal homeostasis, neural protection and psychiatric illness. PACAP is robustly expressed in brain regions regulating hypothalamic-pituitary-adrenal (HPA) axis activation and fear conditioning, including the hippocampus, amygdala and medial prefrontal cortex (mPFC). This study explored the role of mPFC PACAP in regulation of acute stress responses. Adult male Sprague-Dawley rats received bilateral injections of PACAP in a volume of 0.5µl/side into the prelimbic (PL) and infralimbic (IL) junction of the mPFC. Five min after injection, rats were placed in a restrainer for 30 min and tail blood collected. At 120 min, rats were overdosed with pentobarbital and perfused with 4% paraformaldehyde. Rat brains were then removed and sectioned on a microtome to assess the impact of mPFC PACAP infusion on central Fos activation via immunohistochemistry. PACAP infusion decreased Fos activation within the paraventricular nucleus of the hypothalamus (PVN). PACAP also reduced peak corticosterone release at 30 minutes. Given this possible role in stress regulation, the expression of PACAP mRNA in the mPFC was evaluated following 14-day exposure to a chronic social stress regimen via the visible burrow system (VBS). Expression of PACAP mRNA within the PL and IL regions was assessed by semi-quantitative *in situ* hybridization. Analysis of the hybridization data indicated that neither dominance nor subordination affected PACAP mRNA in the IL or PL in this model. These data suggests that PACAP in the mPFC may have a buffering effect on HPA output to acute stress, perhaps by enhancing stress-inhibitory output from the PFC.

Disclosures: **R.A. Makinson:** None. **M. Smeltzer:** None. **K. Lundgren:** None. **R. Sakai:** None. **K. Seroogy:** None. **J. Herman:** None.

Poster

081. Stress Effects on Cortex and Other Brain Regions

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Topic: E.05. Stress and the Brain

Support: NIH grant K22MH097826

VTCRI startup funds

Title: Building prefrontal functional connectivity map at cellular resolution: Application to socially isolated mice

Authors: *A. Y. MOROZOV, C. FARNAN, B. PAUDEL, W. ITO;
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Abstract: (Rationale) Connectivity between brain sites is described by two components: anatomical connectivity and functional connectivity. Popular functional MRI imaging in humans, primates and laboratory animals produces rich datasets of correlated neuronal activities across brain regions during specific behaviors or in mental disease. Nonetheless, there is a gap of knowledge in linking changes in macro-patterns of functional connectivity with alteration at the cellular, synaptic and molecular levels. Thus, description of functional connectivity at cell resolution is necessary. To that end, we began probing functional connectivity of neurons in dorso-medial prefrontal cortex by using local optogenetic stimulation followed by c-Fos mapping of neuronal activity at the targets inside and outside mPFC, investigating which cell-types are recruited in healthy and pathological brain. We use rodent social isolation as a disease model since it elicits behavioral traits relevant to several psychiatric disorders, including deficit in pre-pulse inhibition, which is characteristic of schizophrenia and is attributed to malfunction of the prefrontal cortex. Numerous alterations in molecular composition and morphology of the prefrontal neurons and glia have been documented. Yet, it remains unclear how such alterations affect functional connectivity of the prefrontal neurons within local microcircuits and towards remote targets throughout the brain. (Methods) At p21, mice receive prefrontal injections of adenoassociated virus expressing channelrhodopsin; at p50, optic fiber is implanted above the injected area. From p58 to p60 mice are habituated to optical stimulation setup. On p61, 2 mW

473 nm light stimulation in 2 s 10 Hz bursts repeated every 5 s for 30 min is delivered through the optic fiber. Animals are perfused 90 min after beginning of stimulation, followed by c-Fos analyses of activated areas. For socially isolated mice, animals are singly housed from p21, whereas controls are housed four animals per cage. (Results) Preliminary analyses suggests that post-weaning social isolation increases excitability of neurons in dmPFC and alteres patterns of neuronal activation among cortical layers within dmPFC, with greatest increase in neuronal activation in layer 2/3. In addition, it differentially altered proportion of c-Fos positive cells among distinct subtypes of GABAergic neurons, which suggests reorganization of local dmPFC network and its output to remote targets.

Disclosures: **A.Y. Morozov:** None. **C. Farnan:** None. **B. Paudel:** None. **W. Ito:** None.

Poster

081. Stress Effects on Cortex and Other Brain Regions

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Topic: E.05. Stress and the Brain

Support: NIH Grant MH049698 (JPH)

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Title: Reduced glutamate outflow from infralimbic prefrontal cortex inhibits depression-like behavior and prevents the behavioral consequences of chronic stress

Authors: *B. MYERS[1], J. M. MCKLVEEN[1], W. BEISCHEL[1], J. R. SCHEIMANN[1], R. MORANO[1], S. P. WILSON[2], M. B. SOLOMON[1], J. P. HERMAN[1];
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Abstract: Altered activity of ventromedial prefrontal cortex (vmPFC) correlates with depressive illness in humans. Additionally, studies utilizing electrical or optical stimulation techniques suggest that vmPFC regulates despair-like behavior in rodents. Our previous studies have isolated the infralimbic portion of the vmPFC as key regulator of neuroendocrine responses to

stress. However, despite significant implications for understanding affective disorders, the importance of glutamate outflow from infralimbic cortex for the behavioral consequences of chronic stress is unknown. In the current study, we utilized a lentiviral-packaged gene construct coding for a vesicular glutamate transporter 1 (vGluT1) siRNA. This approach permits the long-term knockdown of glutamate packaging at infralimbic synaptic terminals. Two separate cohorts of rats each received injections of the vGluT1 siRNA-expressing lentivirus or a GFP control virus and were then divided into two groups per cohort that received chronic variable stress (CVS) or remained as unstressed controls. One cohort was assessed for immobility behavior in the forced swim test (FST) prior to stress exposure while a second cohort received the FST after 14 days of CVS. Additional behavioral assays were carried out during the first three and last three days of CVS in each cohort to query the role of infralimbic output in the behavioral changes associated with chronic stress. Our results indicate that knockdown of infralimbic output reduced indices of depression-like behavior in the FST compared to GFP controls, both in animals exposed to chronic stress as well as previously unstressed animals. Further, the effects of CVS to reduce exploratory behavior in the open field test, novel object interaction, and elevated plus maze were prevented by treatment with the vGluT1 siRNA. The effects of vGluT1 knockdown on behavioral responses to CVS were not related to learning deficits, as all groups demonstrated effective associative learning during classical conditioning. These data point to the importance of infralimbic glutamate outflow for regulating depression-related behavior, as well as the behavioral changes associated with chronic stress. These findings, coupled with previous findings related to the role of the infralimbic cortex in physiological responses to stress, identify this area as a potential nexus for chronic stress-related pathologies.

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Poster

081. Stress Effects on Cortex and Other Brain Regions

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Program#/Poster#: 81.06/LL8

Topic: E.05. Stress and the Brain

Support: NIH grant MH41256

Title: BAG-1 levels alter stress-induced gene expression in the prefrontal cortex

Authors: *T. G. RUBIN, J. D. GRAY, B. S. MCEWEN;
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Abstract: Bcl-2 associated athanogene-1 (BAG-1), an established anti-apoptotic chaperone, binds the glucocorticoid receptor (GR) to alter GR-dependent signaling and therefore may serve as a novel molecular switch between GR-dependent and NFkB-dependent gene transcription. While BAG-1 has been shown to directly interact with the NFkB transcription factor p50 in hippocampus, this has not been demonstrated in the prefrontal cortex (PFC). Stress-induced neuroplasticity in the PFC, a region essential for executive cognitive functions, has been observed in rodent models of chronic stress and these changes parallel those observed in patients with stress sensitive mental health disorders. Understanding how BAG-1 levels and stress alter gene expression in the PFC will provide important insight into the molecular mechanisms associated with certain mental illnesses. The present study will characterize the effects of stress on gene expression using transgenic mice overexpressing BAG-1 (BAG-1 TG) and BAG-1 heterozygous knockout (BAG-1 KO) mice, which have been previously shown to have altered stress reactivity. Adult BAG-1 TG, BAG-1 KO, and wild type (WT) littermates were subjected to a 21 day chronic restraint stress (CRS) and sacrificed 24 hrs following the final stress. Brains were quickly removed and the PFC was dissected and flash frozen. RNA was extracted from the tissue and a cDNA library was generated for qRT-PCR analysis. Immunoprecipitations were performed on PFC tissue to probe for an interaction between BAG-1 and NFkB gene products. qRT-PCR revealed BAG-1 KO mice had significantly increased basal GR levels, whereas BAG-1 OE had unchanged basal GR levels compared to WT controls. Further, GR levels in both transgenic lines appeared insensitive to the effects of CRS, suggesting BAG-1 levels may impact stress-induced neuroplasticity in the PFC. Immunoprecipitations indicated BAG-1 interacts with the p50 subunit of NFkB in the PFC. These results suggest that BAG-1 can alter the gene expression response to stress by modulating GR levels, but that it may also act through the NFkB pathway and other pathways to modulate stress reactivity. High throughput analysis of the gene expression networks in BAG-1 mutant mice would identify changes in these other pathways and elucidate possible therapeutic targets for stress-related mood disorders. Studies using the BAG-1 mutant mice to assess the effect of BAG-1 levels on stress-induced morphological neuroplasticity are underway. Future studies will also seek to characterize epigenetic mechanisms that might underlie these stress-induced transcriptional changes.

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Poster

081. Stress Effects on Cortex and Other Brain Regions

Location: Halls A-C

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Program#/Poster#: 81.07/LL9

Topic: E.05. Stress and the Brain

Title: Acute stress-induced prefrontal cortex dysfunction is mediated by the CRH-CRHR1 system

Authors: *A. URIBE, B. SOLFRANK, G. BALSEVICH, C. DOURNES, S. SANTARELLI, M. MASSANA, D. HARBICH, A. CHEN, M. V. SCHMIDT;
Max Planck Inst. of Psychiatry, Munich, Germany

Abstract: The prefrontal cortex (PFC) plays a fundamental role in adaptive responses and has been ascribed to the cognitive hallmarks that characterize different psychiatric disorders. Furthermore, stress has been shown to affect the function of the PFC and is also associated with neuropsychiatric conditions such as depression. Among numerous candidates CRH, through the activation of CRH receptor 1 (CRHR1), constitutes one of the main effector systems by which stress unchains deleterious effects upon different brain regions. However, the contributions of this system to the effects of stress in the PFC are still unclear. The present project aims to characterize the role of the CRH-CRHR1 system on the impact of stress in the PFC. In all experiments, C57Bl6 mice were employed. In experiment 1, animals underwent acute social defeat stress and were tested 8 hours later either in a temporal order memory task (TOM) or a reversal learning test. In experiment 2, animals were submitted to a single episode of social defeat stress and 8 hours later were sacrificed. PFC sections of these animals were submitted to an *in situ* hybridization protocol to assess changes in CRHR1 mRNA levels. In experiment 3, a guide cannula was implanted in the PFC and animals received intra-PFC microinjections of either vehicle or CRH, and 8 hours later were submitted to either the TOM or reversal learning task. In experiment 4, CRHR1lox/lox mice received intra-PFC microinjections of either empty-AAV or Cre-AAV to induce a PFC-specific CRHR1 KO. One month later, they were submitted to an acute social defeat stress and after 8 hours were tested in either the TOM or reversal learning tasks. The results obtained show that a single episode of stress impaired cognitive flexibility and the recognition of objects with different recency. Also, significant increases in CRHR1 mRNA levels were observed in different subregions of the PFC 8 hours after stress. In addition, acute-stress induced prefrontal cortex-mediated cognitive dysfunctions were mimicked by intra-PFC CRH microinjections. Moreover, the CRHR1 knockout in the PFC completely reversed the effects of acute stress in these prefrontal cortex dependent tasks. Taken together, our results suggest a molecular mechanism that links stress to behavioral dysfunctions, thereby opening new intervention strategies for patients suffering from stress-related diseases, such as depression.

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Poster

081. Stress Effects on Cortex and Other Brain Regions

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Topic: E.05. Stress and the Brain

Support: NSF Grant IOS 1146853

Title: Modulation of medial entorhinal cortex layer II principal cell circuitry by glucocorticoids

Authors: *J. HARTNER, L. A. SCHRADER;
Tulane Univ., New Orleans, LA

Abstract: Previous research has shown that stress impairs rodent performance on spatial memory tasks, but the underlying mechanisms are unclear. Spatial memory processing is mainly localized to a loop between the hippocampus and the entorhinal cortex. Stellate cells in layer II of the medial entorhinal cortex (MEC - LII) are spatially-tuned and necessary for establishing an internal grid-like representation of the environment. Our preliminary data show that chronic stress causes dendritic atrophy of stellate cells of layer II, suggesting that stress may functionally modulate these cells. MEC - LII is also composed of pyramidal cells, which are anatomically organized in grid-like fashion around the stellate cells and have a seemingly distinct network of inputs and inhibition. The functional interaction between these two principal cells' networks and modulation of these networks by environmental factors, such as stress, are not studied to date. Here we use stereotaxic injection of fluorescent retrograde tracers into the dentate gyrus and contralateral MEC as a means of real-time identification of MEC - LII principal cell type. We also use whole-cell patch clamp electrophysiology of MEC slice preparations in mice, paired with stimulation of inputs to MEC - LII from the parasubiculum, to elucidate the functional connectivity of MEC - LII principa l cells implicated in spatial processing. We test synaptic changes of layer II principal cells in response to bath application of dexamethasone, a synthetic glucocorticoid, and show that synaptic activity may be differentially affected by dexamethasone application based on cell type.

Disclosures: **J. Hartner:** None. **L.A. Schrader:** None.

Poster

081. Stress Effects on Cortex and Other Brain Regions

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Topic: E.05. Stress and the Brain

Support: NSF Grant IOS1146853

Louisiana Board of Regents LEQSF(2012-17)-GF-15

Title: Stress-induced molecular and structural plasticity in the entorhinal cortex

Authors: *D. R. HOMIACK[1], J. A. FARUQI[2], A. H. MAHNKE[3], C. L. COMBE[1,4], F. M. INGLIS[3], L. A. SCHRADER[1,3];
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Abstract: Psychological stress activates the hypothalamic-pituitary-adrenal (HPA) axis to produce glucocorticoids which mobilize energy for the organism to attend to the stressor. Circulating glucocorticoids act through both rapid and prolonged mechanisms to alter neuronal properties in the hippocampus. Exposure of male rodents to chronic variable stress induces depressive-like symptoms, including anhedonia, increased circulating glucocorticoids, weight loss, and impairments in spatial memory. In contrast, acute stress can facilitate the formation of spatial memory, but impairs working memory and memory recall in male rodents. Fewer studies investigate the effects of acute and chronic stress in female rodents, and varying results are reported. A wealth of literature focuses on molecular and cellular plasticity caused by stress and sex related hormones in the hippocampus; however, the effects of these hormones in the entorhinal cortex, the primary excitatory input into the hippocampus, have been largely unexplored. In this study, we used Golgi Cox impregnation to visualize camera lucida reconstructions of pyramidal and stellate neurons in layer II of the entorhinal cortex. Following exposure to two weeks of chronic variable stress we observed a reduction in dendritic complexity in stellate cells in stressed male rats. Additionally, we investigated the molecular cascades in homogenate from the entorhinal cortex following a 30 minute exposure to acute predator odor (trimethylthiazoline or TMT) in intact male and female rats. We found that TMT exposure caused a rapid decrease in ERK/MAPK activation and increased PKA activity in male, but not female rats. Our results suggest that the entorhinal cortex exhibits structural plasticity in response to chronic variable stress, and that acute stress alters canonical signaling cascades in a sex-

dependent manner. These findings implicate the entorhinal cortex as a brain region in which plasticity may mediate some of the cognitive changes caused by both acute and chronic stress. Future studies will investigate the specific role of corticosterone and estradiol on the structure and function of the principal cells of the entorhinal cortex.

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Poster

081. Stress Effects on Cortex and Other Brain Regions

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Topic: E.05. Stress and the Brain

Support: NSF grant IOS 1146853

Title: SIRT1 activity affects granule cell excitability and synaptic activity in the dentate gyrus of mouse hippocampus

Authors: *D. YU, L. A. SCHRADER;
Tulane Univ., New Orleans, LA

Abstract: The sirtuins are a family of NAD+ dependent protein deacetylases that are involved in metabolic processes and have various functions in neurons, such as DNA repair, stress resistance, and neuronal survival. Recent research indicates that Sirtuin 1 (SIRT1) may also play a critical role in hippocampus function, particularly learning and memory. Our lab and others have shown that chronic stress affects hippocampus structure and function, including causing memory impairments. We found that chronic stress significantly increased SIRT1 activity in the dentate gyrus (DG) of chronically stressed rats. Direct infusion of sirtinol, a SIRT1 and 2 inhibitor, into the DG prevented CVS-induced memory impairment and depressive-like behavior. The specific function of SIRT1 in the DG, however, is unknown. We hypothesized that SIRT activity may affect intrinsic properties or synaptic inputs of the DG granule cells. In this study, we investigated effects of pharmacological activation and inhibition of SIRT1 on the electrophysiological properties of granule cells of the DG. Whole-cell patch-clamp recordings were made from granule cells in acute mouse hippocampus slices. We found that both activation and inhibition of SIRT1 increased granule cell excitability. Analysis of spike properties of granule cells showed that both threshold and afterhyperpolarization (AHP) of spikes significantly

decreased, which led us to focus our future study on modulation of potassium channels. In addition, we found that SIRT1 inhibitor can increase spontaneous excitatory postsynaptic current (sEPSC) frequency in the DG granule cells with no effect on sEPSC amplitude. Further study indicated that this effect was action potential-dependent as miniature EPSC frequency, recorded in tetrodotoxin, was not affected by SIRT1 inhibition. We are now testing the effect of SIRT1 on ATP-dependent potassium channels (K (ATP) channels), A-type potassium current and delayed rectifying potassium current, respectively. In addition, future studies will investigate SIRT1 function in chronically stressed mice. These studies suggest a novel, rapid mechanism for SIRT1 effects in DG neurons.

Disclosures: D. Yu: None. **L.A. Schrader:** None.

Poster

081. Stress Effects on Cortex and Other Brain Regions

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Topic: E.05. Stress and the Brain

Support: Knut and Alice Wallenberg foundation

Title: High cortisol levels are associated with atrophy of cingulate and middle frontal gyrus in healthy men and women

Authors: *A. STOMBY[1], C.-J. OLSSON[2], A. NORDIN[3], L.-G. NILSSON[6], R. ADOLFSSON[3], T. OLSSON[1], L. NYBERG[4,5];
[1]Dept. for medicine, [2]Ageing and Living Conditions programme, [3]Dept. of Clin. Sci., [4]Integrative Med. Biol., [5]Umeå Ctr. for Functional Brain Imaging, Umeå Univ., Umeå, Sweden; [6]Dept. of Psychology, Stockholm Univ., Stockholm, Sweden

Abstract: Glucocorticoids causes dendritic atrophy and decrease plasticity in the hippocampus (HC) and prefrontal cortex (PFC). Our hypothesis was that circulating cortisol levels are associated with HC and PFC volumes in men and women without cognitive impairments. Individuals from the population based Betula prospective cohort study of memory and aging in Umea were included. The sample consisted of 102 men and 103 women with a mean age of 67 years (range 55-80) and a mean score of 28 on the Mini Mental State Examination. A MRI scan of the brain with volumetric analyzes of PFC and HC regions was performed. Cortisol levels in 4 saliva samples collected at 0700, 1100, 1600 and 2300 h during one day were analyzed with a

Chemiluminescence immunoassay. Episodic memory, verbal fluency, visuospatial ability and working memory were tested and participants were asked about diseases and drug use during the preceding 5 years. Multivariable linear regression analyses were used to test whether cortisol levels at 2300 h and area under the curve (AUC) for cortisol levels in all 4 saliva samples were associated with brain volumes and cognitive functions. Age, gender, total intracranial volume and level of education were used as covariates. In the multivariable analysis AUC for cortisol levels in saliva was negatively associated with volume of the left cingulate gyrus (b=-0.15, p=0.011), right cingulate gyrus (b=-0.11, p=0.045), left middle frontal gyrus (b=-0.16, p=0.012) and right middle frontal gyrus (b=-0.20, p=0.001). There were no significant associations between AUC for cortisol levels and volume of superior frontal gyrus, inferior frontal gyrus or the HC in neither hemisphere. Cortisol level at 2300 h was not associated with brain area volumes. There were no significant association between cortisol levels and cognitive measures. Performance on the visuospatial task was positively associated with volume of left middle frontal gyrus (b=0.19, p=0.008) and cingulate gyrus in left (b=0.24, p=0.002) and right (b=0.27, p<0.001) hemisphere. In addition, performance on the 2-back test of working memory was positively associated with volume of right cingulate gyrus (b=0.18, p=0.046). The association between AUC for cortisol and right middle frontal gyrus as well as visuospatial ability and right and left cingulate gyrus remained significant after Bonferroni correction while the other associations became non-significant. Our results add to the growing amount of data suggesting that the association between cortisol levels and brain volume is stronger for PFC than HC brain areas. It is unclear whether the observed brain atrophy is a cause or consequence of increased cortisol levels.

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Poster

081. Stress Effects on Cortex and Other Brain Regions

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Topic: E.05. Stress and the Brain

Support: FCT Grant - PTDC/SAU-NMC/118971/2010

Title: The impact of chronic stress on the rat brain lipidome

Authors: *T. G. OLIVEIRA[1], R. B. CHAN[2], F. V. BRAVO[1], A. MIRANDA[1], B. ZHOU[2], F. MARQUES[1], V. PINTO[1], J. J. CERQUEIRA[1], G. DI PAOLO[2], N. SOUSA[1];
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Abstract: Chronic stress is a major risk factor for several human disorders that affect modern societies. The brain is a key target of chronic stress; in fact, there is growing evidence indicating that exposure to stress affects learning and memory, decision making and emotional responses, and may even predispose for pathological processes, such as Alzheimer's disease and depression. Lipids are a major constituent of the brain, and specifically signaling lipids have been shown to regulate brain function. Here, we used a mass spectrometry-based lipidomic approach to evaluate the impact of a chronic unpredictable stress paradigm and chronic administration of exogenous corticosterone (CORT) on the rat brain in a region-specific manner. We found that the prefrontal cortex (PFC) was the area with the highest degree of changes induced by chronic stress. Specifically, sphingolipid metabolism was profoundly affected, showing an increase in ceramide and a decrease in sphingomyelin and dihydrosphingomyelin levels. Furthermore, the fatty acyl profile of phospholipids and diacylglycerol revealed that chronic stressed rats had higher 38 carbon(38C)-lipid levels in the hippocampus and a decrease in 36C-lipid levels in the PFC. Finally, lysophosphatidylcholine levels in the PFC were found to be correlated with blood CORT levels. In summary, lipidomic profiling of the effect of chronic stress allowed for the identification of dysregulated lipid pathways, revealing putative targets for pharmacological intervention that may potentially be used to modulate the stress-induced deficits.

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Poster

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Topic: E.05. Stress and the Brain

Support: DARPA - W911NF-10-1-0059

Title: Locally synthesized growth hormone enhances spine density in the amygdala

Authors: *B. GISABELLA[1,2], J. YAO[1,2], K. A. GOOSENS[1,2];
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Abstract: Growth hormone (GH) exerts strong trophic effects in multiple tissues of the body. Although GH is synthesized by the pituitary gland, where it is released into the circulating bloodstream, it is also synthesized within limbic brain areas such as the amygdala, a brain region that regulates emotional memory. However, very little is known about the role of locally synthesized GH in regulating emotional processing. Our recent studies show that chronic stress increases GH protein expression in the basolateral complex of the amygdala (BLA) and is both necessary and sufficient for chronic stress-related enhancement of fear memory in rodents, an animal model of post-traumatic stress disorder (PTSD). Others studies have shown that chronic stress enhances spine density in the BLA (Vyas et al., 2002). We hypothesize that stress-related changes in GH may drive the morphological changes observed in the BLA following stress exposure. Here, we characterize the distribution of GH and its receptor (GH secretagogue receptor, or GHS-R) in the rat BLA using florescent *in situ* hybridization. Our preliminary findings suggest that GH and GHS-R are widely expressed in neurons of the BLA, demonstrating that GH is poised to exert potent and widespread effects. To explore the hypothesis that GH regulates neuronal morphology, we used an adeno-associated viral vector to overexpress either GH with green florescent protein (GFP) or GFP alone in the BLA in rats. Dendritic spine density was quantified by combining confocal imaging with three-dimensional dendritic analysis. We find that GH overexpression dramatically enhances spine density on both the primary and secondary branches of BLA pyramidal neurons. These findings support the idea that chronic stress may produces vulnerability to enhanced fear via GH-driven changes in neuronal morphology.

Disclosures: **B. Gisabella:** None. **J. Yao:** None. **K.A. Goosens:** None.

Poster

081. Stress Effects on Cortex and Other Brain Regions

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 81.14/LL16

Topic: E.05. Stress and the Brain

Support: Inserm AVENIR-RSE13004FSA

NIH MH086651-01A1

Title: Characterization of a novel neurotrophic-sensing mechanism of glucocorticoid actions

Authors: M. ARANGO-LIEVANO[1], M. J. GARABEDIAN[2], S. D. GINSBERG[3], M. V. CHAO[4], *F. D. JEANNETEAU[1];
[1]Physiol., Inst. of Functional Genomics, Montpellier, France; [2]NYU, New York, NY; [3]Nathan Kline instutite of psychiatric research, Orangeburg, NY; [4]Skirball institute of Biomolecular Med. NYU, New York, NY

Abstract: Neuropsychiatric disorders are often associated with abnormal glucocorticoid receptor (GR) and neurotrophin signaling. Current knowledge suggests reciprocal actions of glucocorticoids and brain-derived neurotrophic factor (BDNF) on neuronal growth, survival and behavior that suffers from a lack of mechanistic understanding. We report that priming BDNF signaling at the time of glucocorticoid exposure contributes to neuronal allostasis to stress by way of phosphorylation of the glucocorticoid receptor. Manipulations aiming at blocking BDNF-induced GR phosphorylation *in vivo* prevent the allostatic overload of stress on post-synaptic dendritic spine plasticity in a cortico-cortical network. Mechanistically, BDNF-induced GR phosphorylation did not alter GR nuclear translocation, turnover or DNA-binding, but rather fosters cofactor recruitment to promote a novel gene expression signature involved in cellular networks (Lambert et al. MCB 2013). We further assess the allostatic functions of two of these genes using gain- and loss-of-function in *in vivo* experiments. We find at least one of these genes is critical for dendritic spine plasticity to stress in a mouse cortico-cortical network, and ectopically expressed in human BA9-10 cortex as a function of age and cognitive impairment. Thus, we report that glucocorticoid actions may diverge as a function of BDNF signaling to serve allostatic functions at least in cortical networks.

Disclosures: **M. Arango-Lievano:** None. **M.J. Garabedian:** None. **S.D. Ginsberg:** None. **M.V. Chao:** None. **F.D. Jeanneteau:** None.

Poster

081. Stress Effects on Cortex and Other Brain Regions

Location: Halls A-C

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Program#/Poster#: 81.15/LL17

Topic: E.05. Stress and the Brain

Support: T32 DA07288

DoD W81XWH-11-1-1245 Subaward 803-237

Title: Epigenetic alterations in rats with traumatic stress exposure treated with oxytocin prior to reinstatement of methamphetamine-seeking

Authors: *C. L. FERLAND, J. F. MCGINTY;
Neurosciences, Med. Univ. of South Carolina, Charleston, SC

Abstract: Previous research has shown a strong link between post-traumatic stress disorder (PTSD) and substance abuse disorders (SUD). In individuals diagnosed with PTSD, the incidence of SUD is higher than in the general population and treatment outcomes for individuals with co-morbid SUD and PTSD are generally poorer. Moreover, traumatic stress exposure independent of PTSD development can precipitate relapse to abuse in recovering SUD individuals. While alterations in several neural systems are implicated in increased vulnerability to SUD following traumatic stress exposure, the endogenous oxytocin system is regulated in response to both stress and substance use. The goal of these studies is to examine whether stimulating the endogenous oxytocin system attenuates methamphetamine (Meth) seeking in a preclinical model of traumatic stress. We examined (1) reinstatement of Meth-seeking induced by cues or the predator odor, trimethylthiazoline (TMT) paired with Meth self-administration (SA), in rats injected with 1.0 mg/kg, i.p. oxytocin (OXT) or saline 30 min before reinstatement and (2) neuroendocrine alterations and mRNA expression in the dorsomedial prefrontal cortex (dmPFC) and the paraventricular nucleus of the hypothalamus (PVN). TMT-pretreated animals injected with saline exhibited increased lever pressing to both cues and stress (TMT) compared to vehicle-pretreated animals injected with saline. In contrast, OXT treatment suppressed cue or TMT-induced Meth-seeking in both control and TMT-exposed rats. After reinstatement, a significant decrease in *Oxt* and *OxtR* mRNA was seen in the PVN of TMT-pretreated animals compared to saline-pretreated controls. OXT prevented the decrease in *Oxt* mRNA and resulted in a trend toward an increase in *OxtR* mRNA in both control and TMT-exposed animals. In the dmPFC after reinstatement, TMT-pretreated rats treated with saline showed a significant reduction in the epigenetic mark *Hdac5* and a corresponding increase in *Bdnf*IV mRNA compared to saline-pretreated animals. OXT attenuated the *Hdac5* decrease and normalized *Bdnf*IV mRNA in the dmPFC of TMT-pretreated rats. The results from these studies could represent a novel therapeutic strategy for SUD with concurrent with PTSD. Supported by T32 DA07288 and DoD W81XWH-11-1-1245 Subaward 803-237

Disclosures: **C.L. Ferland:** None. **J.F. McGinty:** None.

Poster

081. Stress Effects on Cortex and Other Brain Regions

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 81.16/LL18

Topic: E.05. Stress and the Brain

Title: Effects of chronic stress on methamphetamine-stimulated behaviors and striatal dopamine release in female rats

Authors: *E. M. ANDERSON, M. MCWATERS, L. MATUSZEWICH;
Psychology, Northern Illinois Univ., Dekalb, IL

Abstract: Previous research in humans and animals suggests that exposure to chronic stress alters the susceptibility and behavioral responses to drugs of abuse, such as methamphetamine. Our lab has found that male rats exposed to chronic stress showed an increase in locomotion and dopamine release in the striatum following an acute injection of methamphetamine or cocaine, compared to unstressed control rats (Matuszewich & Yamamoto, 2004; Moenk & Matuszewich, 2012). Interestingly, sex differences have been reported in response to stress and stimulants. Non-stressed female rats exhibited an increase in locomotion and stereotypy following a single injection of methamphetamine compared to male rats (Milesi-Halle et al., 2007). These findings indicate that females may be more sensitive to the effects of stimulants and supports a growing literature characterizing sex differences in stimulant use. Therefore, we were interested in determining whether prior exposure of female rats to chronic unpredictable stress would potentiate methamphetamine-stimulated behaviors and dopamine release in the dorsal striatum compared to unstressed female rats. Female rats were exposed to unpredictable stress or no stress (control) for 10 days and then tested either in the open field or had extracellular dopamine measured through microdialysis two weeks later. In the open field, females that had received unpredictable stress showed an increased response immediately following an injection of methamphetamine as measured by distance traveled ($p=.045$) and speed ($p=.045$) compared to control rats, but had lower distance and speed at later time points. Stereotypy scores showed no differences between rats exposed to stress and rats not exposed to stress following an injection of methamphetamine. Preliminary microdialysis data suggest that rats exposed to chronic unpredictable stress have an increased dopamine response following an injection of methamphetamine compared to non-stressed female rats. Taken together, these findings indicate that chronic stress sensitizes the behavioral and dopaminergic response to an acute methamphetamine injection in female rats similar to the effects of chronic stress in male rats. Where sex differences have been observed in the magnitude of the change following stimulant exposure (Milesi-Halle et al., 2007; Anderson et al., 2014), stress acts in a similar manner to potentiate the response to an acute methamphetamine injection.

Disclosures: **E.M. Anderson:** None. **M. McWaters:** None. **L. Matuszewich:** None.

Poster

081. Stress Effects on Cortex and Other Brain Regions

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 81.17/LL19

Topic: E.05. Stress and the Brain

Title: Effect of heat acclimatization on monoaminergic neurotransmitters in the caudate putamen in rats

Authors: *H. NAKAGAWA[1], T. MATSUMURA[2], K. SUZUKI[2], C. NINOMIYA[2], S. YANAGITA[3], H. HASEGAWA[4], T. ISHIWATA[2];
[1]Rikkyo Univ., Niza / Saitama, Japan; [2]Grad. Sch. of Community Human & Services, Rikkyo Univ., Niza/Saitama, Japan; [3]Dept. of Sci. & Technol., Tokyo Univ. of Sci., Chiba, Japan; [4]Grad. Sch. of Integrated Arts & Sciences., Hiroshima Univ., Hiroshima, Japan

Abstract: Brain monoaminergic neurotransmitters, such as serotonin (5-HT), dopamine (DA), and noradrenaline (NA), play crucial roles in several physiological functions and behavior including exercise. In addition, it has been reported that levels of neurotransmitters are rapidly changed by various thermal conditions. Although many studies have observed heat acclimatization in rats, few studies have assessed neurotransmitters in brain areas that participate in exercise during chronic thermal exposure. The present study was designed to determine changes in the levels and balance of 5-HT, DA, and NA in the caudate putamen (CPU) after heat exposure using homogenate techniques. In addition, we observed core body temperature (Tc), heart rate (HR) and locomotor activity (Act) in order to determine the timing of heat acclimatization. Firstly, we observed physiological indices of the rats. Male Wistar rats (250-300 g body weight) were implanted with a telemetry device. Rats were kept under normal conditions for 1 week (temperature 23 ℃,humidity 50%, 12-h/12-h light/dark cycle with lights on between 6:00 and 18:00 h) , and were then kept in a heated room for up to 4 weeks (temperature 32 ℃ and humidity 50%) . They had free access to food and water. Although Tc and Act rapidly increased and decreased, respectively, after heat exposure, they both returned to control levels at two weeks. We concluded that this timing was due to heat acclimatization. Secondly, we determined the levels of neurotransmitters in the rat CPU after heat exposure for different periods. Male Wistar rats (250-300 g body weight) were kept in a heated room for 3 hours, 1 day, 1 week, 2 weeks, and 1 month. Each group consisted of 6 rats. After each specified period, the rat brains were quickly removed and sliced into 300-μm sections. Samples were cut using a disposable 1-mm-diameter micro-punch, homogenized, and deproteinated in 0.2 M perchloric acid. The levels of 5-HT, DA, and NA in the CPU were analyzed using high-performance liquid

chromatography. DA was decreased when rats were kept in the heated room for 1 day, 1 week, 2 weeks, and 1 month. 5-HT and NA were increased at 3 hours and 1 month. These results suggest that the activity of heat acclimatized rats was regulated not by DA, but by 5-HT and NA.

Disclosures: **H. Nakagawa:** None. **T. Matsumura:** None. **K. Suzuki:** None. **C. Ninomiya:** None. **S. Yanagita:** None. **H. Hasegawa:** None. **T. Ishiwata:** None.

Poster

081. Stress Effects on Cortex and Other Brain Regions

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

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Topic: E.05. Stress and the Brain

Support: NSERC Grant

Title: Distribution of mineralocorticoid and glucocorticoid receptor immunoreactivity in a songbird brain

Authors: ***M. R. HASSTEDT**[1], S. A. MACDOUGALL-SHACKLETON[2];
[2]Psychology, [1]Univ. of Western Ontario, London, ON, Canada

Abstract: Stress affects numerous aspects of physiology, brain and behaviour. In birds, stress research has focused on seasonal changes in the stress response, chronic stress, and the effects of stress on breeding. In addition, recent studies have documented the effects of developmental stress on song learning and hippocampal-dependent spatial cognition. Despite this, there is little research examining where corticosterone (CORT) binds in the brain. CORT binds to two receptor subtypes, mineralocorticoid (MR) and glucocorticoid (GR) receptors with high and low affinity respectively. In the brain these receptors are thought to have opposing effects on the stress reaction. It is thus important to know where in the brain these CORT receptors are located. Previous studies have looked at receptor distribution in the brain but have only looked at glucocorticoid receptors or only the song control nuclei. In this study we have documented the distribution of both GR and MR throughout the entire songbird brain. Four European starling (Sturnus vulgaris) brains were cut coronally at 40um and stained in alternating series for MR and GR using immunohistochemistry. Areas implicated in stress response and regulation in mammals showed immunoreactivity for GR including: hippocampus, hypothalamus and nucleus taenia (avian homolog to amygdala). MR immunoreactivity was seen in several song-control regions though darkest staining occurred in the hippocampus. GR immunoreactivity was more ubiquitous

throughout the brain but was strongest in areas involved in negative feedback in mammals. This distribution of GR and MR is critical for our understanding of how CORT is having its effect on bird behavior and physiology.

Disclosures: **M.R. Hasstedt:** None. **S.A. MacDougall-Shackleton:** None.

Poster

082. Human Long-Term Memory: Medial Temporal Lobe I

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 82.01/LL21

Topic: F.01. Human Cognition and Behavior

Support: ERC-StG 261177

NWO-Vidi 452-12-009

Title: Memory for multiple associations in early visual cortex

Authors: ***S. E. BOSCH**, C. F. DOELLER;
Donders Institute, Radboud Univ. Nijmegen, Nijmegen, Netherlands

Abstract: During successful recall, the hippocampus is thought to interact with the cortex to support mnemonic reinstatement. Previous work in our lab showed that the identity of two recalled associations could be predicted from the activity pattern in early visual cortex. The strength of this reinstatement in visual cortex was further modulated by hippocampal BOLD activity. In the current study, we go beyond this simple two-association paradigm by using multiple associations and parametric behavioural measures to reliably assess variability in memory performance. We combined fast functional magnetic resonance imaging, multivariate analyses and a cued recall paradigm to rigorously investigate how early visual cortex and the hippocampal formation support mnemonic reinstatement. Human participants learned tone-grating associations and subsequently performed a recall task in which they were cued with tones and adjusted a line to match the remembered orientation of the associated grating. Analyses of behavioural data indicate that there is considerable variability in memory performance between different associations within as well as across participants. To examine memory-specific reinstatement in visual cortex we developed an analysis approach employing a forward modelling framework, in which hypothetical orientation channels, weighted by data from an orthogonal task, can be used to predict voxel responses in early visual cortex during recall. In

ongoing analyses, this approach allows us to link fine-grained behavioural measures and neural estimates of the participants' mnemonic representations.

Disclosures: **S.E. Bosch:** None. **C.F. Doeller:** None.

Poster

082. Human Long-Term Memory: Medial Temporal Lobe I

Location: Halls A-C

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Topic: F.01. Human Cognition and Behavior

Support: German Research Council (DFG MO930/4-1)

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Studienstiftung des deutschen Volkes

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Title: Single-unit activity in the human medial temporal lobe during an episodic memory task and subsequent sleep

Authors: *J. NIEDIEK[1], T. REBER[1], H. GAST[1], J. BOSTRÖM[2], V. A. COENEN[2,3], C. E. ELGER[1], F. MORMANN[1];
[1]Dept. of Epileptology, [2]Dept. of Neurosurg., Univ. of Bonn, Bonn, Germany; [3]Dept. of Stereotactic and Functional Neurosurg., Univ. of Freiburg, Freiburg, Germany

Abstract: Episodic memory can be defined as our ability to mentally recall personal experiences, including contents, time, and place of experienced events. Lesion studies, as well as extensive research using functional MRI, have shown that the hippocampus is a necessary structure for the encoding of episodic memory, but the mechanisms by which neuronal ensembles enable episodic memory processes remain unknown. One hypothesis is based on research in rodents, where place-cells (spatially selective cells) in the hippocampus have been found to contribute to spatial memory: Sequences of place-cell activity during slow-wave sleep resemble sequences of place-cell activity during previous exploration of an environment. Experimental disruption of these re-activations severely impairs spatial memory. Our current research focuses on "semantic neurons" in the human medial temporal lobe (MTL). These neurons are tuned to semantic concepts: They respond to the presentation of different pictures or

the written name of, for example, a certain celebrity, but not to any other pictures or names. It has been speculated that these neurons constitute a more abstract version of rodent place-cells, representing semantic concepts instead of locations. We hypothesized that the mechanism by which semantic neurons in the human MTL contribute to episodic memory resembles, to a certain extent, the re-activation of place-cell firing sequences in rodents. We recorded single- and multi-unit activity from different subregions of the MTL of 20 epilepsy patients who had been implanted with intracerebral electrodes for presurgical monitoring. The patients learned a simple story, involving up to 10 concepts for which semantic neurons had previously been identified. These concepts were represented in the stories both as pictures and as written names. Learning consisted of several re-iterations of reading and free recall. Similar to previous findings, we observed internally generated activations of semantic neurons during recall prior to vocalization. Furthermore, we used polysomnography to identify sleep stages. We found increased activity of semantic neurons during slow wave sleep compared to REM sleep. Furthermore, reactivations of semantic neurons were linked to sharp-wave ripple activity. These findings give first, indirect evidence in favor of the hypothesis that semantic neurons contribute to episodic memory.

Disclosures: **J. Niediek:** None. **T. Reber:** None. **H. Gast:** None. **J. Boström:** None. **V.A. Coenen:** None. **C.E. Elger:** None. **F. Mormann:** None.

Poster

082. Human Long-Term Memory: Medial Temporal Lobe I

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 82.03/LL23

Topic: F.01. Human Cognition and Behavior

Title: The schema effect and sleep-dependent memory consolidation

Authors: N. HENNIES, *M. LAMBON RALPH, J. COUSINS, P. A. LEWIS;
Univ. of Manchester, Manchester, United Kingdom

Abstract: Objectives The acquisition of knowledge is more effective if the new information can be incorporated into an associative schema. This advantage for schema-linked learning is thought to be driven by accelerated consolidation mechanisms. Whether or not sleep-dependent consolidation is involved in this process remains unclear. Here, we explore the interaction between sleep-dependent consolidation and schema-linked learning. Methods After establishing a schema over several days, participants encoded new facts that were either related to the schema (schema memories) or unrelated (non-schema memories). The encoding was done in two

sessions, 24 hours apart (consolidated and unconsolidated memories). Overnight sleep was polysomnographically monitored, and memory was tested in an MRI scanner directly after the second encoding. Results Participants showed a greater advantage for schema-linked learning after consolidation (p=0.003). Interestingly, this interaction between schema and consolidation correlated positively with the spindle density (r=0.6, p=0.006), and negatively with the percentage of stage 4 (S4) sleep (r=-0.62, p=0.003). A median split analysis further suggested that the spindle density was associated with the retention of schema memories (p=0.003), while S4 sleep was associated with the retention of non-schema memories. Functionally, we observed two separate clusters in the left medial temporal lobe, including hippocampus and parahippocampus, where the interaction between schema and consolidation was predicted by spindle density and time in slow wave sleep. Conclusion Our results provide initial evidence for an association between schema-linked learning and sleep-dependent memory consolidation, and suggest that deep sleep and sleep spindles may play differential roles.

Disclosures: **N. Hennies:** None. **M. Lambon Ralph:** None. **P.A. Lewis:** None. **J. Cousins:** None.

Poster

082. Human Long-Term Memory: Medial Temporal Lobe I

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Program#/Poster#: 82.04/LL24

Topic: F.01. Human Cognition and Behavior

Support: ERC-StG 261177

NWO-Vidi 452-12-009

Title: Grid cell representations in humans align to a common reference frame

Authors: ***T. NAVARRO SCHROEDER**, C. F. DOELLER;
Donders Institute, Radboud Univ. Nijmegen, Nijmegen, Netherlands

Abstract: Since the seminal discovery that entorhinal grid cells fire at lattices of a triangular grid in freely-moving rodents, the common notion had been that the orientation of the grid is relatively stable within animals, but random between animals. Recently, Stensola et al. [1] reported non-random grid-orientations across rats in a square environment. Currently, it remains elusive what the underlying mechanisms of this effect are and whether it would generalize to

non-square environments. In the present study, we sought to test if human six-fold symmetrical grid-representations align to a common reference frame in circular environments. By combining fMRI proxy-measures of cellular activity and navigation in virtual environments with well-controlled visual cues, we investigated grid-cell representations in humans. We observed an alignment of grid-representations in entorhinal cortex to a common reference frame in a circular virtual environment. Furthermore, we replicated this alignment effect in a second scanning session with the same participants and also in a different group of participants in a modified virtual environment with a different reference frame. The present finding of a common alignment of grid-representations in humans might suggest that, under specific environmental conditions, visual information is being used for grid formation in a stereotypical manner. This could be an important constraint for computational models of spatial navigation and could help to elucidate the network mechanisms leading to stable representations of spatial context. [1] T. STENSOLA, H. STENSOLA, M.-B. MOSER, E. I. MOSER. Environmental constraints on grid cell orientation. Society for Neuroscience meeting 2013

Disclosures: **T. Navarro Schroeder:** None. **C.F. Doeller:** None.

Poster

082. Human Long-Term Memory: Medial Temporal Lobe I

Location: Halls A-C

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Program#/Poster#: 82.05/LL25

Topic: F.01. Human Cognition and Behavior

Support: Biotechnology and Biological Sciences Research Council Grant BB/1007091/1

Title: Domain sensitive responses in medial temporal lobes examined with a large stimulus set

Authors: ***B. B. HARRY**[1], K. UMLA-RUNGE[2], K. GRAHAM[2], P. DOWNING[1];
[1]Sch. of Psychology, Bangor Univ., Bangor, United Kingdom; [2]Sch. of Psychology, Cardiff Univ., Cardiff, United Kingdom

Abstract: Representational models of medial temporal lobe (MTL) function propose that different sub-regions within the MTL are differentially sensitive to stimulus content. Evidence of domain sensitivity has been shown in cortical medial temporal regions, with object sensitivity found in the perirhinal cortex and scene sensitivity in the posterior parahippocampal gyrus. However, to date, sensitivity to stimulus content in medial regions has been demonstrated in studies that presented only a small number of categories (e.g., scenes and faces). The aim of the

present study was to measure MTL activity evoked by stimuli drawn from a broad range of categories to better characterise content sensitivity in this region. We measured brain activity with fMRI while participants (N = 20) performed a one-back task on stimuli from 20 categories in an event related design. A separate odd-one-out task consisting of faces, scenes, objects and shapes was used to localise category selective voxels in the occipito-temporal and medial temporal lobes, independently of the main experimental scans. We compared the response profile for the perirhinal cortex with category specific regions in the occipito-temporal cortex with multidimensional scaling to examine whether the responses observed in the medial temporal regions were typical of other category specific regions. All face and object sensitive regions - including the fusiform face area, occipital face area, lateral occipital, perirhinal cortex and amygdala - clustered together, whereas scene selective regions - including the parahippocampal place area and retrosplenial cortex - formed a separate cluster. To follow up recent reports that the perirhinal cortex may also respond to scenes (e.g., Preston et al., 2009), we analysed all voxels within a perirhinal mask (Holdstock et al., 2009). Face and object selective voxels (as defined in the localiser) showed the strongest response in the main experiment to faces and to other objects, respectively. In contrast, scene selective perirhinal cortex voxels (as defined in the localiser) did not show the strongest response to scenes in the main experiment, indicating that previous reports of scene selectivity in the perirhinal cortex may have been the result of using a restricted stimulus set. Specifically, these results provide a richer characterisation of category-related responses in MTL than previous work, and favour a model in which perirhinal cortex processes objects and faces (but not scenes) in concert with similar inferotemporal regions.

Disclosures: **B.B. Harry:** None. **K. Umla-Runge:** None. **K. Graham:** None. **P. Downing:** None.

Poster

082. Human Long-Term Memory: Medial Temporal Lobe I

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Program#/Poster#: 82.06/LL26

Topic: F.01. Human Cognition and Behavior

Support: ERC-StG 261177

NWO-Vidi 452-12-009

Title: Investigating the spatio-temporal organization of episodic memory within a virtual world

Authors: *L. DEUKER, J. BELLMUND, T. NAVARRO SCHROEDER, C. F. DOELLER; Donders Institute, Radboud Univ. Nijmegen, Nijmegen, Netherlands

Abstract: Both time and space are defining characteristics of everyday experience, and the question how spatial and temporal aspects are represented in episodic memory has been the focus of intensive research in recent years. While it has long been known that the local environment is encoded by spatially tuned cells in the hippocampal formation, specialized hippocampal neurons have been recently discovered in rodents, which seem to encode the temporal properties of specific episodes ("time cells"). However, it remains unclear how spatial and temporal information is integrated in the human brain. To investigate this, we created a novel complex virtual reality environment of the size of a downtown city area. In this virtual world, participants encountered several everyday objects as they repeatedly navigated along a predefined route. Critically, we dissociated the temporal distance between objects (how long it took participants to navigate from one object to the next) and the spatial distance between objects (how far objects were apart in the city). This allowed us to investigate the impact of spatial and temporal context on memory performance in a 2-by-2 design, with factors spatial distance (high vs low) and temporal distance (high vs low). Preliminary behavioural results indicate that participants were able to learn the routes and the locations of the objects with ease and could reproduce the relative spatial and temporal distance between pairs of objects in a subsequent memory test. Furthermore, in a free recall test, the order in which objects were reproduced reflected knowledge about the routes in the virtual environment. In a next step, simultaneously acquired fMRI data will be analysed with respect to the spatial and temporal structure of the newly formed episodic memories.

Disclosures: L. Deuker: None. **J. Bellmund:** None. **T. Navarro Schroeder:** None. **C.F. Doeller:** None.

Poster

082. Human Long-Term Memory: Medial Temporal Lobe I

Location: Halls A-C

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Topic: F.01. Human Cognition and Behavior

Support: ERC-StG 261177

NWO-Vidi 452-12-009

Title: Functional parcellation of the human entorhinal cortex

Authors: *N. I. ZARAGOZA JIMENEZ, T. NAVARRO SCHROEDER, E. VAN OORT, C. F. BECKMANN, C. F. DOELLER;
Donders Institute, Radboud Univ. Nijmegen, Nijmegen, Netherlands

Abstract: The entorhinal cortex (EC) is the interface between the hippocampus and the neocortex. Since the discovery of grid cells in the rodent medial EC, it has been recognized as a crucial hub for spatial coding and navigation in rodents. Traditionally, the EC has been subdivided into a medial (MEC) and a lateral (LEC) portion. Postmortem comparative anatomical evidence, however, suggests broader subdivisions among rodents, non-human primates and humans. Cytoarchitectonically, up to nine subdivisions of the human EC have been distinguished. Several cytoarchitectonic features also suggest a modular arrangement, such as periodic bundling of pyramidal cell dendrites and axons. Furthermore, tract tracing studies in rodents and non-human primates suggest a wide range of projections along the EC, with medial portions of the EC projecting to the temporal portion of the hippocampus, while lateral and caudal portions connected to the septal hippocampus. Differential connectivity pattern of EC subregions could thus lead to distinct intrinsic signal fluctuations, visible to functional magnetic resonance imaging (fMRI) at high spatial resolution. However, knowledge about the fine-grained, in-vivo fMRI-based parcellation of the EC has been scarce. Here, we aimed to parcellate the human EC based on its intrinsic signal fluctuations during a virtual navigation task combined with high-resolution, submillimetre fMRI at 7T. We generated a functional parcellation of the EC by leveraging a novel instantaneous correlation analysis approach, termed instantaneous connectivity parcellation (ICP) [1]. ICP provides information on how the functional connectivity within a region evolves over time. Subsequently, independent component analysis (ICA) was used across subjects to create three-dimensional parcellations of the EC based on its intrinsic functional properties. The resulting parcellations showed strong correspondence to topographies from postmortem cytoarchitectonic studies. These findings allow us to test connectivity fingerprints of human EC subregions *in vivo* and could open up the possibility to investigate neural mechanisms of spatial cognition at an unprecedented level of detail. References[1] Van Oort et al. in preparation

Disclosures: **N.I. Zaragoza Jimenez:** None. **T. Navarro Schroeder:** None. **E. van Oort:** None. **C.F. Beckmann:** None. **C.F. Doeller:** None.

Poster

082. Human Long-Term Memory: Medial Temporal Lobe I

Location: Halls A-C

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Program#/Poster#: 82.08/LL28

Topic: F.01. Human Cognition and Behavior

Support: ERC-StG 261177

NWO-Vidi 452-12-009

Title: Prospective event representation in the hippocampus depends on contextual certainty and time

Authors: *P. W. SMULDERS, B. MILIVOJEVIC, S. E. BOSCH, F. P. DE LANGE, C. F. DOELLER;
Donders Institute, Radboud Univ. Nijmegen, Nijmegen, Netherlands

Abstract: Recent evidence from single cell recordings in rodents and neuroimaging work in humans suggests that the hippocampus represents events in a prospective manner. However, it remains unclear on what temporal scales prospective events are coded by the hippocampus and whether such prospective coding can facilitate decision making. Here, we used functional MRI to record brain activity while participants viewed pictures of faces, houses and objects during three experimental phases. During a learning phase, participants performed an object-categorisation task in which stimuli were organized into triplets so that a face or a scene was always preceded by two objects: the context. The contextual pairs of objects were either fully predictable of the upcoming 'final' stimulus (predictable context) or they created uncertainty as to whether the upcoming stimulus would be a face or a scene (uncertain context). Before and after the learning phase, participants viewed the same images in a random order. We hypothesized that neural-pattern similarity between the 'final' item and the 'context-defining' items would change as a consequence of learning to discriminate between the predictable and uncertain contexts. Over the course of learning, we found that reaction times decreased for the critical 'final' item in predictable contexts compared to uncertain contexts indicating that participants were able to successfully use context to facilitate their behavioural responses. Hippocampal activity gradually increased from the onset of the first item in the triplet to presentation of the 'final' item for predictable but not uncertain contexts. Using representational similarity analysis, we compared neural-pattern similarity before and after learning and found an increase in similarity between the predictable 'final' item and the 'context-defining' items and a decrease in similarity between the uncertain 'final' item and the 'context-defining' items in the medial temporal lobe and the striatum. Furthermore, we found that association between the 'final' item and the preceding context depended on time whereby posterior hippocampus represented associations on shorter timescales (i.e. between the second 'context-defining' item and the 'final' item) while anterior hippocampus was sensitive for the association on longer timescales (i.e. between the first 'context-defining' item and the 'final' item). These preliminary

data suggest that the hippocampus uses contextual information to represent upcoming events simultaneously at multiple timescales resembling a well-known gradient in the representation of spatial distance along the long-axis of the hippocampus in rodents.

Disclosures: P.W. Smulders: None. **B. Milivojevic:** None. **S.E. Bosch:** None. **F.P. de Lange:** None. **C.F. Doeller:** None.

Poster

082. Human Long-Term Memory: Medial Temporal Lobe I

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 82.09/MM1

Topic: F.01. Human Cognition and Behavior

Support: ERC-StG 261177

NWO-Vidi 452-12-009

Title: Dynamic representation of multi-event narratives in the medial temporal lobe

Authors: *S. H. COLLIN, B. MILIVOJEVIC, C. F. DOELLER;
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Abstract: One of the key features of episodic memory formation is the integration of multiple events and experiences into coherent mnemonic representations. We have previously shown that linking two events into one narrative is accompanied by increased neural pattern similarity in the hippocampus and medial prefrontal cortex, which suggests the formation of a shared memory representation coding for linked events. However, it remains unclear how flexible and malleable these novel narrative representations are. In the current fMRI study, we aimed to address this outstanding question by extending the narratives. To do so, we presented the participants with three-event narratives, which were sequentially built up over time, as well as control events that were never integrated into the narratives. Behavioural results show that participants are able to make direct associations between the events with high accuracy. Based on evidence for anatomical and functional specialization within the medial temporal lobe, we hypothesized that the resolution of narrative representations would differ within the medial temporal lobe. Preliminary results of multi-voxel pattern analyses indicate that the representation of direct associations between events, and the representation of the entire multi-event narrative coexist in the medial temporal lobe. These findings suggest that episodic memories might be organized

hierarchically, with representation of individual associations and multi-event narratives co-localized within the hippocampal formation.

Disclosures: **S.H. Collin:** None. **B. Milivojevic:** None. **C.F. Doeller:** None.

Poster

082. Human Long-Term Memory: Medial Temporal Lobe I

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 82.10/MM2

Topic: F.01. Human Cognition and Behavior

Support: ERC-StG 261177

NWO-Vidi 452-12-009

Title: Hippocampal-prefrontal theta oscillations support memory integration in humans

Authors: ***A. R. BACKUS**[1], S. SZEBÉNYI[1,2], J. M. SCHOFFELEN[1,3], S. HANSLMAYR[4,2], C. F. DOELLER[1];
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Abstract: The integration of separate memories forms the basis of inferential reasoning - an essential cognitive process that enables complex behaviour. Considerable evidence from lesion studies in rodents and neuroimaging studies in humans suggest that both the hippocampus and medial prefrontal cortex play a crucial role in memory integration. Although electrophysiology indicates that theta oscillations govern the communication between hippocampus and prefrontal cortex, the oscillatory mechanism underlying memory integration in humans remains elusive. To bridge this gap, we recorded magnetoencephalography data while participants performed an associative inference task. Source power analyses suggest that theta oscillations in medial temporal lobe during encoding predict subsequent memory integration. Moreover, we observe increased encoding-related theta coherence between hippocampus and medial prefrontal cortex for later successfully integrated associations. In sum, our preliminary results suggest that integrated memory representations arise through theta oscillations that engage the medial temporal lobe and might help to orchestrate communication with the medial prefrontal cortex.

Disclosures: **A.R. Backus:** None. **S. Szebényi:** None. **J.M. Schoffelen:** None. **S. Hanslmayr:** None. **C.F. Doeller:** None.

Poster

082. Human Long-Term Memory: Medial Temporal Lobe I

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Topic: F.01. Human Cognition and Behavior

Support: NIH Grant NS19632

NIH Grant MH062500

Title: Brain regions supporting memory processes: using voxelwise lesion-symptom mapping to identify the neural correlates of performance on neuropsychological tests of memory

Authors: *D. E. WARREN[1], J. BRUSS[1], J. GLÄSCHER[2], D. TRANEL[1];
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Abstract: Declarative memory for facts and events depends on the medial temporal lobe (MTL) and hippocampus, but the degree to which specific, widely-used neuropsychological tests of memory are specifically related to MTL or other brain damage has not been conclusively established. Drawing on the Iowa Neurological Patient Registry, we applied voxelwise lesion-symptom mapping (VLSM) in order to identify the neural correlates of performance on common neuropsychological tests of memory in a large sample of patients with focal brain injury (max N=450, with sample size varying somewhat by test). The Registry contains data for patients with focal, stable brain lesions who have undergone neuropsychological testing at least 3 months after symptom onset. Brain lesions were identified using structural neuroimaging (CT or MRI) and mapped in a common template space using the MAP-3 method. These lesion maps, along with memory test performances, were submitted to VLSM analysis. Several distinct memory processes were probed including semantic memory (Wechsler Adult Intelligence Scale, Vocabulary and Information subtests; Wide Range Achievement Test, Spelling subtest), short-term visual memory (Benton Visual Retention Test), delayed visual memory (Complex Figure Task, recall), short term verbal memory (Rey Auditory Verbal Learning Task, trial 1), and delayed verbal memory (Rey Auditory Verbal Learning Task, delay). Composite memory indices from the Wechsler Memory Scale (e.g., General Memory Index, Immediate Memory

Index, Delayed Memory Index) were also analyzed. Our results reinforce the critical role of the MTL and hippocampus in supporting the declarative memory operations exercised by these neuropsychological tests, and also demonstrate significant lateralization of function within that region while highlighting the importance of other brain regions for normal memory processes. These findings provide important insight into the relationship between neuropsychological tests of memory and the necessity of certain brain regions by contributing empirical support to the validity of widely-held assumptions about behavior, cognition, and brain injury in the domain of memory. This work should positively impact clinical practice by further informing brain-behavior relationships.

Disclosures: **D.E. Warren:** None. **J. Bruss:** None. **J. Gläscher:** None. **D. Tranel:** None.

Poster

082. Human Long-Term Memory: Medial Temporal Lobe I

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Support: ERC-StG 261177

NWO-Vidi 452-12-009

Title: Investigating the relationship between head direction representations and spatial cognition

Authors: ***J. BELLMUND**, L. DEUKER, T. NAVARRO SCHROEDER, C. F. DOELLER; Donders Institute, Radboud Univ. Nijmegen, Nijmegen, Netherlands

Abstract: In order to be able to successfully navigate its environment, an organism requires information about its location and orientation. While place cells code for location, head direction cells provide information about the animal's spatial orientation in the environment. In rodents, head direction cells have been found throughout Papez circuit and recent recording work demonstrates that the firing of head direction cells directly impacts spatial behaviour. Despite previous evidence for head direction representations in humans during scene viewing and virtual navigation, the behavioural relevance of the head direction system in humans remains elusive. To investigate this, we combined fMRI with a virtual reality (VR) task in which participants freely navigated a virtual arena with a circular layout and learned the locations of objects over multiple trials. Here, we show that entorhinal cortex exhibits heading direction specific

representations during virtual navigation. Furthermore, the strength of this directional effect was predictive of spatial memory performance. Our preliminary findings shed light on the relationship between head direction representations and spatial cognition in humans. In an ongoing follow-up study, we investigate the relationship between head direction related fMRI responses and a continuous measure of directional behaviour in a realistic, large-scale VR city. Preliminary behavioural results show that participants successfully represented directions between different locations of the VR environment.

Disclosures: **J. Bellmund:** None. **L. Deuker:** None. **T. Navarro Schroeder:** None. **C.F. Doeller:** None.

Poster

082. Human Long-Term Memory: Medial Temporal Lobe I

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Topic: F.01. Human Cognition and Behavior

Support: MRC Grant G1000854

Wellcome Trust Grant 095811

Title: Opposing effects of negative emotion on item and associative memory are predicted by activity in amygdala and hippocampus

Authors: ***J. A. BISBY**[1,2], A. J. HORNER[1,2], L. D. HORLYCK[1], N. BURGESS[1,2]; [1]UCL Inst. of Cognitive Neurosci., London, United Kingdom; [2]UCL Inst. of Neurol., London, United Kingdom

Abstract: The formation of associations between items in memory is thought to rely on hippocampal-dependent mechanisms which go beyond those supporting memory for a single item. Whilst memory for emotionally negative items is enhanced, associative memory can be reduced (e.g., Bisby & Burgess, 2014, Learning & Memory, 21:760-6). Enhanced memory for negative items is thought to rely on amygdala-dependent processes, but the way in which negative emotion affects hippocampal-dependent associative memory remains unclear. We aimed to further understand the contributions of the amygdala and hippocampus to item and associative memory, and the effect on these of negative affect. Using fMRI, we examined encoding and retrieval of paired associates of neutral and negative images. At encoding,

participants viewed pairs of images presented as pure pairs (neutral-neutral or negative-negative) or mixed pairs (neutral-negative). At test, participants were cued with one image and instructed to retrieve the associated (target) image. All combinations of neutral and negative cue and target items were used. Results showed enhanced memory for negative images and reduced associative memory for pairs that included a negative image. Subsequent memory for items was correlated with amygdala activity during encoding, while subsequent associative memory was associated with hippocampal activity during encoding. We also saw a reduction in hippocampal activity in the presence of a negative image compared to viewing neutral images, during encoding trials and during correct associative retrieval trials. Finally, our results showed increased activity in the amygdala for correctly retrieved negative images, even when cued by a neutral image. Our findings suggest negative emotion can enhance amygdala-dependent item memory but disrupt hippocampus-dependent associative memory.

Disclosures: **J.A. Bisby:** None. **A.J. Horner:** None. **L.D. Horlyck:** None. **N. Burgess:** None.

Poster

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NTU 10R80918

NTU 10R8091803

Title: Memory formation in figure-noun association increased functional connectivity between the hippocampus and inferior frontal gyrus

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Abstract: Formation of declarative memory may alter connectivity in the cortical network and such change could be initiated by influence from the medial temporal lobe, particularly the hippocampal formation. To detect functional connectivity changes in the memory network

during human verbal learning, we examined the neural correlates of memory formation for figure-word pairs in human participants by functional magnetic resonance imaging (fMRI) and resting-state functional connectivity. Sixteen participants were recruited to learn 24 pairs of figure-noun associations and the retention was tested by recognition 24 hours later. The participants were subjected to brain scans before, during and after the acquisition trial in the following order: a pre-learning baseline resting-state scan, the paired association learning along with fMRI scan, an interpolated task, and a post-learning resting-state scan. During the resting-state scans, all participants were instructed to close their eyes but remain awake and think of nothing. The learning task required participants to memorize 24 pairs of Fourier figure-Chinese noun association while they underwent fMRI scans. The interpolated task was an attention task that lasted for 1 hour in order to prevent participants from active rehearsal of the learned materials during the memory formation period. Two major findings came out from analyses of the available data. First, learning figure-word association was accompanied with greater bilateral activation in the visual cortices and inferior frontal gyrus (IFG), which was presumably related to the stimulus processing. Second, a contrast of the post-learning resting scan versus the pre-learning resting scan revealed an increase in the functional connectivity between the hippocampus and the right IFG and also between the hippocampus and left inferior parietal lobule (IPL). These findings of increased resting-state functional connectivity suggest the presence of an interaction between the hippocampus and certain cortical areas during the memory formation period. This increased functional connectivity may play a critical role for the hippocampus to modulate memory formation in the neocortex.

Disclosures: **K. Liang:** None. **Y. Wang:** None. **J. Chen:** None. **T. Chou:** None.

Poster

082. Human Long-Term Memory: Medial Temporal Lobe I

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Topic: F.01. Human Cognition and Behavior

Support: CIHR Grant 301763

Title: Sleep interruptions impair learning in a virtual navigation task

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Abstract: Aim: Researchers have established a relation between sleep, and learning and memory. In the rodent hippocampus (HPC), the same group of place cells activated in a navigation task were reactivated during sleep (Wilson and McNaughton, 1994). In humans, sleep was shown to reorganize brain activity such that spatial memory was mediated more by the striatal network than HPC structures (Orban et al, 2006). Sleep deprivation, as well as stress, impairs HPC-dependent memory. Interestingly, stress hormones impair memory only during sleep but improve memory in the wake state (Kelemen, et al, 2014). In this study, we studied the relation between sleep and navigational strategies dependent on the hippocampus and caudate nucleus. Methods: We tested 20 healthy older adults on a 4-on-8 virtual maze (4/8VM) designed to differentiate between the two strategies. Each trial consisted of 8 arms branching out from the center. Participants must retrieve objects at the end of the arms. In part 1 of the learning stage, 4 arms were blocked, and objects were located in the open arms. In part 2, all arms were open and participants had to retrieve objects from the 4 previously blocked arms. A probe trial was administered after participants reached the criterion in the learning stage. The criterion was making zero errors on part 2 of a trial. In the probe, all landmarks were removed allowing for the dissociation of the two strategies. The sleep data were obtained with an Actiwatch that detected counts of body motion in two separate nights. The metrics included duration of sleep/wake period, the ratio of sleep/wake duration over the entire rest period, wake time after sleep onset (WASO), and the time spent before sleep onset. Results: WASO was significantly correlated with the number of trials needed to reach the criterion on the 4/8VM ($r=.49$, $p < .05$), indicating that the more people woke up after sleep, the more trials they needed to reach the criterion. No significant differences were found between the two strategies at $p <.05$ level on any measures of sleep. As per our previous findings (Iaria et al, 2003), spatial learners required more trials to reach the criterion and made more errors in both the learning stage and probe trial. Conclusion: Consistent with previous studies, sleep seems to be associated with general learning ability. Sleep interruptions were associated with slower learning on a navigation task.

Disclosures: **Y. Yang:** None. **K. Konishi:** None. **R. Gruber:** None. **V.D. Bohbot:** None.

Poster

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Topic: F.01. Human Cognition and Behavior

Support: MRC Grant G1000854

Wellcome Trust Grant 095811

Title: Pattern completion for episodic events in the human hippocampus

Authors: *A. J. HORNER[1,2], W.-J. LIN[1,2], J. BISBY[1,2], N. BURGESS[1,2];
[1]UCL Inst. of Cognitive Neurosci., London, United Kingdom; [2]UCL Inst. of Neurol., London, United Kingdom

Abstract: The multiple elements of episodic events, represented in distinct neocortical regions, are thought to be bound into coherent 'event engrams' by the hippocampus (Damasio, 1989; Tulving, 1983). These engrams should allow for the retrieval of all event elements via pattern completion, resulting in their 'reinstatement' in the neocortex (Marr, 1971). We have previously shown behavioural dependency between retrieval of different elements of the same event (Horner & Burgess, 2013). For example, for location-person-object events, if we remember the location of a specific event we should be more likely to remember the person and object from that same event. We have further shown that this behavioural dependency is due to pattern completion at retrieval, rather than fluctuations in encoding strength (Horner & Burgess, 2014). Thus, pattern completion was seen even when the overlapping pairwise associations comprising an event were presented separately, but was only seen when all possible within-event pairwise associations were encoded, forming a 'closed-loop' associative structure, but not when incomplete 'open-loop' sets of associations were encoded. Here we asked whether pattern completion is related to hippocampal activity and whether it results in the reinstatement of event elements in the neocortex. In an fMRI experiment, participants learned events consisting of multiple distinct elements (locations, people and objects/animals) via separate presentation of the pairwise associations. At retrieval, all pairwise associations from each event were tested. During encoding of paired associates from the closed-loop condition, hippocampal activity predicted subsequent memory performance for all within-event elements, supporting the idea that the hippocampus binds the multimodal elements of events into event engrams. During retrieval of a paired associate hippocampal activity was greater for 'closed-loop' relative to 'open-loop' events, and correlated with reinstatement activity corresponding to all within-event elements in the neocortex. Thus, we provide evidence for the reinstatement of event elements in the neocortex following hippocampal pattern completion.

Disclosures: **A.J. Horner:** None. **W. Lin:** None. **J. Bisby:** None. **N. Burgess:** None.

Poster

082. Human Long-Term Memory: Medial Temporal Lobe I

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Topic: F.01. Human Cognition and Behavior

Support: CIHR Grant 8464

FRSQ Grant 26608

Title: The BDNF val66met polymorphism is associated with decreased use of landmarks and decreased fMRI activity in the hippocampus during virtual navigation

Authors: *K. KONISHI[1], R. JOOBER[1], J. BREITNER[2], V. D. BOHBOT[1];
[1]Psychiatry, Douglas Mental Hlth. Univ. Institute, McGill Univ., Verdun, QC, Canada; [2]Dept. of Psychiatry, McGill Univ., Ctr. for Studies on Prevention of Alzheimer's Dis. (StoP-AD), Douglas Mental Hlth. Univ. Inst., Montreal, QC, Canada

Abstract: Aim: People can navigate in a new environment using one of two strategies. A series of studies have dissociated between hippocampus-dependent "spatial" navigation and habit-based "response" learning mediated by the caudate nucleus. The val66met polymorphism of the brain-derived neurotrophic factor (BDNF) gene leads to decreased secretion of BDNF in the brain, including the hippocampus. In young adults the BDNF val66met polymorphism is associated with the decreased use of spatial strategies during virtual navigation and decreased fMRI activity in the human hippocampus. Here, we aim to investigate the role of the BDNF val66met polymorphism on virtual navigation behaviour and brain activity in healthy older adults. Methods: 139 healthy older adult participants (mean age=65.8 ± 4.4 yrs) were tested in this study. Blood samples were collected and BDNF val66met genotyping was performed. Participants were divided into two distinct genotype groups: val/val and met carriers. The 4on8 Virtual Maze (4/8VM) consists of an 8-arm radial maze, in which 4 arms are accessible and 4 are blocked. Participants have to retrieve objects located at the end of the 4 accessible arms. Then, all 8 arms become accessible and participants have to retrieve objects now located in the 4 arms that were previously blocked. After participants have learned the task, a probe trial is given in which all landmarks are removed. The probe trial allows us to dissociate spatial and response learners, as spatial learners make more mistakes compared to response learners. A subset of the participants (n=66) were scanned in a 3T MRI scanner while engaging in a virtual navigation task called the Concurrent Spatial Discrimination Learning Task (CSDLT). The CSDLT is a virtual 12-arm radial maze in which participants have to learn the location of objects within 6 pairs of arms. Results BDNF val/val individuals made more errors on the probe trial of the 4/8VM compared to BDNF met carriers, confirming that val/val individuals relied more on landmarks to remember target locations (i.e. increased use of the spatial strategy). Additionally, BDNF val/val individuals had more fMRI activity in the hippocampus compared to BDNF met carriers during performance of the CSDLT. Conclusion: Our results are consistent with findings in young adults, showing that, even with aging, the BDNF val/met polymorphism is associated with dysfunction of the hippocampus.

Disclosures: **K. Konishi:** None. **R. Joober:** None. **J. Breitner:** None. **V.D. Bohbot:** None.

Poster

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Topic: F.01. Human Cognition and Behavior

Support: CIHR Grant 8464

Title: Non-depressed older adult response learners score slightly but significantly lower than spatial leaners on a depression scale

Authors: *Z. K. CHAUDHARY[1], K. KONISHI[2], V. D. BOHBOT[2];
[1]Psychiatry, McGill Univ., Montreal, QC, Canada; [2]Douglas Mental Hlth. Univ. Inst., Montreal, QC, Canada

Abstract: Aim: People spontaneously use different strategies when they navigate in an environment and these strategies rely on different memory systems. The spatial strategy involves remembering the locations of objects in relation to landmarks in the environment, while the stimulus-response strategy, relies on making a series of stimulus-response associations (e.g., right/left turns). These strategies depend on the hippocampus and the striatum, respectively. The use of these strategies has implications for other cognitive processes. Namely, the use of striatum-dependent response strategies is associated with an increased use of drugs of abuse and alludes to heightened sensitivity to reward. Given their reward-seeking tendencies, we asked whether non-depressed response learners would also have more positive affect compared to spatial learners. Methods: 139 healthy older adults (mean age: 66.4) completed the Geriatric Depression Scale (GDS), a self-report questionnaire used to assess depression in the elderly. They were also tested on the 4-on-8 virtual maze, a task that requires remembering the locations of 4 objects in an 8-arm radial maze. Participants retrieved objects located at the end of four accessible arms in a landscape embedded with an array of landmarks after which they were instructed to avoid these previously visited paths to retrieve the objects among eight accessible arms. They completed this task until they reached criterion (100% accuracy) for at least one trial and moved onto a probe trial in which landmarks were removed, rendering spatial learners more prone to making errors. Participants' spontaneous navigation strategies were then assessed and they were classified as either spatial or response learners using both probe trial errors and verbal reports. Results: When differences in scores on the GDS were compared across navigational

strategies, response learners (mean = 2.61) had significantly lower scores compared to spatial learners (mean = 3.66; t = 2.34, p = 0.021). Conclusions: GDS has been shown to correlate inversely with positive affect and to correlate directly with negative affect. Furthermore, there is a distinction between two clusters of depression-related symptoms (increased negative affect, decreased positive affect), and these are mediated primarily by different neurotransmitter systems (serotonergic and dopaminergic, respectively). It is then possible that a disparity in the level of positive affect between spatial and response learners could reflect: a) their previously reported differential reward seeking tendencies and b) tendencies towards different personality traits among spatial and response learners.

Disclosures: **Z.K. Chaudhary:** None. **K. Konishi:** None. **V.D. Bohbot:** None.

Poster

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Program#/Poster#: 82.19/MM11

Topic: F.01. Human Cognition and Behavior

Support: CIHR Grant 8464

Title: Luteal phase of the menstrual cycle positively correlates with use of a spatial strategy in a human virtual navigation task

Authors: ***S. HANAFI**[1], D. HUSSAIN[2], K. KONISHI[1], W. BRAKE[2], V. D. BOHBOT[1]; [1]Douglas Mental Hlth. Univ. Inst., Montreal, QC, Canada; [2]Concordia Univ., Montreal, QC, Canada

Abstract: Aim: People spontaneously use a spatial or response strategy when learning to navigate in an environment. These strategies depend on the hippocampus and the caudate nucleus, respectively. Estrogen (E) is known to enhance dendritic spine density in hippocampal CA1 pyramidal cells (Gould, et al., 1990). Furthermore, in women, concentrations of E flux in a regular manner over the course of the menstrual cycle. Notably, there is a surge of E during the second half of the cycle (i.e. the luteal phase). In addition, the hippocampus has been shown to undergo an increase in gray matter as women progress from the late-luteal (low E) to late-follicular (high E) phases of the menstrual cycle (Protopescu et al., 2008). Thus we hypothesized that women tested during the luteal phase of their menstrual cycle would be more likely to use a spatial strategy on a virtual navigation task, as previously found in rodents (Korol, 2004).

Methods: We tested 44 young women (ages 18-35) on the 4-on-8 virtual maze (4/8VM). Participants were grouped for phase of menstrual cycle on the date of testing based on data gathered via a questionnaire. Eighteen participants were in the luteal phase and 26 participants were in the follicular phase of their menstrual cycle. The 4/8VM consists of an 8-arm radial maze, in which 4 arms are accessible and 4 are blocked. Participants have to retrieve objects located at the end of the 4 accessible arms. Then, all 8 arms become accessible and participants have to retrieve objects now located in the 4 arms that were previously blocked. Participant's strategies are assessed with a verbal report and a probe trial whereby all landmarks are removed, leading to errors only in participants who had previously used these landmarks. Results: As per our hypothesis, 66.7% of women tested during their luteal phase used a spatial strategy in contrast to only 38.5% of women tested during their follicular phase. Consistent with our previous work showing that spatial strategies are more cognitively demanding (Iaria et al., 2003), women tested during their luteal phase required significantly more trials to reach criteria and made more errors when compared to women tested during their follicular phase. Conclusion: We concluded that the luteal phase, during which E is high, is associated with increased use of a spatial navigational strategy. This is congruent with Qiu et al. (2013), who found that hippocampal volume in rats increased during the proestrus (i.e. high E) phase, and that these increases were positively correlated with the use of a spatial strategy on a dual solution T-maze. This evidence suggests that the association of phase and navigational strategy may be due to elevated E levels.

Disclosures: **S. Hanafi:** None. **D. Hussain:** None. **K. Konishi:** None. **W. Brake:** None. **V.D. Bohbot:** None.

Poster

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CIHR Grant 86727

CIHR Grant 230771

Title: Patients with mild cognitive impairment show increased fMRI activity in the hippocampus following a spatial memory intervention program

Authors: *D. SODUMS[1], K. KONISHI[1], V. NAIR[1], H. CHERTKOW[2], S. GAUTHIER[1], L. KOSKI[3], L. BEHRER[4], V. D. BOHBOT[1];
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Abstract: Aim: Atrophy of the hippocampus has been observed in patients with Mild Cognitive Impairment (MCI) and has been shown to be associated with an increased probability of conversion to Alzheimer's disease (AD). Hence, it is relevant to explore methods that could stimulate the hippocampus and potentially delay the onset of dementia among people who are at a high risk of developing AD. Previous research in our laboratory has shown that healthy participants spontaneously use different strategies when navigating in a virtual environment. More specifically, the use of spatial memory strategies has been found to be associated with increased activity and grey matter in the hippocampus while the use of stimulus-response strategies has been found to be associated with increased activity and grey matter in the caudate nucleus. Since AD affects the function and volume of the hippocampus, we developed a computerized spatial memory intervention program (SMIP) to specifically stimulate this region. This program, developed and validated over the course of 5 years, has been designed to promote extensive use of spatial memory strategies, taking particular attention to minimize the use of stimulus-response strategies. Methods: MCI participants underwent the SMIP. Each participant underwent a functional Magnetic Resonance Imaging (fMRI) scan before and after the SMIP. During this scan participants performed a virtual navigation task, called the Concurrent Spatial Discrimination Task (CSDLT), to assess potential change in fMRI activity in the hippocampus. The CSDLT is a virtual 12-arm radial maze in which participants have to learn the location of objects within 6 pairs of arms. The SMIP is comprised of 16 one-hour spatial memory training sessions administered to participants twice a week during the course of eight weeks. The participants navigate in 46 different virtual environments that vary in size and complexity in which the relative positions of objects, landmarks, or rooms need to be learned. Results: After intervention, there was a significant increase in the functional activity in the hippocampus during the first experimental trial of the virtual navigation task, consistent with early activity in the hippocampus established in previous studies. Conclusion: The results suggest that promoting spatial strategies leads to increased fMRI activity in the hippocampus during virtual navigation tasks. Though these results are preliminary, they are promising and suggest the potential effectiveness of SMIP restoring function in the hippocampus of patients at risk for AD.

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Poster

082. Human Long-Term Memory: Medial Temporal Lobe I

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 82.21/MM13

Topic: F.01. Human Cognition and Behavior

Support: Wabash College

CIHR #265167

Title: Assessing navigation performance in virtual environments on mobile devices

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Abstract: Aim: Navigation paradigms are useful for the study of memory in non-human animals, and virtual navigation tasks have allowed for comparative and neuroimaging studies in humans. However, virtual navigation tasks typically collect data using desktop and laptop computers, while mobile devices and tablets are becoming increasingly prevalent. The aim of this study was to assess navigation performance using standard virtual navigation tasks adapted for mobile devices. **Methods:** A set of virtual navigation tasks (Barnes maze, Morris water maze, and radial maze task) were developed for Android tablets. The tasks were released as a game in an online store. Upon the first use after installation, participants were randomly assigned to a Barnes maze, Morris water maze, or the 4-on-8 virtual radial maze (based on Iaria, Petrides, Dagher, Pike, Bohbot, 2003). More participants were assigned to the Barnes maze task than to the other tasks. After the first attempt, users could select additional tasks to complete. Participants were also asked to submit their sex and age after each task. **Results:** A total of 10,973 installations of the game were obtained. From all of the installations, 10,490 Barnes maze sessions, 819 water maze sessions, and 652 radial maze sessions were submitted. A total of 5,721 sex responses and 5,255 age responses were submitted. Each task included a probe trial, used to assess spatial learning (water maze), or the use of spatial versus response strategies (Barnes and radial maze). Behavioral performance across the tasks was not well related, with some exceptions. Across all three tasks, reaction times on the probe trial and the trial preceding the probe were more consistently related than other measures. Also, participants who made fewer errors on the radial maze task probe trial were also more likely to use a response strategy on the Barnes maze probe. In the water maze, men had better performance than women early in the

task. However, sex was not associated with performance or strategy on the Barnes or radial maze. Additionally, age was associated with longer latencies in the water maze, and with the use of response strategies on the Barnes maze probe trial. Data were also collected using a desktop version of the task under controlled laboratory conditions, and largely replicated the findings in the mobile sample. **Conclusion:** While these mobile tasks are currently being validated against standard virtual navigation tests, our results demonstrate the utility of remote data collection from mobile devices, and the potential of such devices to collect data from large samples for clinical and epidemiological studies.

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Poster

082. Human Long-Term Memory: Medial Temporal Lobe I

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Program#/Poster#: 82.22/MM14

Topic: F.01. Human Cognition and Behavior

Support: CIHR Grant 8464

Title: Higher dietary vitamin K intake is associated with increased use of the spatial strategy in healthy older adults

Authors: *N. ANDRUCHOW[1], K. KONISHI[2], B. SHATENSTEIN[3], V. D. BOHBOT[2]; [1]Dept. of Psychiatry, Douglas Mental Hlth. Univ. Institute, Dept. of Psychiatry, McGill Uni, Verdun, QC, Canada; [2]Douglas Mental Hlth. Univ. Inst., Montreal, QC, Canada; [3]Inst. universitaire de gériatrie de Montréal, Univ. de Montréal, Montreal, QC, Canada

Abstract: Aim: Vitamin K deficiency potentially plays a role in Alzheimer's disease (AD) pathology. Vitamin K can improve survival of neurons and has important functions such as preventing apoptosis of neurons in the brain (Allison, A. C. 2001). In AD, the hippocampus is one of the first structures to degenerate. We previously demonstrated that healthy participants spontaneously use different strategies to navigate an environment. Spatial memory strategies are associated with increased activity and grey matter of the hippocampus, while stimulus-response strategies are associated with increased activity and grey matter in the caudate nucleus (Iaria et al., 2003; Bohbot et al., 2007). Thus, we investigated the relationship between dietary vitamin K intake and navigational strategies in healthy older adults. Methods: We administered the Food

Frequency Questionnaire (FFQ), validated by Shatenstein et al. (2005), which assesses participants' regular diet over the past year, to a group of healthy older adults (N = 53, mean age = 65.96 ± 4.5 yrs). Participants were also administered the Concurrent Spatial Discrimination Task (CSDLT). The CSDLT is a virtual 12-arm radial maze in which participants have to learn the location of objects within 6 pairs of arms. After participants learn the location of the objects, a probe trial is administered where the arms are recombined but the reward contingency remains the same. Performance on the probe trial indicates whether participants learned the position of the objects in relation to environmental landmarks (i.e., using a spatial strategy), which results in few errors. Results: We found a positive correlation between dietary vitamin K intake and probe scores on the CSDLT ($r = 0.289$, $p < 0.05$), indicating that increased dietary vitamin K intake is associated with increased use of the spatial strategy. Conclusion: Prior studies have shown a relationship between vitamin K and AD. In rats, lifetime vitamin K deficiency leads to impaired spatial memory performance on the Morris Water Maze (Carrie et al, 2011) and in healthy older adults, higher vitamin K concentrations correlate with better verbal episodic memory and free recall (Presse et al, 2013). Here, our results suggest that dietary vitamin K is associated with increased use of spatial strategies and may be protective against degeneration of the hippocampus. In fact, in rodents, it has been shown that vitamin K increases the survival of embryonic hippocampal neurons grown in serum-free medium (Nakajima, 1993). Therefore, the current results suggest that a diet higher in vitamin K may contribute to healthy aging.

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Poster

082. Human Long-Term Memory: Medial Temporal Lobe I

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Topic: F.01. Human Cognition and Behavior

Support: CIHR Grant 274766

Title: Navigational strategies in young and older adult Inuit hunters

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Abstract: Aim: Inuit hunters exhibit exceptional navigational skills. For centuries, they have found their way through seemingly homogeneous landscapes without using maps or navigation devices (Aporta, 2009). Although researchers have described the ways in which Inuit hunters navigate, we do not know the specific cognitive strategies that they use to find their way. They use a variety of environmental features to navigate, including the direction of winds, stars, landmarks, and snowdrifts. The fact that they use these ever-changing features implies that they are constantly forming spatial relationships between their current location, these environmental features, and their destination. This process is the hallmark of the "spatial strategy". Different strategies can be used to navigate, but we hypothesized that Inuit hunters use the spatial strategy to a greater extent. We also hypothesize that young Inuit hunters, because they rely more on technology, use the spatial strategy to a lesser extent than older Inuit hunters. Methods: Nine healthy older Inuit men hunters (mean age: 60.13 yrs) and 7 healthy young Inuit men hunters (mean age: 30.86 yrs), tested in Igloolik, Nunavut, took part in the 4-on-8 virtual maze. The task consists of an 8-arm radial maze, in which 4 arms are accessible and 4 are blocked. Participants have to retrieve objects located at the end of the 4 accessible arms. Then, all 8 arms become accessible and participants have to retrieve objects now located in the 4 pathways that were previously blocked. Spatial learners use landmarks to learn the location of the objects in the maze, while response learners use a sequence of right and left turns from a given starting position or stimulus. Results: As per our hypotheses, 87.5% of the elderly Inuit participants used a spatial strategy in contrast to only 39% of Southern Canadians. Further, elderly Inuit participants used spatial strategies to a greater extent than young adult Inuit hunters. Interestingly, the proportion of spatial learners was the same (50%) in young adult Inuit hunters and young adult Southern Canadians. Conclusion: Traditional occupations such as hunting seem to promote hippocampus-dependent spatial memory in the Inuit, a strategy associated with healthy cognition. Further research is needed in order to expand these results in a larger sample, balanced for sex, profession, and environment. Interestingly, contrary to our findings in southern Canadians whereby there is a decrease in the use of spatial strategies with age, older Inuit use spatial strategies to a greater extent than young Inuit, suggesting that growing up in a traditional environment increases the use of spatial strategies.

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Poster

082. Human Long-Term Memory: Medial Temporal Lobe I

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Support: Department of Defense NDSEG Fellowship

NSF CAREER Award (1056019)

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Title: Structural development of hippocampal subfields is related to statistical learning and inference

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Abstract: Recent work suggests that the hippocampus undergoes protracted structural development, which may explain developmental changes in learning and memory. However, the hippocampus is a heterogeneous system comprising several subfields that differ in cellular makeup, anatomical connectivity, and hypothesized function. Little is known about how these subfields develop or how their development relates to behavior. Although the hippocampus is primarily known for its critical role in the encoding and retrieval of individual episodic memories, it has also been implicated in coding relationships that span experiences. For instance, the hippocampus is involved in a form of statistical learning, extracting temporal regularities from the environment by generalizing across events. The hippocampus has also been implicated in novel inference judgments that require consideration of the relationships among multiple episodes. In the present study, we investigated the relationship of individual differences in hippocampal subfield anatomy to statistical learning and inference abilities from ages 6-30. Participants performed statistical learning and associative inference tasks that have previously been shown to require hippocampal function. We then acquired high-resolution T2-weighted structural MR images oriented perpendicular to the main hippocampal axis to quantify changes in subfield volumes across the age range. We found reduced behavioral performance among children relative to adults in both statistical learning and associative inference tasks. Consistent with the notion that hippocampal structures continue to mature into adolescence, we also observed that hippocampal volumes decreased over development, which was primarily driven by changes in the CA fields in the head of the hippocampus. Moreover, after accounting for age effects, we found relationships between hippocampal volumes and behavioral performance on both statistical learning and inference tasks. These results paralleled the volume decreases we observed over development, with smaller CA3 subfield volumes linked to superior behavioral performance. These data show that hippocampal structure relates to individual differences in the ability to integrate related experiences across time. Moreover, our findings suggest that the ability to derive knowledge across learning episodes may not reach maturity until adulthood.

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Poster

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Topic: F.01. Human Cognition and Behavior

Support: NSERC Discovery Grant

QBIN Pilot Grant

FRQNT Team Grant

Title: Habitual action video game playing is associated with caudate nucleus-dependent navigational strategies

Authors: *G. WEWT[1], B. DRISDELLE[2], K. KONISHI[3], M. DIARRA[2], P. JOLICOEUR[2], V. D. BOHBOT[4];
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Abstract: Aim: Action video games are associated with increased grey matter and activity in the striatum (Kuhn et al., 2011; Tanaka et al., 2013). Studies in humans and rodents have shown an inverse relationship between grey matter in the striatum and hippocampus. Therefore, we investigated whether habitual action-video game playing is also associated with increased use of response strategies, known to be dependent on the caudate nucleus of the striatum. Methods: Seventeen action video game players (VGPs) and 18 non-action video game players (NVGPs) were administered the 4-on-8 virtual maze (4/8VM), a virtual navigation task aimed to dissociate between spatial and response navigational strategies. The 4/8VM consists of an 8-arm radial maze, in which 4 arms are accessible and 4 are blocked. Participants have to retrieve objects located at the end of the 4 accessible arms. Then, all 8 arms become accessible and participants have to retrieve objects now located in the 4 arms that were previously blocked. To find the rewarded arms, spatial learners used environmental landmarks, while response learners used a pattern of open and closed arms from a given position. After participants have learned the task, a

probe trial is given in which all landmarks are removed, whereby spatial learners make more errors than response learners, indicating reliance on external landmarks. In addition to this, we used an event related potential (ERP) target detection task to measure group differences in stimulus-response orienting. Results: We found that VGPs had a significantly higher likelihood of using a caudate nucleus-dependent response strategy (83.33%) compared to NVGPs (50%). In contrast, NVGPs used spatial and response strategies in similar proportions to what was observed in past young adult studies using the 4/8VM (Iaria et al. 2003). Further, VGPs showed a larger N2pc component for targets that were furthest away from fixation, consistent with prior studies. Conclusion: Increased use of the response strategy in VGPs is consistent with previously observed increases in striatal volume in VGPs. Response strategies are associated with decreased grey matter in the hippocampus, which, in turn, is associated with increased risk of neurological and psychiatric disorders. While VGPs may have been predisposed to action video game playing due to genetic and experience dependent factors such as early adversity (Schwabe et al., 2011), recent evidence suggests a correlation between time playing action video games and atrophy of the entorhinal cortex (Kuhn and Gallinat, 2013). As such, these results suggest that playing action video games may have an impact on long-term cognitive health.

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Poster

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Support: CIHR Grant 265167

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Title: EEG-Theta modulation is greater in spatial learners than response learners: A scalp-EEG study in young adults tested on a virtual navigation task

Authors: ***T. H. FALK**[1], H. J. BANVILLE[1], S. BISHUNDAYAL[2], R. CASSANI[1], E. CHAN[3], A. CLERICO[1], L. DAHMANI[3], R. GUPTA[1], A. RATHARAJAH[2], N. PHILLIPS[2], V. D. BOHBOT[3];
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Canada; [2]Concordia Univ., Montreal, QC, Canada; [3]Douglas Mental Hlth. Univ. Inst., Montreal, QC, Canada

Abstract: Aim: Using spatial memory when navigating is known to elicit hippocampal function, which in turn activates theta frequency electroencephalography (EEG) modulation for spatial coding purposes. The virtual navigation tasks developed by Bohbot have been shown to correlate with hippocampal fMRI activity for both young and older adults. Previously, hippocampal activity was detected using intracortical EEG. Our aim was to detect hippocampal activity with scalp EEG, which is non-invasive. We tested both spatial and response learners and hypothesized that spatial learners would show more theta EEG activity than response learners. Methods: We performed a pilot experiment with 10 young adults (5 women, 5 men; 3 spatial and 7 response learners). We used the 4-on-8 virtual maze to investigate EEG activity during virtual navigation. Participants performed a series of control and experimental tasks. The task consists of an 8-arm radial maze, in which 4 arms are accessible and 4 are blocked. In the experimental trials, participants have to retrieve objects located at the end of the 4 accessible arms. Then, all 8 arms became accessible and participants had to retrieve objects now located in the 4 pathways that were previously blocked. After the task was learned, a probe trial was given in which all landmarks were removed. The probe trial allows us to dissociate spatial and response learners, as spatial learners make more mistakes then response learners. In the control trials, participants explored the virtual environment while counting down by 3 from 1000. The experimental task is known to elicit fMRI activity in the hippocampus relative to the control task. We recorded 64-channel EEG; amplitude modulation and cross-frequency interaction analysis was performed. Results: A pair-wise comparison test showed that theta amplitude modulation was significantly higher for the spatial learners during the experimental task compared to the control task, response learners in the experimental task, and lastly response learners in the control task. Conclusion: Theta activity, an EEG band previously shown to be associated with hippocampal function, is differentially modulated in spatial vs. response learners. These preliminary findings suggest that the proposed EEG features are sensitive to hippocampal function. Interestingly, the control condition of the spatial learners elicited greater theta than the experimental condition of the response learners. Assuming that theta rhythm is associated with spatial learning, these results suggest that spatial learners have a general brain state or readiness for acquiring spatial information that is not as present in response learners.

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Poster

082. Human Long-Term Memory: Medial Temporal Lobe I

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Program#/Poster#: 82.27/MM19

Topic: F.01. Human Cognition and Behavior

Support: NIH R01 EY021755

Title: Focusing on what matters: Modulation of the human hippocampus by relational attention

Authors: ***N. I. CORDOVA**, M. ALY, N. B. TURK-BROWNE;
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Abstract: Relations are essential for long-term memory, helping us remember where and when things occurred within past episodes. The hippocampus is known to play a critical role in such relational memory. However, relations are also important over much shorter time periods, when perceiving the current environment. For example, how do we see the relative positions of objects, or their temporal order, or the relationship between their features? Here we investigate the role of the hippocampus in such online relational processing by manipulating visual attention to different kinds of relations in a dynamic display. During high-resolution fMRI, participants were presented with a series of trials in which two images appeared very rapidly. Orthogonally, the images differed in relative spatial position, temporal onset, and size; in addition, each of the images was rotated independently of the other on a subset of trials. Afterwards, a post-cue appeared that pointed to the location of one image. This enabled four tasks: (1) spatial task: was the cued image to the left or right of the other image? (2) timing task: did the cued image appear before or after the other image? (3) size task: was the cued image smaller or bigger than the other image? (4) tilt task: was the cued image tilted or not (irrespective of the other image)? The spatial, timing, and size tasks oriented attention to the corresponding relations between the images, while the tilt task provided a control in which attention was oriented to an individual image and between-image relations were task-irrelevant. Critically, we used a staircasing procedure to equate behavioral performance across tasks (i.e., by changing the spatial, temporal, and size offsets, and amount of tilt), eliminating explanations of the neural data based on task difficulty. We examined attentional modulation in three hippocampal subfield regions-of-interest: CA1, CA2/CA3/dentate gyrus, and subiculum. All subfields showed reliable deactivation across tasks, but deactivation was greater when participants attended to relational vs. item information. This effect was most strongly driven by attention to temporal relations. One possible interpretation is that this deactivation simply reflects disengagement of the hippocampus. To investigate this possibility, we performed pattern similarity analyses, with the idea that disengagement would reduce information content and result in noisier and less reliable patterns of activity. Instead, pattern similarity was robust over runs of the timing task, and

stronger than in any other task. These findings suggest a general role for human hippocampus in the rapid online extraction of relational information.

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Poster

083. Human Emotion: Perception and Expression

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Program#/Poster#: 83.01/MM20

Topic: F.01. Human Cognition and Behavior

Title: Neural correlates of dynamic emotional facial expressions in infants

Authors: ***N. ROTEM-KOHAVI**[1,2], A. ROSE[4], C. G. E. HILDERMAN[3], T. F. OBERLANDER[6], N. VIRJI-BABUL[3,5];
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Abstract: Infants' ability to discriminate between different facial expressions is necessary for social and emotional development. Recognizing facial expressions supports the detection of another person's emotional state, and provides cues on how to respond in different social situations. Simulation theories for the perceptual processing of emotional faces assert that in order to recognize the emotions and infer the feelings of others, observers recruit the neural circuitry involved in creating their own emotional facial expressions. The electroencephalography (EEG) mu rhythm is a sensorimotor oscillation hypothesized to index the activation of the mirror neuron system. The mirror neurons have been shown to fire during both action performance and during action observation. Recently, it was suggested that observing emotional faces involves simulation of the emotional facial expression, which may elicit mu rhythm desynchronization. However, to date, the EEG mu response to viewing different facial expressions, in infants has not been studied. In this study we recorded EEG responses in 8-10 month old infants (n=11) while they observed dynamic facial expressions of neutral, sad, pain and happiness. Mean mu desynchronization was calculated for each expression in the central brain regions. The response to all expressions was significantly greater than zero ($p<0.001$ for all of the expressions). Our data show that the magnitude of mu desynchronization in response to observing pain was significantly larger than for happy and sad facial expressions in the left

central regions (p=0.038, and p=0.021 respectively). Our preliminary results suggest that between the ages of 8-10 months infants respond differentially to dynamic facial expressions and that the perception of painful expressions may evoke a stronger response. These results may provide insights into the developmental processes associated with empathy.

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Poster

083. Human Emotion: Perception and Expression

Location: Halls A-C

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Topic: F.01. Human Cognition and Behavior

Support: KAKENHI（25330311）

Title: Involvement of bilateral inferior frontal gyri in emotional recognition with social context: A TMS study

Authors: ***Y. YAMASHITA**[1,2,3], H. MAESHIMA[2,4], M. ABE[1,6], M. HONDA[1], M. OKADA[2,5], K. OKANOYA[2,3,4];
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Abstract: In order to investigate the involvement of both left and right inferior frontal gyri (IFG) in emotional recognition with social context, we developed a novel behavioral task to quantitatively measure an effect of contextual information on the recognition of facial expression changes. Then, by applying repetitive transcranial magnetic stimulation (rTMS) over left and right IFG, we examined whether these brain regions are critically involved in the processing of emotional context. The task trial consisted of sequential presentation of three pictures of a pre-face, a context scene, and a post-face, selected from the facial expression continua that were morphed in 6 steps from happy to fearful faces of a same model identity. Inserted scene images

were pleasant, unpleasant scenes describing social context, and meaningless images were used for control conditions. The participants were asked to discriminate quickly and accurately the direction of facial expression changes (positive/negative) from the pre-face to the post-face. The behavioral experiment demonstrated that the detection of subtle facial expression change was highly sensitive to the context scene. That is, when a facial expression change was "compatible" (i.e. direction of a change in facial expression was consistent with the context stimulus), subjects were able to recognize significantly more precisely than "incompatible" changes of facial expressions (context effect). In addition, when pairs of the pre and the post faces belonged to different emotional category, subjects were able to recognize significantly more quickly and precisely than those from same category (category effect). Eighteen subjects, in whom the left IFG was successfully identified using TMS-induced speech arrest (SA) method, participated in the rTMS experiment. Fifteen minute-rTMS of 1Hz was applied to the left and right IFG to interfere cortical functions of these areas and the performances of the above task were measured before, immediately after and 30 minutes after the rTMS. As a result, the context effect was affected by the TMS applied over both the right and the left IFG. That is, TMS temporarily increased the error rate for the contextual trials, but not for the control, suggesting that bilateral IFG may be critically involved in the processing of emotional context. By contrast, the category effect was selectively affected by the TMS applied over the left IFG, which increased response latencies for the different category trials but not for the same category trials. This suggests that the left IFG plays a critical role in the categorical perception of emotional facial expression.

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Poster

083. Human Emotion: Perception and Expression

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Support: María del Carmen Castro González was granted by DGAPA Project IN224414-2

Title: Different widespread networks are activated by musical prosody: An EEG and subjective evaluation study

Authors: ***M. CASTRO GONZÁLEZ**[1], B. CORONA-DZUL[1], M. CORSI-CABRERA[1], E. FLORES-GUTIÉRREZ[2,3];

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Abstract: Musical prosody communicates different emotional feelings, when melody is influenced by consonant or dissonant chords, and major or minor chords. The aim of this study was to analyze brain response to musical prosody in conjunction with subjective ratings of feelings expressed by music. We used ten musical pieces, each one in four piano versions: major consonant, minor consonant, major dissonant and minor dissonant (40 pieces in total). Musical pieces (about 16 s each) were presented in a classical block design alternating white noise in four counterbalance runs, one for each group of musical version. After each run subjects (n = 20) rated the subjective feelings communicated by the music (Likert scales). Spectral power (1-50 Hz) and current density sources were obtained. Significant differences were found between music and noise and between musical pieces depending on their musical structure and feelings expressed by them. Music compared to noise, induced higher theta and alpha absolute power over widespread brain regions of the two hemispheres and decreased beta and gamma mainly in the right hemisphere. Significant differences in current source density and spectral power were found between consonance and dissonance that were also different for major and minor modes involving alpha, beta and gamma frequencies in the right hemisphere in frontal lobe, orbitofrontal, cingulate, insular and superior and middle temporal cortex. The left hemisphere showed only higher alpha current density in orbital and cingulate gyrus and higher gamma in superior parietal lobule, superior temporal lobe, angular gyrus and precunneus. Alpha, beta2 and gamma current densities were higher for major consonant compared to major dissonant pieces in right frontal lobe and lateral orbital region. Feelings communicated by musical pieces, pleasant for consonants and major, and unpleasant by dissonant and minor, were corroborated. The right hemisphere responds stronger to musical prosody, and the left for consonance and major mode only in posterior regions fast frequencies.

Disclosures: **M. Castro González:** None. **B. Corona-Dzul:** None. **M. Corsi-Cabrera:** None. **E. Flores-Gutiérrez:** None.

Poster

083. Human Emotion: Perception and Expression

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 83.04/MM23

Topic: F.01. Human Cognition and Behavior

Support: Daito Bunka University Research Grant

Title: Is the cognition of emotional face expression influenced by menstrual cycle?

Authors: *M. YAMAZAKI[1], S. SUZUKI[1], K. TAMURA[1], Y. UGAWA[2];
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Abstract: Women in reproductive age experience dynamic changes of sexual hormones level and also serotonin which influences emotion and mood with their menstrual cycle. However, it remains still poorly understood that whether the brain response in face processing is affected by the menstrual cycle or not. The purpose of this study was to investigate the influence of the menstrual cycle and gender on the emotional face cognition. We employed event-related potentials (ERP) with scalp 19channels during a judgment task of emotional expression (happy or fear) / non-emotional expression (neutral) selected from the Karolinska Directed Emotional Faces (KDEF). Twenty (10 males and 10 females) university students were participated in this study. Female subjects were examined during both the preovulatory (high estrogen) stage and premenstrual (low estrogen) stage. We analyzed the peak amplitude, latency of ERP components and the scalp topographies of the ERP. The fear expression elicited higher amplitude in premenstrual stage females compared to men and preovulatory stage females. Scalp topographies showed gender difference female but not in menstrual stage. The present data suggest that we should take account of the influence of the menstrual cycles when we discuss the gender differences in emotional face cognition, such as the emotional content of stimuli.

Disclosures: **M. Yamazaki:** None. **S. Suzuki:** None. **K. Tamura:** None. **Y. Ugawa:** None.

Poster

083. Human Emotion: Perception and Expression

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Topic: F.01. Human Cognition and Behavior

Support: FWO Grant 1265714N

PF 10/008

Title: Common network for the processing of dynamic emotional bodies contains information to discriminate individual basic emotions

Authors: *J. JASTORFF[1,2], M. A. GIESE[3], M. VANDENBULCKE[1];
[1]Res. Group Psychiatry, [2]Res. Group Neurophysiol., KULeuven, Leuven, Belgium; [3]Dept. of Cognitive Neurol., Univ. Clin. Tübingen, Tübingen, Germany

Abstract: Mechanisms underlying the processing of basic emotional expressions have been proposed to reside in distinct gross anatomical locations. Recent meta-analytical studies, however, provide conflicting results with some in favor (Vytal & Hamann 2010) and others disagreeing (Lindquist et al. 2012) with this proposition. Here, we used dynamic stimuli of emotionally expressive gaits to address the question whether common or distinct networks subserve processing of different emotions displayed by human bodies. Furthermore, instead of relying on general activation differences between emotions, we investigated which regions contain information to reliably discriminate between different emotions. Stimuli were generated from a motion capture database of actors performing neutral and emotionally expressive (anger, happiness, sadness, fear) gaits. A custom-built volumetric avatar model was used for animation. This procedure has been shown to result in highly recognizable emotional stimuli (Röther & Giese 2009). Six stimuli of each of the four emotions and the neutral gaits were selected for the event-related fMRI experiment, resulting in 30 stimuli. These were presented twice within one run (8 & 5 vis. degrees respectively) for two consecutive gait cycles in a pseudo-random order. To identify regions commonly activated by all emotions, we contrasted each emotion individually with the neutral condition and selected voxels significantly activated in all 4 contrasts. These were located in the posterior STS, the temporal poles, posterior, middle and anterior cingulate cortex, the left parahippocampal gyrus and the medial orbitofrontal cortex. Investigating the information present in these ROIs, SVM classification showed above chance performance for at least 3 out of the 4 emotions in the left posterior STS, left parahippocampal gyrus, medial orbitofrontal cortex and middle cingulate cortex. Contrasting each emotion separately with the three others did not yield any significant voxels common to all 3 contrasts. Furthermore, information about the specific emotion was not limited to the emotion network, but was also present in the posterior MTG, left inferior frontal sulcus, and the fusiform gyrus; all parts of the action observation network. We did not obtain evidence for discriminable brain correlates of individual basic emotions. Nevertheless, several regions commonly activated for all emotions also contained information about the specific emotion displayed. In our ongoing work, including patients with neurodegenerative diseases, we intend to identify the contribution of the localized emotion network for the processing of emotional body expressions.

Disclosures: **J. Jastorff:** None. **M.A. Giese:** None. **M. Vandenbulcke:** None.

Poster

083. Human Emotion: Perception and Expression

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Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 83.06/MM25

Topic: F.01. Human Cognition and Behavior

Title: Infants' brain responses to subliminal emotional eyes

Authors: *S. JESSEN, T. GROSSMANN;
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Abstract: Emotion perception is a fundamental aspect of social interaction and as such operates extremely fast and efficient. Increasing evidence suggests that emotional information can influence neural and behavioral responses even in the absence of conscious awareness. While this subliminal emotion perception has been studied extensively in adults, its development in ontogeny is poorly understood. The present electroencephalographic (EEG) study therefore investigated the brain responses to subliminally presented eyes in 7-month-old infants. Infants were presented with fearful and happy eyes, as well as a polarity-inverted version of these stimuli. All stimuli were displayed for 50 milliseconds, which is well below the perceptual threshold established for this age group, and followed by a mask consisting of a neutral facial expression. We observed differential brain responses for fearful and happy eyes within 200 milliseconds at occipital electrodes, suggesting an emotional distinction early in visual processing. Furthermore, neural response at frontal electrodes clearly differed between happy and fearful eyes from 400 milliseconds onwards, which points to an influence of subliminal emotional content on the allocation of attention. Crucially, both effects were only observed for the original stimuli and not for the polarity-inverted control condition. Our results therefore highlight the role of eyes in emotion perception by showing that they are sufficient to elicit emotion specific neural activations, even when not consciously perceived. The present study is the first to show that this mechanism operates not only in adults, but that differential brain responses to subliminal emotional stimuli can already by observed in infants.

Disclosures: **S. Jessen:** None. **T. Grossmann:** None.

Poster

083. Human Emotion: Perception and Expression

Location: Halls A-C

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Program#/Poster#: 83.07/MM26

Topic: F.01. Human Cognition and Behavior

Support: National Research Foundation of Korea (NRF) grant funded by the Korea government (MEST) (No. 2012R1A2A2A03).

Title: Power spectral analysis of EEG from emotional auditory stimuli

Authors: *H. LEE[1,2], R. DU[1];
[1]Computer Sci. & Engin., Chonbuk Natl. Univ., Jeonju, Jeonbuk, Korea, Republic of; [2]Ctr. for Advanced Image and Information Technol., Jeonju, Korea, Republic of

Abstract: Purpose: Many researchers have investigated various psychological and physiological phenomena because of the importance of emotions. Emotion recognition process is the important part in the auditory BCI system. Power spectral analysis is a useful way to investigate the emotional information from EEG signals. The purpose of this abstract is to explore cortical oscillation properties in the emotional auditory experiment by analyzing the significant effects of the power spectrum. Method: We have collected the 18 channel EEG signals over 30-male subjects under the standard. International affective digitized sounds clips are classified in three emotional states, happy, neutral and fear. Power spectrum were calculated from the four frequency bands namely theta (4-8 Hz), alpha (8-13 Hz), beta (13-30 Hz) and gamma (30-50 Hz). In order to discover significant effects, a significance level of $p < 0.01$ is combined with the power spectrum to analyze the data. Repeated measures ANOVA with Bonferoni correction were employed to analysis. Results: The results showed only three bands have the significant relations between power and emotion. High alpha band waves ranged 10.9 and 12.2 Hz showed differences with significant effects in the prefrontal, central, temporal and parietal regions. Power differences of beta band between 13.7 and 29.6 Hz were found in the all cerebral areas except prefrontal region. In addition, the gamma band has one narrow band between 30.2 and 38.6 Hz with power differences in the frontal and central regions, and another narrow band between 40 and 46.8 Hz in the parietal and occipital regions. Conclusion: Interesting findings are 1) there had no significant effects of response in the theta band. 2) the narrow frequency bands between 10.9 and 12.2 Hz showed differences in the prefrontal, central, temporal and parietal regions. 3) the whole beta band ranged 13.7 and 29.6 Hz was found in all cerebral areas except prefrontal region. 4) gamma band have the narrow bands of 30.2-38.6 Hz with power differences in the frontal and central regions.

Disclosures: **H. Lee:** None. **R. Du:** None.

Poster

083. Human Emotion: Perception and Expression

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Program#/Poster#: 83.08/MM27

Topic: F.01. Human Cognition and Behavior

Support: SNF Grant No 320030B_138668

Title: Decoding music-evoked emotions from brain activity

Authors: *L. ROGENMOSER, S. ELMER, L. JÄNCKE;
Dept. of psychology, Div. neuropsychology, Univ. of Zurich, Zurich, Switzerland

Abstract: Music contributes enormously to our daily emotional experiences. Nevertheless, relatively little is known about the underlying mechanisms. Hindering factors are, on the one hand, the complexity and the length of the researching stimulus and, on the other hand, the individuality of the subjects' preferences. The present study aims to investigate the underlying mechanisms of music-evoked emotions in an ecologically valid manner by taking these hindering factors in consideration. To address this, we recorded the electroencephalography (EEG) in 20 subjects. For each subject, 24 excerpts (60 sec) were individually selected, based on their previous ratings, and were presented in the EEG session. Independent component (IC) analysis, spectrogram and IC clustering were applied. Specific ICs with specific spectra were linked to the valance (happy vs. sad) and the arousal experienced during music listening.

Disclosures: **L. Rogenmoser:** None. **S. Elmer:** None. **L. Jäncke:** None.

Poster

083. Human Emotion: Perception and Expression

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Program#/Poster#: 83.09/MM28

Topic: F.01. Human Cognition and Behavior

Title: Perception of happiness affected by exposure to the life histories of others

Authors: *K. MOGI;
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Abstract: The elucidation of cognitive mechanisms involved in the perception of happiness has recently become an important research topic (Lyubomirsky & Lepper 1999, Diener 2000,

Killingsworth & Gilbert 2010). Subjects are known to assume that their perceived happiness is influenced by a certain set of elements, when in fact the perceived utility is determined by a broader set of parameters (Kahneman et. al 2006). It is an interesting question, from scientific and practical points of view, to study how people can be induced to break free from the cognitive bias of anchoring (Wilson et al. 1996), to arrive at a balanced assessment of their own happiness. Evidence suggests that prefrontal areas are involved in the formation of cognitive bias (Podell et al. 1995, Browning et al. 2010). Activities in the ventral mesial frontal cortex correlate with positive emotions (Lane et al. 1997), while greater left superior frontal activation has been associated with higher levels of perceived well-being (Urry et al. 2004). These results suggest the possibility that we may be able to change a subject's perception of happiness through a manipulation of the bias parameters and contexts. Here I study how the exposure to descriptions of the life history of other subjects with similar or different backgrounds lead to a "defocusing" of the focusing illusion (Kahneman et. al 2006). Subjects were recruited to give estimates of their individual happiness, together with elements of life that they consider are important in determining the measure of happiness. In addition, the subjects were asked to provide generic and anonymous descriptions of the conditions in their lives they consider are important in determining happiness. Changes in the subjects' perceived levels of happiness after exposure to life accounts of subjects with backgrounds of various similarities are analyzed. Personal connection to other people is known to enhance the level of perceived happiness (Diener & Seligman 2002). Based on the results, I discuss how the perception of happiness can be made more robust through the exchange of life history accounts with other people.

Disclosures: K. Mogi: None.

Poster

083. Human Emotion: Perception and Expression

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

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Topic: F.01. Human Cognition and Behavior

Support: NIMH grant RO1MH091848

Title: Intertwined affective and semantic representations of the world around us: Applying voxel-wise encoding models to studying the cortical representation of emotional natural images

Authors: *S. A. ABDEL-GHAFFAR[1], A. G. HUTH[2], D. E. STANSBURY[3], A. S. COWEN[1], S. J. BISHOP[1,2];
[1]Psychology, [2]HWNI Neurosci., [3]Vision Sci., UC Berkeley, Berkeley, CA

Abstract: It has been argued that emotional stimuli receive prioritized processing. This is especially held to be the case for 'biologically prepared' stimuli. Prior fMRI studies have investigated the brain circuitry that encodes image affective value, but there has been little investigation of how this interacts with image semantic content. Regions throughout occipital and temporal cortex are known to be highly selective for semantic categories such as faces, bodies, and places. If these regions also store information as to the affective value of stimuli, this might enable quick identification of prepared stimuli. This raises the questions: Is there representation of image affective content within these regions? How does this interact with semantic tuning? To address these questions, we used voxel-wise encoding models (Naselaris, Kay, Nishimoto, Gallant, 2011) to investigate the brain-based representation of complex emotional images. Participants viewed a large set of emotional images (>1500), categorizing each image by valence (negative, neutral, or positive). Post scan ratings of image arousal and the emotion engendered by each image were also acquired. Encoding models of image structural properties, semantic and affective content were used to predict the BOLD response to new images from a held out 'validation' data set. In line with prior findings, a gabor model best predicted the BOLD response in early retinotopic visual regions, while semantic models predicted well in anterior occipital and ventral temporal regions. An interaction model (semantic category x emotional valence x arousal level (high, low)) significantly improved BOLD response prediction accuracy across anterior occipital, ventral temporal and other cortical regions. A principal components analysis was conducted upon the features weights from this model. The first 3 components explained >50% of variance across voxels, primarily encoding animacy, emotional intensity (arousal) and negativity, respectively. Plotting each voxel's response as characterized within this PC space onto flat maps of each subject's brain showed a similar structure across hemispheres, as well as certain commonalities between subjects. Within semantically selective regions, we find additional tuning for image valence and arousal, with voxel response profiles varying in a smooth and consistent manner across both hemispheres. These maps allow us to visualize the complex interactions between semantic and affective value encoded within visual cortex. Similarities across subjects may reflect common representations allowing for fast recognition of high survival-value stimuli.

Disclosures: S.A. Abdel-Ghaffar: None. **A.G. Huth:** None. **D.E. Stansbury:** None. **A.S. Cowen:** None. **S.J. Bishop:** None.

Poster

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Support: NNSF Grant (31271092)

Chinese Academy of Sciences Knowledge Innovation Project Grant (KSCX2-EW-Q -18)

Title: Different empathic responses to painful expressions versus needle-pricked body parts: Evidence from behavioral and electrophysiological study

Authors: *Y.-B. SUN, J.-Y. WANG, F. LUO;
Inst. of Psychology, Chinese Acad. of Scienc, Beijing, China

Abstract: Empathy, a basic social cognitive function, plays an vital role in everyone's daily life. Two kinds of stimulus materials (painful facial expressions and body parts receiving painful stimulation) are widely used as representations of other's pain in these research. However, what is always ignored by previous studies is that the two kinds of stimuli may result in different psychological processes of the observers, e.g., one may imagine he himself in pain when viewing injured body parts while do not implicate himself when seeing painful faces. The present study focused on the differences between empathic responses elicited by painful expressions and needle-pricked body parts. Highly ecological validity video clips were prerecorded depicting people being injected (n=10, 4 males). The videos were split and trimmed into two different clips, consisting of faces and injected arms respectively. The control video clips were recorded from additional 10 subjects showing neutral expressions with arms being wiped by a Q-tip. A total of 28 video clips are assigned to 3 blocks. Thirty-six graduate and undergraduate subjects (males 12, age 22.0±1.8) participated in this experiment. Facial EMG and pulse rate were recorded to assess the emotional responses elicited by visual stimuli. Subjective ratings of pain intensity and unpleasantness as well as IRI were measured after the termination of video stimuli. The results showed that, compared to the neutral videos, viewing pain videos elicited more corrugator activity, lower pulse rate, and higher pain ratings. Further more, stronger empathetic reactions (more corrugator activity, lower pulse rate, and higher pain ratings were also found when watching arm-pain videos than face-pain ones. These results suggest that observing injured body may lead to more self involvement relative to observing painful faces. Using the event-related potential approach, we examined the differences between brain responses elicited by visual images of painful faces versus needle-pricked arm. The time-course of empathic reactions to painful condition and nonpainful condition was investigated and compared. The preliminary data showed face-locked ERP components (N1, P2 and N2) recorded at electrode site Cz. The arm-locked ERPs displayed longer latency and lower amplitude as compared to the face-locked

ERPs. Painful faces elicited N1 and P2 of greater amplitude than neutral faces. Further analysis of electrophysiological data is being done relating to the late, emotion-related components as well as the source location.

Disclosures: **Y. Sun:** None. **J. Wang:** None. **F. Luo:** None.

Poster

083. Human Emotion: Perception and Expression

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Topic: F.01. Human Cognition and Behavior

Support: Instrumentation, Bridge, Instruction, Seed (IBIS) Funding, Emory College

Giles Robertson Alcohol Fund

Title: Sleep duration and fMRI measures of emotional reactivity in children

Authors: *B. L. REIDY[1], P. A. BRENNAN[1], S. B. HAMANN[1], C. INMAN[1], K. C. JOHNSON[2];
[1]Emory Univ., Atlanta, GA; [2]Dept. of Psychiatry & Behavioral Sci., Emory Univ. Sch. of Med., Atlanta, GA

Abstract: In adults and children, sleep loss is associated with affective dysregulation and increased reactivity to negative stimuli (Franzen et al., 2009, Walker & van der Helm, 2009; Legenbauer et al.2012; Berger et al., 2011). Adult functional neuroimaging (fMRI) studies have also demonstrated associations between sleep deficits and increased activation in the amygdala and reward systems when viewing emotional picture and face stimuli (Yoo et al, 2007; Gujar et al., 2011). However, few studies have examined the associations between sleep and emotional reactivity in school-aged children and no studies have investigated this relationship using fMRI. In the current study, we examined the relationship between sleep quality in pre-adolescents and fMRI responses to emotional faces. Fifteen male child subjects (ages 7-11) were recruited from a larger study (N=88) investigating fear processing and child behavior problems (Sylvers et al., 2011). Information about child sleep duration was collected via maternal report. Children were scanned with fMRI in an event-related paradigm in which they viewed and made perceptual judgments about negative, neutral, and positive emotional faces. We predicted that shorter sleep duration would be associated with increased activation in the amygdala and other regions

associated with emotional response, in line with prior studies in adults. Neuroimaging results revealed that when viewing negative (i.e., fearful, disgust) vs. neutral emotional faces, shorter sleep duration was associated with increased activation in the bilateral amygdala, left insula, and left temporal pole ($p<.05$, corrected for multiple comparisons). Additionally, shorter sleep duration was associated with increased right orbitofrontal and bilateral prefrontal activation when viewing disgusted vs. neutral faces, as well as increased bilateral orbitofrontal, right anterior cingulate, and left amygdala activation when viewing happy vs. neutral emotional faces. In contrast, as predicted, longer sleep duration was not specifically associated with increased activation in emotion-related brain regions. Taken together, these findings indicate that among school-aged children, shorter sleep duration may contribute to neural alterations of circuitry involved in the regulation of emotion and reward processing. Future studies should extend this work to examine the relationship between child sleep deficits and emotion regulation on both neural and behavioral levels.

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Poster

083. Human Emotion: Perception and Expression

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Topic: F.01. Human Cognition and Behavior

Title: Variability in human anterior insula gray matter volume predicts awareness for perithreshold backward masked fearful faces

Authors: *R. TORRENCE, J. CARLSON;
Psychology, Northern Michigan Univ., Marquette, MI

Abstract: The threshold for conscious perception of stimuli within the environment varies from individual to individual. For example, behavioral research has noted that when administering an awareness task with fearful vs neutral faces to normal healthy individuals, some perform above chance level indicating that they have better perceptual awareness. Functional neuroimaging studies suggest that the anterior insular cortex (AIC) positively correlates with perceptual awareness. However, few have examined the structural differences among individuals. The purpose of this study is to examine neural differences in perceptual awareness. This study hypothesizes that there will be a positive correlation with AIC gray matter volume and scores on

the awareness task. The awareness task was designed to assess awareness for the presence and location of backward masked fearful and neutral faces, masked with neutral faces. The participants responded by indicating on which side the masked fearful face appeared, or whether there were two neutral faces. The task included a total of 60 trials. T_1-weighted magnetic resonance images were collected to measure gray matter volumes. A whole brain threshold of $p < .001$ and 20 voxels were used with the insular as the regions of interest. In SPM8, a regression analysis was used with awareness as the predictor variable, gray matter as the dependent variable, and age and total gray matter volume as the control variables. The results indicated that there was a relationship between greater awareness and greater gray matter volume in bilateral AIC: left ($t(38) = 3.85$, $p < .001$), $k = 21$ and right ($t(38) = 4.49$, $p < .001$), $k = 108$. Individuals that were more aware of backward masked fearful faces had greater gray matter volume in their AIC. Future research could examine if practicing tasks that require greater perceptual awareness causes structural differences in the brain.

Disclosures: **R. Torrence:** None. **J. Carlson:** None.

Poster

083. Human Emotion: Perception and Expression

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Topic: F.01. Human Cognition and Behavior

Support: NIMH grant RO1MH091848

Title: Individual differences in detecting changes in face identity and emotional expressions depend on two distinct mechanisms

Authors: *A. L. ACHAIBOU, S. J. BISHOP;
Dept Psychology, UC Berkeley, UC Berkeley, Berkeley, CA

Abstract: Despite the importance of detecting changes in our environment, people often fail to notice changes when they occur after a brief visual disruption. In an early fMRI study, Beck and colleagues showed that change detection was associated with increased activity in fronto-parietal networks and category selective areas of the ventral visual stream, concluding that these activations indexed awareness of change (Beck et al., 2001). However, a subsequent EEG study revealed that frontal activity associated with change detection actually occurred prior to the change itself, suggesting that this prefrontal activity may reflect preparatory attentional function

as opposed to conscious change detection (Pourtois et al., 2006) Faces convey information not only about identity but also expression. It has been suggested that processing of these two aspects of faces are relatively independent from one another (Bruce & Young, 1986) and that fearful facial expressions are processed via a fast subcortical thalamo-amygdala pathway (de Gelder, 2011), and may facilitate 'bottom-up' stimulus driven attention. Here, we adapted Beck's change detection paradigm to investigate the hypothesis that detection of changes in identity would be associated with increased prefrontal activity (presumed to index preparatory activity), but that successful detection of changes in expression (from neutral to fearful) would be primarily linked with amygdala activation. Participants saw two consecutive displays, separated by 1s, each with two faces or two houses either side of fixation presented for 250 ms, and reported if there was a change on the left side or right side or if no change had occurred. Morphing was used to create both small and large identity and expression changes. After controlling for general change detection ability (indexed by performance on house trials), we found that there was no relationship between performances on identity and expression change trials but strong commonality in performance within each type of change condition (but across difficulty levels). FFA activity tracked change detection regardless of conditions. In contrast, increased activity in ventro-lateral prefrontal cortex was associated with detection of identity changes, but not expression changes, while amygdala activity was associated with performance on expression change but not identity change trials. Overall, our results support the idea that the ability to detect changes in face identity and emotional expressions depends on two distinct neural mechanisms.

Disclosures: **A.L. Achaibou:** None. **S.J. Bishop:** None.

Poster

083. Human Emotion: Perception and Expression

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 83.15/MM34

Topic: F.01. Human Cognition and Behavior

Title: Sustained, not habituated, activity in the human amygdala during threat-elicited attention

Authors: *M. A. WEBER, W. RIZER, K. MORROW, K. KANGAS, R. TORRENCE, J. M. CARLSON;
Northern Michigan Univ., Marquette, MI

Abstract: The human amygdala processes conscious and nonconscious fearful facial expressions and directs spatial attention to these faces. Additional research has shown that amygdala activity decreases or habituates after repeated exposure to fearful faces in passive viewing tasks. However, it is unclear to what extent the amygdala habituates during biologically relevant amygdala-mediated behaviors such as the orienting of attention to environment threat signals. This study investigated amygdala habituation to backward masked fearful faces compared to neutral faces when attention is directed and undirected. Participants performed a dot probe task while amygdala activity was recorded using functional magnetic resonance imaging (fMRI). The dot-probe task required participants to locate a dot either to the left or right of a fixation cross after two face stimuli were presented. Directed attention trials consisted of one fearful and one neutral face equally appearing the left or right visual field followed by a neutral mask. Half of these trials were congruent (fearful face-dot) or incongruent (neutral face-dot). Subject response times to dot location were significantly faster for congruent than incongruent trials because attention was oriented to that location, $p = 0.006$. Undirected trials consisted of two fearful (FF) or two neutral (NN) faces presented to either side of the fixation cross followed by a neutral mask. Subject response times were not different between FF and NN trials because attention is equally divided across the two visual fields ($p > 0.1$). Sustained and habituation amygdala activity was analyzed in SPM8 by looking for elevated activity across the task or for a relative decline in activity across the task. There was significant sustained activity in the left amygdala for directed attention (vs. NN, $t = 3.34$, $p = 0.004$, $k = 78$) and undirected attention (FF vs. NN, $t = 3.08$, $p = 0.006$), but no overall habituation pattern was observed for directed ($p > 0.05$) or undirected ($p > 0.05$) attention. This study suggests that the amygdala does not habituate during the presentation of backward masked fearful faces during dot-probe task. The findings suggest that the demand for attention and action during the task leads to sustained, rather than habituated, amygdala activation.

Disclosures: **M.A. Weber:** None. **W. Rizer:** None. **K. Morrow:** None. **K. Kangas:** None. **R. Torrence:** None. **J.M. Carlson:** None.

Poster

083. Human Emotion: Perception and Expression

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Program#/Poster#: 83.16/MM35

Topic: F.01. Human Cognition and Behavior

Support: NRF-2006-2005112

Title: Gender and task-context modulate the LPC amplitudes related to facial preference

Authors: *S. KIM[1], J.-H. KANG[2], S.-P. KIM[4], Y. CHO[3];
[1]Korea Univ., Korea, Republic of; [2]Brain and Cognitive Engin., [3]Cognitive Psychology, Korea Univ., Seoul, Korea, Republic of; [4]Design and Human Engin., Ulsan Natl. Inst. of Sci. and Technol., Ulsan, Korea, Republic of

Abstract: A great number of previous works with various research methods has been conducted on the topic of facial attractiveness or preference (Johnston & Oliver-Rodriguez, 1997; Werheid et al., 2007, Zhang et al., 2011). Specifically, in the ERP studies, Late Positive Complex (LPC) is one of the representative components sensitive to the attractiveness of face stimulus in latency after 400 ms. Here, we investigated the effect of facial preference on LPC component with two different tasks. Participants were to perform a passive viewing task and an active choice task with the same face images sequentially. In the first passive task, each face stimulus was presented one at a time and participants simply looked at the face stimuli without any explicit response. After a few minute breaks, a pair of same-gender face images, which were used in the passive viewing task, was presented sequentially and participants were asked to actively choose one image among the two, within the context of preference, "which face do you prefer more?" On the basis of the behavioral data from the second task, the face images were classified into three preference groups, "preferred" (33%), "neutral" (33%), "non-preferred" (33%) groups and ERP analyses were conducted based on this preference categorization. The LPC time range was 600-1,000 ms after the onset of a stimulus and the ERP waveforms were acquired from the parietocentral site (Pz). There were significant different patterns of LPC among three preference groups in both tasks. Non-preferred images elicited the lowest positivity. The LPC amplitude gradually increased with the level of preference. In the passive task, participants' gender differences was observed. Female participants showed the greatest mean LPC amplitude toward preferred images than the other images, whereas male participants showed the lowest mean LPC amplitude toward non-preferred image and no difference between preferred and neutral images. In the active choice task, both genders of participants showed the greatest LPC amplitude to preferred images than the other images, suggesting that the preference related a neural component could possibly be modulated by the participants' gender and task context. Also, both genders of participants had a tendency to show greater LPC amplitudes toward preferred "female" faces than preferred male faces, suggesting that people react somewhat more sensitively and are more likely to be tuned toward attractive female faces.

Disclosures: **S. Kim:** None. **J. Kang:** None. **S. Kim:** None. **Y. Cho:** None.

Poster

083. Human Emotion: Perception and Expression

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 83.17/MM36

Topic: F.03. Motivation and Emotion

Title: Effects of inconsistency between facial and verbal expressions on degree of trust and brain activity

Authors: *S. MORIOKA[1], M. OSUMI[1], M. OKAMOTO[2], M. HIYAMIZU[1], H. MAEOKA[1], Y. OKADA[1], A. MATSUO[1];
[1]NeuroRehabilitation, Kio Univ., Kitakatsuragi-gun/Nara, Japan; [2]Rehabil., Kitade Hosp., Gobo, Japan

Abstract: [Objective] Social communication is performed smoothly using both verbal and non-verbal language. The present study aims to determine the degree of trust and brain activity when verbal and facial expressions are inconsistent. [Methods] The subjects were 14 healthy volunteers (age: 21.7±0.8). The task used photographs of 8 people with pleasant expressions (smile) or unpleasant expressions (frown). For verbal expression, the 2 most positive and 2 most negative expressions were chosen from 50 sentences in a preliminary experiment; rescuing a child from a fire and keeping a promise with a friend were considered positive, whereas running over and killing a friend and bullying a friend were considered negative. A verbal [positive/negative] and a facial [smile/frown] expression were combined in each trial. Four types of representative images were randomly shown 80 times on a PC screen. Verbal expressions were initially presented alone for 5 s, followed by verbal expressions and photographs concurrently for 2 s. A donation was established as an index for the degree of trust: subjects were asked how much they would offer (amount of offer) when told the person in the photograph was troubled financially. Positive emotions and degree of trust were evaluated using the visual analogue scale (VAS). Using electroencephalography (EEG), event-related potentials (ERPs) were obtained by averaging the wave patterns appearing 170~240 ms after viewing the photograph. From these ERPs, the area of brain activity during inconsistency conditions was identified using standardized low-resolution brain electromagnetic tomography (sLORETA). One-way analysis of variance, multiple comparison tests, and Pearson's correlation were used for statistical analysis (significance level 5%). [Results] The VAS scores for positive emotions and degree of trust for [positive × smile] were significantly higher than those for the other conditions($p < 0.05$). Positive correlations were seen between all positive emotions and degree of trust ($r = 0.54$~0.84, $p < 0.05$). The amount of offer was significantly lower for inconsistencies between verbal and facial expressions, particularly for [negative × smile]. EEG showed more activity in the parietal lobe (BA7) with [inconsistency] than with [consistency]. [Conclusion] A negative verbal expression with a positive facial expression [negative × smile] elicited the least positive emotion, degree of trust, and amount of offer. Also our result indicated that parietal lobe

was activated in inconsistency of sensory information in social communication. It was suggested that this activity was a base of mentalizing.

Disclosures: **S. Morioka:** None. **M. Osumi:** None. **M. Okamoto:** None. **M. Hiyamizu:** None. **H. Maeoka:** None. **Y. Okada:** None. **A. Matsuo:** None.

Poster

083. Human Emotion: Perception and Expression

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 83.18/NN1

Topic: F.03. Motivation and Emotion

Title: The emotional response to social gaze is a domain specific cognitive mechanism

Authors: *N. LAPOLLA[1], B. BISHOP[1], J. CIBOTTI[1], S. STEDNITZ[1], E. GAHTAN*[2]; [2]Psychology, [1]Humboldt State Univ., Arcata, CA

Abstract: Eye contact with another person (social gaze) reliably produces an emotional response, accompanied by amygdala and autonomic nervous system activation, which is measurable using the skin conductance response method (SCR). While social gaze is known to produce an SCR, we investigated whether 'self-gaze' (gazing at one's own eyes in a mirror) also elicits an SCR. In cognitive psychology, domain specific cognitive mechanisms have narrow responses properties and domain general mechanisms have broad response properties, and this difference has implications for understanding how diverse cognitive functions evolved. We reasoned that if SCRs were different on social gaze trials relative to self-gaze trials that would support a domain specific model of the social gaze response mechanism. We compared SCRs between episodes of social gaze and self-gaze in 40 Humboldt State University student pairs. Each participant engaged in ten, 20 second eye contact trials, alternating between social gaze and self-gaze. Stimuli were presented using an automated panel on which a mirror (for self-gaze trials), open window (for social gaze trials), or opaque panel (for inter trial intervals) could be displayed. SCR data was collected using an ADInstruments amplifier and stainless steel finger electrodes. Self-gaze was found to elicit SCRs, however social gaze produced significantly larger responses compared to self-gaze, ($p<.001$). SCRs decreased across trials ($p=.021$), suggesting habituation. Eye contact between opposite sex partners was predicted to yield larger SCRs, but this effect was not found. These results suggest that the social gaze response mechanism is domain specific and evolved for social communication outside of the mating domain.

Disclosures: **N. Lapolla:** None. **B. Bishop:** None. **J. Cibotti:** None. **S. Stednitz:** None. **E. Gahtan*:** A. Employment/Salary (full or part-time):; Humboldt State University.

Poster

084. Human Decision-Making: Perceptual Processes

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 84.01/NN2

Topic: F.01. Human Cognition and Behavior

Support: AFOSR/ARFL Grant FA9550-10-1- 0385

Title: Sensory evidence is predictive of certainty in perceptual decisions

Authors: ***G. A. BUZZELL**[1], D. M. ROBERTS[1], J. R. FEDOTA[2], E. P. SHAW[1], C. G. MCDONALD[1];
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Abstract: Human perceptual decision-making is a complex process involving attention, the accumulation of sensory evidence, the comparison of this evidence, and ultimately the arrival at a decision outcome. In addition, stochastic variations in this process invariably lead to variations in both the accuracy of discriminations, as well as the subjective experience of certainty associated with a given response. A longstanding body of research within primate models has elucidated a general process by which decision-making is achieved, and recent neuroimaging work has begun to extend this work to humans. Nonetheless it remains unclear exactly how and when uncertainty arises in the decision-making process. In the present study, we recorded EEG while participants performed a difficult perceptual decision-making task and rated their subjective certainty of stimulus identity following each trial. In this way, we were able to investigate the neural changes that give rise to uncertainty when participants respond correctly, but are uncertain of their response. Critically, sensory evidence was assessed by comparing the occipital-temporal N1 component for correct, sure trials to those in which participants were correct, but unsure. In addition, pre-stimulus attentional state was assessed by measuring oscillations in the alpha band of the EEG, and confidence in the outcome of the decision was assessed using the P3 component, in addition to behavioral measures. Our data suggest that in a difficult perceptual decision-making task uncertainty arises from a cascade of events, beginning with reduced attention prior to stimulus onset (indexed by increased pre-stimulus alpha), which leads to impoverished sensory evidence (indexed by a reduced N1 component). These events were predictive of a reduction in the P3 component and slower response times. Our findings

demonstrate that when perceptual decisions are difficult uncertainty detected later in the processing stream can be attributed to lapses in proactive executive control that lead to impoverished sensory representations.

Disclosures: **G.A. Buzzell:** None. **D.M. Roberts:** None. **J.R. Fedota:** None. **E.P. Shaw:** None. **C.G. McDonald:** None.

Poster

084. Human Decision-Making: Perceptual Processes

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 84.02/NN3

Topic: F.01. Human Cognition and Behavior

Support: European Research Council, ERC, 260424

DFG Grant VE 675/1-1

Title: Transcranial direct current stimulation over ventro-medial prefrontal cortex changes human value-based decision making: A computational neurostimulation study

Authors: ***D. HAEMMERER**[1,2], M. KLEIN-FLÜGGE[3,4], J. BONAIUTO[4], M. BIKSON[5], S. BESTMANN[4];
[1]Inst. of Cognitive Neurosci., London, United Kingdom; [2]Lifespan Psychology, MPI for Human Develop., Berlin, Germany; [3]Wellcome Trust Ctr. for Neuroimaging, [4]Inst. of Neurol., Univ. Col. London, London, United Kingdom; [5]Biomed. Engin., The City Univ. of New York, New York, NY

Abstract: When deciding between options with different expected outcomes, the ventromedial prefrontal cortex (vmPFC) tracks the value of the expected net gain from this decision. Mechanistically, value tracking is conceived as a competition between neural populations coding for the value of individual options via mutual inhibition. However, this assumption is largely based on correlational evidence. Here we asked how causally influencing neurotransmission in vmPFC through non-invasive brain stimulation (NIBS) impacts decision making. We used a novel computational neurostimulation approach to generate behavioural predictions for transcranial direct current stimulation (tDCS) of vmPFC. Neural activity during decision making was modelled in a well-established and biophysically realistic model of recurrently connected spiking neurons that predicted choices by competition via mutual inhibition. We then fit the

choice predictions of the biophysical model with a simplified reinforcement learning (RL) model. Simulated anodal stimulation in this model reduced the inverse temperature parameter (a measure for the impact of value expectations on choices) but had no effect on learning rate. To test these model predictions, we used tDCS with the anode over medial or lateral PFC during value-based decision making. Current modelling confirmed induced current peaks at target site. The same RL model was used to fit subject's behavioural data. In line with model predictions, stimulation over vmPFC reduced the inverse temperature parameter but not learning rates. Stimulation over lateral PFC had no such effect. We demonstrate that a causal manipulation of the vmPFC activity pattern impairs value choice processes but not learning. Importantly, modelling suggests that this is caused by depolarization of response-specific neural populations and a hyperpolarization of their mutual inhibition via interneurons. Behaviorally, this resulted in a shift from selecting the option that has the highest expected value to selecting options more randomly. This novel computational neurostimulation approach outlines how mechanistic predictions about the consequences of NIBS in value-based decision making can be obtained, with relevance for applications that seek to augment cognitive function through NIBS.

Disclosures: **D. Haemmerer:** None. **M. Klein-Flügge:** None. **J. Bonaiuto:** None. **M. Bikson:** None. **S. Bestmann:** None.

Poster

084. Human Decision-Making: Perceptual Processes

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Program#/Poster#: 84.03/NN4

Topic: F.01. Human Cognition and Behavior

Support: ERC Grant 260424

Title: Predicting the behavioral impact of tDCS on perceptual decision making with computational neurostimulation

Authors: ***J. J. BONAIUTO**, A. DE BERKER, S. BESTMANN;
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Abstract: Transcranial direct current stimulation (tDCS) allows for non-invasive and reversible modulation of neural activity in widespread cortical and subcortical networks. These network changes can generate both beneficial and detrimental changes in behavior, depending on the

specific placement of electrodes on the scalp and stimulation parameters. However, there are currently no mechanistic frameworks capable of predicting or explaining these behavioral changes. Here we introduce a computational neurostimulation approach in which we use a theoretically established computational network model that generates realistic behavior in the random dot kinetogram (RDK) task, a classic perceptual decision making test. We then perturb various parameters in this biophysically realistic model of recurrently connected populations of spiking neurons to generate principled predictions about the behavioral impact of tDCS. Specifically, we simulated the effects of tDCS on the model by altering the resting membrane potential of its neurons based on values suggested by an *in vitro* study of the effects of transcranial stimulation on sensory evoked potentials (Molaee-Ardekani, 2013). These simulations predict that anodal stimulation decreases and cathodal stimulation increases reaction times (by -20.2% and 60.6% respectively), but importantly that both forms of stimulation reduce the accuracy of performance by 12.3- 25.9% on difficult trials. We then tested the behavioral predictions of the model in healthy human subjects performing the task. tDCS was applied over dorsolateral prefrontal cortex (dlPFC), thought to integrate sensory evidence for decision making independent of response modality (Heekeren, 2006). Finite element modeling of current flow was used to guide electrode placement such that induced currents peaked in left dlPFC. The size of the model effects scales with the intensity of the simulated stimulation, however ongoing analyses show the qualitative changes in response time and performance predicted by our model match those observed in human subjects: anodal stimulation over dlPFC improves reaction time on the RDK task but at the cost of decreased performance, with ongoing testing for cathodal stimulation. Our model suggests that this is due to an increase in pyramidal cell firing coupled with a decrease in inhibitory interneuron firing, which results in task options being less differentiable but causing the network to reach the response threshold faster. More generally, our novel computational neurostimulation approach demonstrates how one can obtain mechanistic predictions about the consequences of tDCS at the appropriate level of description.

Disclosures: **J.J. Bonaiuto:** None. **A. de Berker:** None. **S. Bestmann:** None.

Poster

084. Human Decision-Making: Perceptual Processes

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 84.04/NN5

Topic: F.01. Human Cognition and Behavior

Support: Penn State in-kind MRI scanner hours

Title: Characterizing functional brain networks associated with speed vs. accuracy emphasis in simple decision-making

Authors: *A. S. WEIGARD[1], S. WILSON[2], C. HUANG-POLLOCK[2];
[2]Psychology, [1]The Pennsylvania State Univ., University Park, PA

Abstract: When engaging in simple cognitive tasks, individuals can choose to emphasize either response speed or accuracy depending on individual characteristics, instructions on performance strategies, and/or the specific demands of the task. This function been explained in the context of formal models of decision-making as changes in response threshold, or the amount of evidence an individual deems appropriate to make a decision (Brown & Heathcote, 2008). Recent research on the neural basis of this function has supported the "striatal hypothesis", which contends that response thresholds are modulated when cortical regions involved in motor control (e.g., the pre-SMA) increase activity in the striatum to disinhibit the motor system under speed emphasis (Bogacz et al, 2010, Forstmann, et al, 2010; 2008). However, the role that other top-down control regions (DLPFC, ACC) play in this process, and whether the same connections between the striatum and pre-SMA are essential for cautious responding that emphasizes accuracy, as well, is not currently known. The current study aims to use unified structural equation modeling (uSEM) approach for directed functional connectivity analysis, which can indicate both contemporaneous and time-lagged connections between regions (Gates & Molenaar, 2012), to further clarify this. It is predicted that, when speed is emphasized, the DLPFC and ACC will display functional connections with the pre-SMA, which will, in turn, be related to increases in striatal activity. In contrast, when accuracy is emphasized, DLPFC activity will be negatively associated with baseline striatal activity. In an ongoing study, young adult participants (target N = 20) are asked to complete a simple perceptual decision-making task while fMRI data were collected, in which they must decide whether a briefly presented letter is a vowel ("A", "U", "E") or a consonant ("P", "H", "F") under speed or accuracy instructions. Behavioral analyses on data already collected (N=6) indicate that, as expected, individuals were significantly faster during the speed emphasis condition, $t(5)=3.595$, $p=.02$, and displayed a non-significant trend toward being less accurate in this condition, $t(5)=1.190$, $p=.29$. Neuroimaging analyses will be presented, which will utilize peak coordinates from prior studies to select ROIs for the DLPFC, pre-SMA, ACC and striatum. Functional connectivity between these ROIs will be estimated using the GIMME model (Gates & Molenaar, 2012), a technique that can identify group-level connectivity maps using uSEM while incorporating individual variation into these estimates.

Disclosures: **A.S. Weigard:** None. **S. Wilson:** None. **C. Huang-Pollock:** None.

Poster

084. Human Decision-Making: Perceptual Processes

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 84.05/NN6

Topic: F.01. Human Cognition and Behavior

Title: Cortical representations of confidence in a visual perceptual decision

Authors: *L. ZIZLSPERGER[1], T. SAUVIGNY[2], B. HÄNDEL[3], T. HAARMEIER[1]; [1]Dept. of Neurol., RWTH Aachen Univ., Aachen, Germany; [2]Dept. of Neurosurg., Univ. Med. Ctr. Hamburg-Eppendorf, Hamburg, Germany; [3]Ernst Strüngmann Inst. (ESI) for Neurosci. in Cooperation with Max Planck Society, Frankfurt, Germany

Abstract: Neuronal recordings in behaving monkeys and rats have detected representations of decision formation and the degree of certainty in it. Surprisingly, to date there is little electrophysiological evidence on human choice certainty. To dissociate electrophysiological representations of objective performance and subjective confidence, in a first experiment we asked one group of subjects to indicate the perceived direction of moving random dots and the confidence in that perceptual decision while stimuli were cued validly and invalidly. In the second experiment we did not cue selective attention but the motor effector to identify electrophysiological correlates of decision confidence in motor preparation. Motion stimuli, i.e. moving random dot patterns of varied coherence levels, were presented and subjects indicated global motion direction in a 4-alternative forced-choice design and the confidence in their decision via 4 numerical certainty ratings using a 4-button controller. We demonstrate electrophysiological correlates of choice certainty that evolve as early as 300 ms after stimulus onset and resemble the primary visual motion representations in early visual cortex. These correlates do not emerge unless or until the subject unambiguously knows which of the competing visual stimuli is actually relevant to behavior. They extend beyond stimulus presentation up to the motor response but are independent of the motor effector. Our findings suggest that perceptual confidence evolves in parallel with representations of stimulus properties and is dedicated to one specific aspect of the visual world. Its electroencephalographic correlates can be disentangled from representations of sensory evidence, objective discrimination performance, and overt motor behavior.

Disclosures: **L. Zizlsperger:** None. **T. Sauvigny:** None. **T. Haarmeier:** None. **B. Händel:** None.

Poster

084. Human Decision-Making: Perceptual Processes

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Topic: F.01. Human Cognition and Behavior

Support: NSF Grant (BCS 0955037)

Title: The salience network in multisensory perception

Authors: *B. LAMICHHANE[1], M. DHAMALA[1,2];
[1]Physics and Astronomy, [2]Neurosci. Inst., Georgia State Univ., Atlanta, GA

Abstract: Recent evidence suggests that the anterior insula (AIN) and the dorsal anterior cingulate cortex (dACC) are a part of "salience network (SN)". dACC is known to play a role in detecting interference between competing responses and in selecting appropriate behavioral responses. AIN is known to be involved in accumulating sensory evidence in perceptual decision independent of the motor response. How these nodes in SN contribute to the decision process from segregation of stimuli to the generation of appropriate behavioral response remains to be understood. Here, we performed functional magnetic resonance imaging (fMRI) experiments and fMRI data analysis using the dynamical causal modeling (DCM) to investigate the functional relationship between the activity of SN and the audiovisual-asynchrony perception. Thirty-three subjects were scanned in fMRI and asked whether the presented pairs of a brief tone and a flash were synchronous or asynchronous. Response time was observed in asynchrony percepts were longer compared to the time in synchrony percepts. Synchrony and asynchrony percepts both resulted strong activation in the SN. Brain activation of asynchrony percept was found significantly higher compared to synchrony percept in SN. The DCM analyses determined that the input into SN is most likely via the right insula (RI) and found to be intrinsically connected with the other nodes of SN. The dACC received causal inputs from both the left and right insula. These results suggest that INSs might provide perceptual evidence and trigger dACC for detecting interference between competing responses to generate an appropriate response.

Disclosures: **B. Lamichhane:** None. **M. Dhamala:** None.

Poster

084. Human Decision-Making: Perceptual Processes

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 84.07/NN8

Topic: F.01. Human Cognition and Behavior

Support: NSF BCS 0955037

Title: Cortical network oscillations during perceptual decision-making of visual objects

Authors: *G. CHAND[1], B. LAMICHHANE[1], M. DHAMALA[1,2];
[1]Physics and Astronomy, [2]Neurosci. Inst., Georgia State Univ., Atlanta, GA

Abstract: Previous neuroimaging studies have shown that perceptual decisions of human-faces and houses involve distributed brain networks that include the ventral temporal cortex and prefrontal cortex. The field still lacks a systematic investigation of how perceptual decisions are formed and related to the large-scale brain networks and network activity. We monitored brain activity with millisecond time resolution by recording electroencephalographic (EEG) responses while participants decided whether the presented picture was human-face or house. We found that the peak activity appeared in the areas of ventral temporal cortex and in the prefrontal cortex at latencies ~130-180 ms and ~190-220 ms respectively. We found that the beta and gamma-band oscillatory networks and their network activity were at work to carry out perceptual decisions. These findings suggest that beta and gamma band oscillatory networks coordinate activity between cortical regions mediating sensory and cognitive processing to arrive at perceptual decisions of visual objects.

Disclosures: **G. Chand:** None. **B. Lamichhane:** None. **M. Dhamala:** None.

Poster

084. Human Decision-Making: Perceptual Processes

Location: Halls A-C

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Topic: F.01. Human Cognition and Behavior

Support: NIH Grant MH086492

Title: Prior expectations modulate hemodynamic activity before and during perceptual decisions: Evidence from diffusion modeling and fMRI

Authors: *M. E. WHEELER[1], K. E. DUNOVAN[2], J. J. TREMEL[2];
[1]Psychology, Georgia Inst. of Technol., Atlanta, GA; [2]Univ. of Pittsburgh, Pittsburgh, PA

Abstract: As we gather noisy sensory information from the environment, prior knowledge about can be leveraged to facilitate recognition. However, when violated by observed evidence, strong prior beliefs can lead to errors in perceptual judgment and delay the recognition of unexpected stimuli. In the present study we probed the underlying dynamics of perceptual expectation biases using a combination of diffusion modeling and functional magnetic resonance imaging (fMRI). Human subjects performed a noisy face/house discrimination task in which each stimulus was preceded by either a predictive or neutral cue indicating the likelihood of the upcoming stimulus being a face or house. Predictive cues were valid on 80% of trials, and "catch trials" were included to separate cue-related and decision-related activity in "pre-stimulus" and "post-stimulus" epochs, respectively. Each subject performed the task while whole-brain activity was recorded using fMRI. As expected, face/house decisions were faster and more accurate when cued expectations matched the stimulus category. Behavioral data were modeled as a bounded drift-diffusion process in which cued expectations biased the prior starting-point (i.e., pre-stimulus baseline) and the drift-rate of evidence accumulation (i.e., post-stimulus sensory gain). Critically, this approach allowed us to quantify subject-specific biases and observe their effects on fMRI activity during isolated components of the decision process (i.e. pre- and post-stimulus epochs). Imaging results showed a clear mapping between diffusion model bias estimates and activity in category selective regions of inferotemporal cortex as well as domain-general regions within the frontoparietal decision-making network during pre- and post-stimulus epochs. Furthermore, effects of prior expectation were more apparent for face than house stimuli. These findings highlight a specificity in the effectiveness of perceptual expectations, and provide new insights into how top-down and bottom-up sources of information converge over the course of a decision.

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Poster

084. Human Decision-Making: Perceptual Processes

Location: Halls A-C

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Program#/Poster#: 84.09/NN10

Topic: F.01. Human Cognition and Behavior

Support: Marie Curie ACCDECMEM

Title: Neural correlates of evidence accumulation in perceptual and memory-based decision making

Authors: *M. A. BEULEN, N. A. TAATGEN, M. K. VAN VUGT;
Artificial Intelligence, Univ. of Groningen, Groningen, Netherlands

Abstract: Decision making is well described by evidence accumulation models. This class of mathematical models states that decisions are made by accumulating evidence for each available option until a threshold is reached, at which point the corresponding response is executed. We have previously found that oscillatory activity in the theta band (4-9Hz) in occipitoparietal brain areas shows a ramping pattern of activity during decision periods, consistent with such accumulation models. Most studies so far have focused on perceptual decision making tasks, ignoring the role of additional cognitive processes like memory retrieval in many real-world decisions. However, in a recent intracranial EEG study, we have shown that a similar evidence accumulation process takes place during recognition memory decisions, and that in addition to the previously observed posterior theta oscillations, gamma activity (48-90Hz) in dorsolateral prefrontal cortex also shows dynamics consistent with evidence accumulation. It is unclear, however, whether this result generalizes to scalp EEG, and whether the crucial difference with earlier work is indeed the memory demands of the task. We therefore created a perceptual discrimination task and a recognition memory task that are matched on stimulus material and task difficulty. Using both scalp EEG and intracranial EEG, we investigated the neural correlates of the evidence accumulation process in these matched tasks. This allows us to see whether and how the addition of memory retrieval to the decision process impacts the brain regions accumulating evidence for the decision, and the pattern of accumulation itself. We replicated our earlier finding of a ramping activity pattern in low-frequency oscillations during decision periods. The sets of channels exhibiting this ramping activity was similar between the perception and memory conditions. In contrast to our previous EEG experiments, we also saw ramping activity in 14-28Hz beta oscillations, and this was carried by posterior channels for the perception task and more central channels for the memory task.

Disclosures: **M.A. Beulen:** None. **N.A. Taatgen:** None. **M.K. van Vugt:** None.

Poster

084. Human Decision-Making: Perceptual Processes

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Program#/Poster#: 84.10/NN11

Topic: F.01. Human Cognition and Behavior

Support: Champalimaud Foundation

Fundaçao de Ciencia e Tecnologia

Title: Online behavioral readouts in an auditory perceptual decision making task

Authors: R. MEDINA, J. L. PARDO-VAZQUEZ, *A. RENART;
Champalimaud Neurosci. Programme, Champalimaud Ctr. for the Unknown, Lisbon, Portugal

Abstract: The behavioral evidence typically used to infer the mechanisms underlying decision processes consists of reaction time (RT) and choice, which reflect only the output of the decision process, and is therefore only indirectly informative on the decision process itself. In order to gain more direct access to the decision process from behavior, we have set-up an auditory 2AFC perceptual decision-making with two online behavioral read-outs: motor output and pupil size. In the task, human subjects have to decide whether the overall lateralization of a time-varying white-noise stimulus is left or right. Lateralization is controlled using the inter-aural level difference (ILD) of the stimulus delivered through headphones. The instantaneous ILD is chosen with a given probability every 15-25 ms from a set of ILD values, and the overall difficulty is controlled by rigidly moving this probability function. Depending on the shape of this probability we can alter the temporal correlations in the ILD stream. Subjects report their choices by moving a joystick-controlled dot in a computer screen to either of two lateral locations and are free to move the joystick throughout the stimulus presentation. Pupil size is monitored through the experiment. Stable psychometric functions confirm that the subjects can reliably perform this task. Mean RTs are in the range of 1-2 s, and increase with difficulty. Interestingly, behavioral reverse correlation shows that the subject's strategy seems to change with the stimulus correlation time, with choices relying on evidence accumulation vs. detection of sustained ILD transients for temporally uncorrelated vs. correlated stimuli respectively. Joystick trajectories can be classified into stereotyped (ST) and non-stereotyped (NST). ST trajectories are the majority (75-85%) and consist of a ballistic movement to either of the choice targets. In NST trials displayed large amplitude movements before choice. The proportion of NST trials increases with both difficulty and with the temporal correlations in the stimulus. We have so far been unable to find statistically significant trial-by-trial correlations between the stimulus sequence and the joystick trajectory in NST trials, but the speed of the choice response becomes slower in ST trials as stimulus difficulty increases, consistent with previous work (Selen et al., J. Neurosci., 2012). Preliminary analysis of pupil dynamics shows pupil size increases towards the moment of choice, at a rate which depends on stimulus difficulty. We are currently exploring modifications in the task that will increase the number of NST trials and facilitate their interpretation.

Disclosures: **R. Medina:** None. **J.L. Pardo-Vazquez:** None. **A. Renart:** None.

Poster

084. Human Decision-Making: Perceptual Processes

Location: Halls A-C

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Program#/Poster#: 84.11/NN12

Topic: F.01. Human Cognition and Behavior

Title: New predictions and experimental tests of scale invariance in a diffusion decision/timing model

Authors: *P. A. SIMEN, K. Y. VLASOV, S. PAPADAKIS;
Neurosci., Oberlin Col., Oberlin, OH

Abstract: Weber's law is the canonical scale invariance law in psychology: when the intensities of two stimuli are scaled by any value k, the just-noticeable-difference between them also scales by k. A diffusion model approximating a Poisson spike counting process accounts for Weber's law (Link, 1992), but there exist surprising corollaries of this account that have not been previously described or tested. We show that: 1) the Poisson counting model predicts time scale invariant decision time distributions in perceptual decision making, and time scale invariant response time distributions in interval timing; 2) for two-choice perceptual decisions, the model predicts equal accuracy but faster responding for stimulus pairs with equally scaled-up intensities; 3) the coefficient of variation (CV) of decision times should remain constant across average intensity scales, but should otherwise decrease as a specific function of stimulus discriminability and speed-accuracy tradeoff; 4) for timing tasks, response time CVs should be constant for all durations, and the skewness of response time distributions should always equal 3 times the CV. We tested these predictions using visual, auditory and vibrotactile decision tasks and visual interval timing tasks in humans. The data conformed closely to the predictions in all cases. These results support a unified theory of decision making and timing in terms of a common, underlying Poisson spike counting process, compactly represented as a diffusion process.

Disclosures: **P.A. Simen:** None. **K.Y. Vlasov:** None. **S. Papadakis:** None.

Poster

084. Human Decision-Making: Perceptual Processes

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 84.12/NN13

Topic: F.01. Human Cognition and Behavior

Title: Probing a model of self-consistent perceptual decision making

Authors: *A. STOCKER;
Psychology, Univ. of Pennsylvania, Philadelphia, PA

Abstract: Human perception is context-dependent. The interpretation of sensory information is shaped and biased by the context within which the information is presented to the observer. We have previously conjectured that context can also include preceding decisions of the observer when performing a task sequence involving the same sensory evidence (Stocker and Simoncelli 2008). This hypothesis can be formulated as a probabilistic observer model that performs optimal inference about a stimulus parameter based on sensory uncertainty and prior beliefs, yet is conditioned on the observer's preceding decision. We refer to this as a "self-consistent" observer model. We tested the model using the following psychophysical experiment: After being presented with two orientation stimuli, subjects were asked to judge whether the orientation of the second stimulus was clockwise or counter-clockwise relative to the first one. After the response, a third stimulus was presented, and the subjects were then asked to judge the orientation of the third stimulus relative to the second one. The "self-consistent" model predicts that a subject's first decision reshapes their probabilistic representation of the second stimulus' orientation such that the probability for any orientation that is inconsistent with the first decision is set to zero. Thus, a subject would be more likely to respond "counter clockwise" in the second task after having responded "clockwise" in the first task, and vice versa. This leads to characteristics distortions and shifts of the psychometric functions. In contrast, an "optimal" model assumes that both decisions are performed independently, and thus does not predict any change in subjects' psychometric functions. By measuring subjects' individual sensory uncertainties in a separate discrimination experiment, we were able to make quantitatively predictions for their behavior in the sequential decision task for each of the two models. We found that the data is very well predicted by our "self-consistent" model, including a characteristic distortion and shift of the psychometric curve while the optimal model provided rather poor predictions. We conclude that perception follows a self-consistency principle that guarantees a robust and stable interpretation of the world.

Disclosures: A. Stocker: None.

Poster

084. Human Decision-Making: Perceptual Processes

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 84.13/NN14

Topic: F.01. Human Cognition and Behavior

Title: A common frontoparietal network underlying categorization and perceptual decision-making

Authors: *S. SHANKAR[1], A. S. KAYSER[2];
[1]Ernest Gallo Clin. & Res. Ctr., Emeryville, CA; [2]Neurol., Univ. of California, San Francisco, San Francisco, CA

Abstract: Perceptual decision-making in the laboratory setting typically involves choosing between two or more discrete stimuli. Choice behavior in perceptual decision-making depends to a great extent on stimulus strength (perceptual uncertainty), and activity in the frontoparietal decision network reflects this sensitivity. In comparison, categorization involves a more abstract choice between two or more discrete categories of stimuli in accordance with some rule. Categorization too can be difficult when stimuli are noisy, but it is also affected by proximity of the stimulus to category boundary (categorical uncertainty). In addition, these two kinds of uncertainty can interact to decrease the efficacy of categorization. Previous categorization studies in macaques have revealed category related activity in posterior parietal cortex and lateral prefrontal cortex. Human studies have also implicated prefrontal cortex participation in coding uncertainty. These studies have, however, primarily modulated ambiguity in only one of the domains. To characterize uncertainty effects more completely and to assess the role of the frontoparietal decision network in categorization, we trained subjects to categorize dot motion as left or right of a pre-defined boundary, an oblique line inclined at either 45° or 135° from the horizontal. Perceptual ambiguity was introduced by changing the percentage of dots moving coherently while categorical ambiguity was manipulated by setting motion direction to different distances from the category boundary. The two types of ambiguity were independently modulated on each trial and their levels (4 perceptual, 5 categorical) were determined to provide a consistent set of accuracy levels across subjects, from 50% (chance) to 100%. Subject accuracy and response times varied as a function of not only perceptual and categorical uncertainty but also their interaction; within a given uncertainty level in either domain, performance declined as ambiguity increased in the other domain. Whole brain BOLD data revealed that a frontoparietal network active during categorization was similar to the one recruited in perceptual decision-making. This network showed parametric modulation to dot-motion coherence as well as distance from category boundary. Additionally, it appears to encode an interaction of uncertainties; as a function of distance from category boundary, the parametric effect of coherence seems to change qualitatively in amplitude and/or sign. Further analyses using regions

of interest and timecourses therein will help ascertain the robustness of these results and better characterize the categorization network.

Disclosures: **S. Shankar:** None. **A.S. Kayser:** None.

Poster

084. Human Decision-Making: Perceptual Processes

Location: Halls A-C

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Program#/Poster#: 84.14/NN15

Topic: F.01. Human Cognition and Behavior

Support: NSF Grant BCS-1358955

Title: Neurophysiological correlates of the incorporation of prior information into decisions under perceptual versus temporal demands

Authors: ***S. KELLY**[1], R. G. O'CONNELL[2];
[1]Neurosci., City Col. of New York, NEW YORK, NY; [2]Psychology, Trinity Col. Dublin, Dublin, Ireland

Abstract: Given prior information that one of two alternatives is more likely, observers will respond faster and exhibit a choice bias for perception of the more likely event. In most mathematical models of decision making based on sequential sampling, priors are incorporated by shifting the starting point of a decision variable (DV) closer to the more likely of two opposing boundaries. Little neurophysiological work has been done to directly test this. Here we test the idea that prior information is incorporated in different ways depending on the conditions under which the observer is compelled to utilize it. We exploited a recent finding that supramodal decision formation can be traced in a centro-parietal positivity (CPP) on the human scalp (O'Connell et al 2012). Observers discriminated the motion direction of random dots in discrete trials, with trial-randomized color changes preceding evidence onset indicating whether leftward or rightward were more likely (75% validity) or neither (50%, neutral). This task was performed under three blocked conditions: Easy blocks allowed 1600 ms to discriminate motion at 20% coherence; Low-coh blocks had the same deadline but reduced coherence (7-11% individually titrated); and Deadline blocks had 20% coherence but a strict individually set deadline of 400-500 ms. The nine observers run so far show strong effects of informative pre-cueing on reaction time (RT) and choice bias in both the Low-coh and Deadline conditions. The Easy condition showed weak if any such behavioral effects, and event-related potentials (ERP)

locked to evidence onset were similarly unmodulated. In the Deadline condition, the CPP decision signal reached a higher amplitude at the time of a correct decision report for invalidly cued motion compared to neutral, and reached a lower amplitude for valid trials, consistent with shifting decision bounds. In contrast, in the perceptually demanding Low-coh condition, prior information modulated build-up dynamics in a distinct way that is consistent with selective attentional enhancement of early motion detectors to facilitate perception. ERPs to cue onset showed no trace of a shift in CPP starting point prior to evidence onset according to prior information, but did show interesting differences in anticipatory frontal and occipital signals across blocked conditions: a frontocentral negativity was significantly stronger when a strict Deadline was imposed, whereas anticipatory negative potential over visual cortex was stronger for the Low-coh condition. This work stands to illuminate context-specific modulations and parameter adjustments that enable advantageous information integration.

Disclosures: **S. Kelly:** None. **R.G. O'Connell:** None.

Poster

084. Human Decision-Making: Perceptual Processes

Location: Halls A-C

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Program#/Poster#: 84.15/NN16

Topic: F.01. Human Cognition and Behavior

Support: Irish Research Council

Trinity College Dublin

National Science Foundation

Title: The classic P300 component indexes an accumulation-to-bound decision signal

Authors: ***D. M. TWOMEY**[1], P. R. MURPHY[2], S. P. KELLY[3], R. G. O'CONNELL[4], R. G. O'CONNELL[1];
[1]Psychology/Neuroscience, Trinity Col. Dublin, Ireland, Dublin, Ireland; [2]Dept. of Cognitive Psychology and Leiden Inst. for Brain and Cognition,, Leiden Univ., Leiden, Netherlands; [3]Dept. of Biomed. Engin., City Col. of the City Univ. of New York., New York, NY; [4]Trinity Col. Inst. of Neurosci. and Sch. of Psychology, Trinity Col. Dublin, Dublin, Ireland

Abstract: The P300 component of the human event-related potential has been the subject of intensive experimental investigation across a five-decade period, drawing enduring interest for

its apparent role in a range of cognitive operations and its sensitivity to brain disorders. Yet, its exact contribution to cognition remains unresolved in the absence of conclusive empirical data linking the P300 to a specific neural mechanism. Here, a new analysis of ERPs elicited by auditory and visual targets, combined with computational simulations, reveals that, rather than being the culmination of a unitary neural event, the P300 is a dynamically evolving process that triggers action upon reaching a stereotyped level and whose rate-of-rise determines reaction time at the single-trial level. Thus, the P300 exhibits the critical properties of the theoretical 'decision variable' signals predicted by sequential sampling models and directly observed in monkey neurophysiology. In identifying the P300 as a decision variable signal we place it at the heart of a well-established, explanatory framework that should facilitate more mechanistically principled investigations of sensorimotor transformations in both the typical and atypical human brain.

Disclosures: D.M. Twomey: None. **P.R. Murphy:** None. **S.P. Kelly:** None. **R.G. O'Connell:** None. **R.G. O'Connell:** None.

Poster

084. Human Decision-Making: Perceptual Processes

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 84.16/NN17

Topic: F.01. Human Cognition and Behavior

Title: Perceptual task difficulty and inhibitory control during a Go-NoGo task: An event-related potential study

Authors: D. W. SHUCARD[1], R. CHIN[2], T. J. COVEY[1], *J. L. SHUCARD[1];
[1]Neurol., [2]Psychol., Univ. At Buffalo, BUFFALO, NY

Abstract: Cognitive control mechanisms such as conflict monitoring and inhibitory control are dynamic neural processes that can be influenced by variations in task difficulty. The N2 and P3 components of the event-related brain potential (ERP) are thought to be influenced by these neural processes. One hypothesis is that N2 is an index of conflict monitoring (e.g., the monitoring of competing response options), while the P3 reflects inhibitory processes (e.g., the inhibition of specific response options). Several studies have manipulated task difficulty, most often using speed of response, to examine the independent contributions of the N2 and P3 components during response inhibition tasks. The findings regarding the roles of N2 and P3 have been mixed. The present study, unlike previous studies, used perceptually ambiguous stimuli to evaluate the underlying functional significance of N2 and P3 ERP components in relationship to

conflict monitoring and response inhibition. Eleven healthy adults completed two visual Go/NoGo continuous performance tasks (A-X CPT), the first with regular letters (unambiguous, easy task) and the second with degraded letters (ambiguous, difficult task). ERPs to regular and degraded Go and NoGo stimuli were obtained using a dense-electrode array recording system. Behavioral measures of reaction time (RT) and accuracy were also recorded during both tasks. During the Go condition, RT was slower for degraded compared to the regular stimuli. Both N2 and P3 latencies were longer for the degraded compared to the regular stimuli. The P3 component showed the classic frontocentral maximum amplitude for NoGo trials and a parietal maximum amplitude for Go trials. These P3 findings were present regardless of whether the stimuli were regular or degraded. Thus, the amplitude of the P3 was influenced by the need to carry out or inhibit a response, but not by the perceptual integrity of the stimulus. When greater conflict was introduced by increased perceptual ambiguity of the stimuli, a more prominent frontal-N2 response was elicited compared to the regular stimuli. This greater N2 negativity to degraded compared to regular stimuli was present for both the Go and NoGo conditions. Thus, the N2 negativity was influenced by the conflict created during the evaluation of perceptually ambiguous stimuli and not by the response properties of the stimuli (i.e., the necessity to carry out or inhibit a response; Go or NoGo, respectively). These findings support the notion that P3 amplitude is an index of inhibitory control and that N2 reflects conflict monitoring. This ERP approach may be useful in understanding inhibitory mechanisms in clinical and at-risk populations.

Disclosures: **D.W. Shucard:** None. **R. Chin:** None. **T.J. Covey:** None. **J.L. Shucard:** None.

Poster

084. Human Decision-Making: Perceptual Processes

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Topic: F.01. Human Cognition and Behavior

Support: NEI Grant EY021833

Title: Relating a spiking neural network model and the diffusion model of decision making to investigate neural mechanisms of speed-accuracy tradeoffs

Authors: *A. UMAKANTHA[1], B. A. PURCELL[2], T. J. PALMERI[1];
[1]Vanderbilt Univ., Nashville, TN; [2]New York Univ., New York City, NY

Abstract: Speed-accuracy tradeoffs (SAT) involve strategic adjustments of decision-making processes to optimize desired outcomes. The drift-diffusion model [1] explains decision making as the integration of perceptual evidence over time for alternative choices until a response boundary is reached. According to this model, SAT is commonly implemented through adjustments of a single parameter, the response boundary. The neural circuits underlying decision-making are likely more complex than the elements assumed by this abstract model, and recent neurophysiology suggests that SAT may involve multiple changes to the networks that underlie decision making [2]. Indeed, any single diffusion model parameter could potentially be implemented by many neural mechanisms. To explore the potential neural mechanisms of SAT and related decision-making processes, we simulated perceptual decisions using a biophysically-plausible spiking neural network model [3], and then fit the diffusion model to the simulated data just like we would fit the model to human or animal data. If variation in diffusion model parameters are necessary to explain behavior, then what can we conclude about variation in the underlying neural processes based on diffusion model fits? We systematically examined how variation of a number of spiking neural network parameters cause variation in the best-fitting parameters of the diffusion model. As expected, variation in the baseline firing rate and the response boundary of the spiking neural network were captured by variation in the diffusion model response boundary, implementing SAT as traditionally assumed. More surprisingly, we found that variation in the strength of recurrent excitation as well as variation in NMDA, AMPA, and GABA conductances were also captured by variation in in the diffusion model boundary. This demonstrates how a single psychological phenomena, SAT, captured by a single diffusion model parameter, response boundary, can be implemented by multiple - and sometimes unanticipated - neural processes. This also demonstrates how parameters and mechanisms of complex spiking neural network models can be non-uniquely identifiable based on their predictions of behavior as many different low-level neural changes can all produce the same behavioral change. References: 1. Ratcliff R, Rouder JN (1998). Modeling response times for two-choice decisions. Psychological Science 9, 347-356. 2. Heitz RP, Schall JD (2012). Neural mechanisms of speed-accuracy tradeoff. Neuron 76, 616-628. 3. Wang XJ (2002). Probabilistic decision making by slow reverberation in cortical circuits. Neuron 36, 955-968.

Disclosures: **A. Umakantha:** None. **B.A. Purcell:** None. **T.J. Palmeri:** None.

Poster

084. Human Decision-Making: Perceptual Processes

Location: Halls A-C

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Topic: F.01. Human Cognition and Behavior

Support: NIH/OppNet Grant R01-MH098899

Title: Learning expectations about changing environments for optimal inference

Authors: *C. M. GLAZE, J. I. GOLD;
Univ. of Pennsylvania, Philadelphia, PA

Abstract: Many decisions depend on the accumulation of noisy evidence that arrives sequentially over time. The dynamics of this process can include perfect, leaky, and/or bounded evidence accumulation to identify stable signals and differentiation to detect abrupt signal changes. We recently developed a normative model that naturally encompasses all of these different dynamics. The model is derived from the Bayes-optimal solution to evidence accumulation in environments subject to unpredictable changes that occur at a constant hazard rate. A key feature of the model is that the expected hazard rate governs decision dynamics. When perfect stability is expected, evidence is accumulated perfectly. Otherwise, however, evidence is accumulated with a leak and a bound that both depend on subjective hazard rate. We have previously shown that, for two separate tasks, human subjects can adapt to different objective hazard rates and use decision dynamics prescribed by the model. However, little is known how these dynamics are learned. To address this issue, we used a task in which subjects made trial-by-trial choices about which of two spatially separated triangles on a computer screen was the source of noisy samples of data. The correct source changed at a hazard rate (0.05-0.95) that was constant within a block of trials but varied across blocks. Model fits suggest that subjects were able to effectively adapt to different generative hazard rates when given trial-by-trial feedback on the correct answer, but with a bias towards a prior hazard rate. In sessions without any feedback, subjects showed less learning and more bias towards their prior hazard rate. For sessions in which the presence of feedback was alternated across blocks of trials, hazard rates learned in the presence of feedback were maintained over hundreds of non-feedback trials but slowly drifted back in the direction of the prior. Overall, the data suggest that humans have subjective prior expectations about rates of change that can be adapted to the environment when given appropriate feedback.

Disclosures: **C.M. Glaze:** None. **J.I. Gold:** None.

Poster

084. Human Decision-Making: Perceptual Processes

Location: Halls A-C

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Topic: F.01. Human Cognition and Behavior

Support: NSF Grant 1350786

Title: Self-consistent inference explains bias in sequential perception

Authors: *L. LUU, A. A. STOCKER;
Univ. of Pennsylvania, Philadelphia, PA

Abstract: The perceived motion direction of a random-dot kinematogram is systematically biased when probed in the context of a fine discrimination task (Jazayeri and Movshon, 2007). This bias was thought to represent the combined result of a noisy sensory representation and a neural decoding strategy that uses a weighting profile optimized for the discrimination task. The task-dependent nature of the decoding strategy predicts that no perceptual bias would occur if the discrimination task was omitted or modified such that the optimal strategy does not induce any preferential weighting. We conducted two psychophysical experiments to test this hypothesis. Experiment 1 was aimed to replicate the results of the original study for a different visual stimulus. A white ellipse (length of long axis: 5 degs) was presented at the center of the screen along with black reference marks indicating a reference orientation. Subjects were instructed to indicate whether the orientation of the ellipse was clockwise (CW) or counter-clockwise (CCW) relative to the reference orientation. Subsequently, they were asked to provide an estimate of the ellipse's orientation. For each trial, the reference orientation was randomly chosen and the ellipse's orientation was within +/- 10 degs of the reference orientation. We used three different aspect ratios for the ellipse (1.1, 1.2, and 2) to modulate sensory uncertainty. We found that subjects' orientation estimates were systematically biased away from the reference orientation, and that the bias increases with increasing stimulus uncertainty. Our results confirm the findings of the original study. Experiment 2 was identical to Experiment 1 except that i) subjects were given the categorical information (CW/CCW) before the stimulus was presented, and ii) subjects performed an orthogonal color discrimination task before estimating stimulus orientation. Specifically, the categorical information was provided with a colored (red/green) arc extending 90 degs from the reference mark; and subjects had to discriminate the arc's color that was randomly picked on each trial. We found perceptual biases that were similar to those found in Experiment 1. We conclude that the bias cannot be the result of a task-dependent neural weighting profile. Instead, we show that the results of both experiments are in agreement with a "self-consistent" Bayesian observer model which assumes that subjects' orientation estimates are the result of an inference process that is conditioned on the (given or self-made) categorical decision (Stocker and Simoncelli, 2008).

Disclosures: **L. Luu:** None. **A.A. Stocker:** None.

Poster

084. Human Decision-Making: Perceptual Processes

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 84.20/NN21

Topic: F.01. Human Cognition and Behavior

Title: Role of Attention in Perceptual Decision-Making

Authors: *G. TAVARES, P. PERONA, A. RANGEL;
Caltech, Pasadena, CA

Abstract: When faced with a value-based decision, humans tend to fixate on the items presented to them in order to compare their values and make the best possible choice. Previous work using food items has shown that patterns of fixations have an important role in this type of decision process. In particular, choices and reaction times in binary value-based decisions can be quantitatively described by a modified version of the drift decision model (called the aDDM), in which the value comparison process depends on the pattern of fixations among the two items, and in which relative visual attention have a sizable effect on choices. Here we investigate whether the same mechanism applies to perceptual decision making. Our experiment consists of a simple binary perceptual choice task. On each block of trials, subjects are first trained to recognize a target, which is a bar oriented at a certain angle. Then, on each subsequent trial, they are shown two different bars on the screen, and must decide which of the bars has an orientation closest to the target. During trials we recorded both choices and reaction times, and used an eye-tracking device in order to obtain the subjects' patterns of fixations. We collected data from 25 different subjects (10 female; mean age 23±4), and each one of them completed a total of 1,344 individual trials. Our preliminary data analysis shows several choice biases that are predicted by the aDDM. First, our data shows a last-fixation bias, meaning that subjects are more likely to choose the last item that was fixated in that particular trial. Second, subjects are also more likely to choose items that have been fixated for a longer period of time during the trial. Finally, the first item fixated in a trial does not influence that trial's final choice. Additional investigation with this data will allow us to estimate the parameters of the model at both individual and group levels, then use the estimated parameters to predict choices and reaction times. Our results will generalize the validity of the DDM to perceptual decisions, providing additional evidence of this model's capability to explain the role of attention in a wide range of decision situations.

Disclosures: **G. Tavares:** None. **P. Perona:** None. **A. Rangel:** None.

Poster

084. Human Decision-Making: Perceptual Processes

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

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Topic: F.01. Human Cognition and Behavior

Support: NIH Grant T32HD007151

University of Minnesota Interdisciplinary Doctoral Fellowship

Title: Differential MEG activity patterns for congruent and incongruent spatial relational judgments

Authors: N. M. SCOTT[1], M. SERA[2], *A. P. GEORGOPOULOS[3];
[1]Ctr. for Cogntive Sci., [2]Inst. of Child Develop., Univ. of Minnesota, Minneapolis, MN;
[3]Neurosci, Univ. Minnesota, MINNEAPOLIS, MN

Abstract: Children learn above/below relational judgments earlier than right/left judgments, but there is no explanation for this phenomenon. Relational judgments may exhibit congruency effects and this may complicate learning. For example, encoding above is easier at the top part of the screen than at the bottom - where it is incongruent. Incongruent trials, like right and left judgments, may require inhibition of a prepotent response or additional processing. We investigated the neural correlates of encoding and maintaining spatial relations for 3 seconds in working memory in 20 adults using magnetoencephalography (MEG). When comparing the MEG signal for above/below vs. right/left judgments over the period of stimulus presentation, we found the greatest difference in activity in the left temporal-parietal-occipital junction, right temporal cortex and cerebellum. Similar differential activation was observed during the working memory phase. When comparing congruent to incongruent trials, we found differential neural activity only in left frontal areas. Congruent-incongruent comparisons in working memory indicated differential activity bilaterally in frontal and temporal areas, including the same left frontal area indicated in the encoding phase. These findings are consistent with the idea that right/left relations are represented differently than above/below during both encoding and working memory phases.

Disclosures: **N.M. Scott:** None. **M. Sera:** None. **A.P. Georgopoulos:** None.

Poster

084. Human Decision-Making: Perceptual Processes

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Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 84.22/NN23

Topic: F.01. Human Cognition and Behavior

Support: NSC, Taiwan

Title: Geometrical concepts in the brain: An fMRI study on figure dependency

Authors: *T. CHIANG[1], C.-J. WU[2], D. ANSARI[3];
[1]Univ. of Western Ontario, London, ON, Canada; [2]Dept. of Educational Psychology and Counselling, Taipei, Taiwan; [3]Brain and Mind Inst., London, ON, Canada

Abstract: What kinds of representations are stored in the brain for geometrical concepts we have learnt in the primary school? We can speak out without a second thought some geometrical concepts such as 'triangle inequity theorem'. However, some theorems require more time to respond, such as 'In a triangle, the longest side is across from the largest angle', or 'the opposite sides of a parallelogram are parallel'. To what extent of differences in the brain to deal with the two types of known concepts? Two experiments were executed to clarify the issue. The first experiment was a behavioural study to differentiate the two types of learnt concepts by a priming paradigm in which a congruent/incongrount/no_precue geometrical figure was presented before a math question. In control of background knowledge, subjects were separated from science and arts groups. Math questions were selected for the analysis when the accuracy is above 85% in the group. Ccorrect RTs in 3 precue conditions were cluster axises and the results showed 2 distinctive types of questions, one was primed by geometrical figures, called 'figure questions'; the other was immune from the influence of geometrical figures, called 'rule questions'. The fMRI study selected some of more distinctive questions in the 'figure' and 'rule' category based on the 1st experiment. The contrast, 'figure questions' vs 'rule questions', showed activation in the right angular gyrus, and LOC areas. Meanwhile, the questions with the accuracy above 0.75 activated bilateral hippocampus, bilateral IFL, left angular gyrus, left IPL/IPS, anterior cingulate cortex, compared to the extremely difficult questions with the accuracy below 0.4. The results verifies 2 corresponding systems in the brain and also shed a light on Neuroeducation.

Disclosures: **T. Chiang:** None. **C. Wu:** None. **D. Ansari:** None.

Poster

085. Cognitive Development

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 85.01/NN24

Topic: F.01. Human Cognition and Behavior

Support: RO1 MH069942

Stony Brook Research Foundation

Title: Differential load- and performance-related brain activity during spatial working memory in preadolescent children

Authors: *A. S. HUANG, A. SMALL, A. S. LEE, D. KLEIN, H.-C. LEUNG;
Psychology, Stony Brook Univ., Stony Brook, NY

Abstract: Spatial working memory is commonly associated with activity in a set of frontal and parietal regions, including the dorsolateral prefrontal cortex and posterior parietal cortex, which typically show increasing activity with load in young adults. Children show similar activation of these prefrontal and parietal regions during working memory tasks in comparison to baseline tasks with no memory requirements. However, working memory load effects in the prefrontal cortex were not consistently found in studies using spatial tasks, but were more consistently observed in studies using verbal or visual tasks. These inconsistencies may result from large individual differences between children. Recent studies showed correlations between visual working memory task performance and activity in frontal and parietal regions in children. Here we conducted an fMRI study to examine individual differences in spatial working memory performance and brain activity. We hypothesize that activity in frontal and parietal regions increases with memory demand, and that load-related difference in activity predicts performance. Using a 3 T Siemens Trio system, we collected structural and functional images from 36 preadolescent children (9-12 years old, 16 female) . The children performed a 1-back task where they keep track of either 1 spatial location (Load 1), or 3 spatial locations (Load 3). The two load conditions were presented in blocks of 5 trials. The order of the two load conditions was counterbalanced across two runs, each lasted 4 min 7 sec and contained 4 blocks of each load with 13-sec of fixation in between. During each block, the participants made button presses on each trial to indicate whether any location differed from the previous trial. There was a variable delay of between 2.3 to 4.8 seconds between each trial. Children performed worse on the Load 3 condition compared to the Load 1 condition. Our fMRI data revealed greater activity in the

dorsolateral prefrontal cortex, superior parietal lobule, inferior parietal lobule and intraparietal sulcus in Load 3 compared to Load 1. There was greater activity in the precuneus and supramarginal gyrus in Load 1 compared to Load 3. Activity differences in the supramarginal gyrus comparing Load 3 versus Load 1 is positively correlated with performance differences between the two load conditions across subjects. In sum, our results show load-related increases in brain activations and deactivations in preadolescent children similar to the patterns documented in previous studies of young adults. Further, the differences in load-related activity mainly correlate with individual differences in performance in this age range.

Disclosures: **A.S. Huang:** None. **A. Small:** None. **A.S. Lee:** None. **D. Klein:** None. **H. Leung:** None.

Poster

085. Cognitive Development

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Topic: F.01. Human Cognition and Behavior

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PEst-C/SAU/UI3282/2013

PTDC/QUI-QUI/102049/2008

Title: GABA alterations in the NF1 brain: A multimodal 11C-flumazenil pet and MRS study

Authors: *I. R. VIOLANTE[1], M. PATRICIO[2], I. BERNARDINO[1], J. REBOLA[1], A. J. ABRUNHOSA[3], N. C. FERREIRA[3], M. CASTELO-BRANCO[1];
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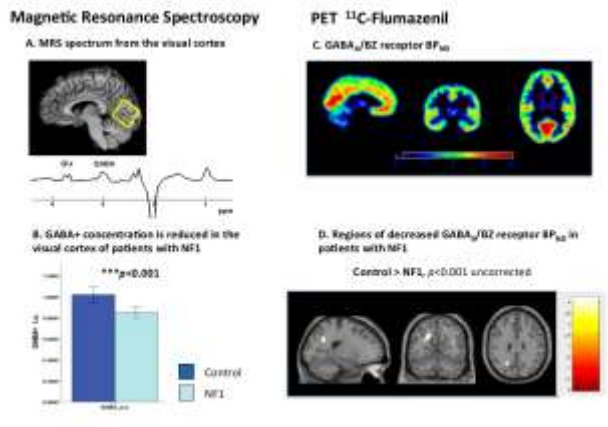
Abstract: Alterations in the push-pull mechanism regulating excitatory and inhibitory neurotransmission might underlie the cognitive deficits found in several developmental disorders. Neurofibromatosis type 1 (NF1) is a genetic disorder associated with cognitive dysfunction. Recently, using magnetic resonance spectroscopy (MRS), it was shown that GABA levels are decreased in the visual cortex of children with NF1. However, it is still to determine

whether GABAergic alterations are present in adults and whether GABA concentration deficits impact the distribution of GABA receptors. Here, we investigated GABA alterations in adult patients using a multimodal strategy combining MRS to determine GABA concentration and PET 11C-Flumazenil (FMZ) to investigate GABAA receptor binding. 14 patients with NF1 (35±7 yo.,4M) and 13 aged and gender matched controls (36±9 yo.,4M) performed PET and MRS in the same day. Our results show that: 1.Patients with NF1 have significantly less GABA+ than controls, $p<0.001$ in visual cortex, Fig1B. 2.Whole-brain analysis of FMZ BPND revealed decreased GABAA receptor binding in the left precuneus and right middle temporal gyrus, $p<0.001$ in patients, Fig1D. 3.We found no difference in GABAA receptor binding in the visual cortex between patients and controls. 4.There was no correlation between GABA concentration and FMZ BPND in the visual cortex, neither in controls nor in patients. In sum, adult patients with NF1 showed reduced GABA in the visual cortex, indicating that GABA deficits do not ameliorate with age. Whole-brain PET analysis revealed reduced FMZ BPND in the precuneus and middle temporal gyrus. Interestingly, precuneus is involved in visuospatial tasks, a hallmark deficit of NF1. Reduced GABA levels do not seem to impact the density of GABAA receptors, as we did not observe altered 11C-FMZ BPND in the visual cortex or a relationship between GABA levels measured by MRS and GABAA receptor density measured by PET. This result has important implications for understanding the disease mechanism, as it suggests that GABA alterations are predominantly pre-synaptic.



Disclosures: **I.R. Violante:** None. **M. Patricio:** None. **I. Bernardino:** None. **J. Rebola:** None. **A.J. Abrunhosa:** None. **N.C. Ferreira:** None. **M. Castelo-Branco:** None.

Poster

085. Cognitive Development

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 85.03/NN26

Topic: F.01. Human Cognition and Behavior

Title: Influence of Perioperative Hypnotherapy on Postoperative Improvement in Cognitive Performance (HYPNOC): preliminary results of a randomized controlled clinical trial

Authors: *C. A. IONESCU, F. BORCHERS, E. WEISS-GERLACH, K.-D. WERNECKE, C. SPIES;
Anesthesiol., Charite Berlin, Berlin, Germany

Abstract: Background: Every third patient develops postoperative delirium (POD) and postoperative cognitive dysfunction (POCD), respectively. POD and POCD increase postoperative morbidity and mortality as well as health care costs due to prolonged hospital stay. In previous studies, hypnosis has been shown to effectively reduce acute pain, stress, and anxiety; all of them contributing to the development of POD and POCD. The aim of this study is to evaluate the effect of perioperative hypnosis on the incidence of POD and POCD. Methods: In this ongoing RCT single center study 59 adult patients scheduled for cardiac surgery or spine surgery (mean age 61.6 years, 64.4% males) have been randomly selected to receive either hypnosis (3 sessions of 60 minutes, the first session 1 day preoperatively followed by 2 sessions postoperatively within 5 days) or to usual care. At baseline all patients were tested for suggestibility using the Stanford Hypnotic Suggestibility Scale. Patents with mild cognitive disorders (< 24 points in the Minimental State Examination, MMSE) or known dementia were excluded. POCD was diagnosed by using the CANTAB™ test battery (visual memory test, attention test, verbal learning test and Stroop colour test) on the day of discharge (primary endpoint) from hospital and three months after surgery. After surgery, patients were screened within the next 7 days for POD using validated delirium scores and the gold standard DSM-IV. In addition pain- and stress levels, emotional and functional status, sleep quality and time on ICU/in hospital were investigated as secondary endpoints. Statistics: Considering the low number of cases we performed a two-sided Fisher's-exact test to evaluate the influence of hypnosis on POCD. Results: POD occurred in 8 out of 59 patients. Three of those patients were in the intervention group and 5 in the control group (10.34% vs. 16.67%, p = 0.71). POCD occurred in 9 out of 59 patients. Three of those patients were in the intervention group and 6 in the control group (10.35% vs. 20.0%, p = 0.47). Conclusions: Preliminary results of this ongoing RCT did not show a significant reduction of postoperative cognitive dysfunction on discharge from hospital through hypnosis. Due to a remarkably low incidence of POCD, randomization of 409 additional patients (alpha error 5%; power 80%) is required.

Disclosures: C.A. Ionescu: None. **F. Borchers:** None. **E. Weiss-Gerlach:** None. **K. Wernecke:** None. **C. Spies:** None.

Poster

085. Cognitive Development

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 85.04/NN27

Topic: F.01. Human Cognition and Behavior

Support: NIMH R01-MH099078

Title: The development of hierarchical cognitive control and rule-guided behavior

Authors: *K. UNGER[1], D. AMSO[1], L. ACKERMAN[2], D. BADRE[1];
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Abstract: As children transition from childhood to adolescence, they make gains in their ability to use abstract rules that define the relationship between context and classes of more concrete actions. For example, behavioral evidence has demonstrated that children have greater difficulty following progressively abstract rules, even when demands on competition and load are controlled (Amso et al., in press). Previous work in adults has indicated that increasing abstraction in rule-guided action recruits progressively rostral portions of lateral frontal cortex along a rostrocaudal axis. Importantly, the frontal cortex undergoes a protracted and heterogeneous developmental course, with different regions reaching maturity at different times. We hypothesized that the functional development of the frontal cortex, from middle childhood to adulthood will reflect the emergence of mature rule-guided behavior. Here, we tested children through young adults (ages 7-28 years) in a task that requires a high degree (3rd order) of rule abstraction, while separately manipulating competition among choice alternatives in working memory. We replicated previous observations that developmental change in rule-guided behavior was evident only in in rule abstraction, whereas increasing competition did not result in developmental differences in task performance. In adults, parametric variation of competition among abstract rule representations was reflected in the level of activation in the inferior frontal sulcus, consistent with prior observations. Preliminary fMRI data from children and adolescents suggest that abstract rule use is associated with less focal activation in lateral frontal cortex than in adults. This pattern is consistent with previous findings showing lower functional specialization in the fronto-parietal control network at younger ages, and may reflect qualitative differences in how children represent and follow abstract rules.

Disclosures: **K. Unger:** None. **D. Amso:** None. **D. Badre:** None. **L. Ackerman:** None.

Poster

085. Cognitive Development

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 85.05/NN28

Topic: F.01. Human Cognition and Behavior

Support: Eunice Kennedy Shriver National Institutes of Child Health and Human Development P50 HD40095

Eunice Kennedy Shriver National Institutes of Child Health and Human Development R01HD056107

Title: Age-dependent sex-specific differences in the functional neuroanatomy of word processing

Authors: *O. A. OLULADE, J. S. NEIMAN, G. F. EDEN;
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Abstract: Studies have demonstrated sex-specific differences in behavioral performance on cognitive tasks (Kimura, 1996), brain structure (Good et al., 2001), and brain function (Shaywitz et al., 1995). Differences in language and reading have been of special interest, given the sex-specific differences in rate of language (Ozçalişkan and Goldin-Meadow, 2010; Eriksson et al., 2012) and reading (Wolf and Gow, 1986) acquisition and the higher incidence of language-based learning disabilities such as dyslexia (Rutter et al., 2004) in males. Previous studies have addressed the role of age (Turkeltaub et al., 2003) and sex (Ihnen et al., 2009) on the functional anatomy of single word processing, but not concurrently. In this study, we examined the interaction of age and sex in modulating neural response to real word processing and to false font processing. Twenty-three children (13 Girls, 7-16 yrs; 10 Boys, 7-13 yrs) and 50 adults (21 Women, 19-30 yrs; 29 Men, 19-42 yrs) were matched on intelligence and reading (Full-scale IQ/WJ-III Word Identification mean ± std: Girls: 121 ± 15/117 ± 14; Boys: 118 ± 10/111 ± 13; Women: 123 ± 7/111 ± 8; Men: 119 ± 9/108 ± 11). fMRI whole-brain images were collected while participants performed feature detection of a tall character within visually presented real words (RW) or false font strings (FF). Analysis was performed using SPM8. Following pre-processing, images were submitted to a whole-brain statistical ANOVA with Sex and Age Group as between-subject factors, and Task as a within-subject factor. Significant clusters emerging from the three-way interaction (Sex x Age Group x Task; $p < 0.005$; $k>30$) were extracted using MarsBar. For each subject, mean percent signal change was computed for each task relative to

baseline, and subsequently averaged for each of the four groups to determine the direction of observed effects. The three-way ANOVA yielded a significant cluster within the left supramarginal gyrus. This area is known to be activated in normal readers during reading and phonological tasks (Pugh et al., 2001) and gray matter volume here is correlated with reading ability (He et al., 2013). We found that Girls had lower activation for RW than for FF, while Women had greater activation for RW than for FF. The opposite trend was observed for males: Boys had greater activation for RW than for FF, while Men had lower activation for RW than for FF. Our results present evidence for age-dependent, sex-specific differences that are specific to word processing.

Disclosures: **O.A. Olulade:** None. **J.S. Neiman:** None. **G.F. Eden:** None.

Poster

085. Cognitive Development

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 85.06/NN29

Topic: F.01. Human Cognition and Behavior

Support: The South African National Research Council (NRF) - Thuthuka grant

Title: Neuro-Electrical bases during encoding and calculation phase of arithmetic Sequential: An electroencephalography investigation

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Abstract: Objective: The study investigated the encoding and subsequent calculation cognitive processes when arithmetic problem are presented in different formats (3 + 4, 3 x 4, three + four, three x four, /three/ + /four/, and /three/ x /four/). Method: Electroencephalography (EEG) data were collected from seventeen first year University students while they mentally process addition and multiplication problems presented in Arabic, audio and word formats. Early and late event-related potentials (ERPs) components where analyzed for the presentation-format effect (PFE), problem-size effect (PSE) and arithmetic-operation effect (AOE) using an exact-statistical method. Results: The PFE, PSE and AOE were observed both at the encoding and calculation phases of problems. Interactions of presentation-format with problem-size and arithmetic-operation at parietal electrode locations were associated with shift in calculation strategies in the

various formats and operations. ERPs latencies where shortest with operand where presented auditory while Arabic operands took intermediate position between auditory and word operands. Conclusion: Results suggests that the effects of auditory versus Arabic or word formats on ERPs components substantially reflect format-related shifts in the use of procedural strategies or codes that contribute to given tasks as early as the encoding phase of problems and continue to calculation phase. Significance: These findings will add insight into how the human brain performs the everyday activities that require mental calculation.

Disclosures: **E.T. Muluh:** None. **E. Muluh:** None. **L. John:** None.

Poster

085. Cognitive Development

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Program#/Poster#: 85.07/NN30

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: Electrophysiological investigation of number and area comparison abilities in children

Authors: *D. SANTANA[1], R. A. ABREU-MENDOZA[2], N. ARIAS-TREJO[2], R. HARO VALENCIA[3];
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Abstract: The salience of number over other magnitudes has been proved across several infant studies. Seven-month-olds are better at discriminating smaller ratio changes in number than area (Libertus et al., 2013). However, this salience tends to disappear: adults are equally good at discriminating number than area (Lourenco et al., 2012). Importantly, number but not area comparison abilities have been explored by means of electrophysiological techniques. Positive amplitudes behave according to the ratio: increased amplitudes for smaller ratio changes than for larger changes (Hyde and Spelke, 2011). The current study aims to contrast the accuracy of

children in discriminating area and number while brain activation was recorded. 19 children participated in two tasks: a Number Comparison Task (NCT) and an Area Comparison Task (ACT). In the first task, children had to decide whether the first or second stimuli had a larger number of dots_the surface area of the stimuli was held constant. In the second, they had to decide whether the first or the second stimuli had bigger or smaller dots_the number of dots was held constant. Children responded by pressing a left button if the first stimulus was larger or bigger and a right button if it was the second. Accuracy across tasks was analyzed by means of a 3x2 repeated measures ANOVA with Ratio (3:4, 5:6, 7:8) and Task (NCT & ACT) as within-subjects factors and mean correct responses as the dependent measure. The analysis only yielded a significant effect of Ratio ($F(2,32)= 10.49$, $p < .001$, $n2 =.39$). Post-hoc analyses indicated that children were better at discriminating 3:4 than 5:6 ($p = .05$) or 7:8 ratio changes ($p < .001$). Visual inspection of the grand average Event-Related Potentials waveforms showed a positive amplitude between 210-310 ms at P3 electrode that behaved accordingly to the ratio changes of number of dots; while a negative amplitude between 100-200 ms at P3 that only behaved according to the ratio changes of area. This was confirmed in two 3x2 repeated measures ANOVAs with Ratio (3:4, 5:6, 7:8) and Task (NCT & ACT) as within-subjects factors, one with the negative amplitudes and another with the positive amplitudes as dependent measures. The analyses yielded significant interactions of Ratio and Task for both measures (Negative $F(2,32)= 4.26$, $p < .05$, $n2 =.21$; Positive amplitudes $F(2,32)= 4.54$, $p < .05$, $n2 =.22$). The Ratio effect was illustrated by positive amplitudes for the number ratio changes, while the Ratio effect in negative amplitudes for area changes. These results indicate that, even though children had the same accuracy in discriminating number and area changes, these two magnitudes are processed differently in childhood.

Disclosures: **D. Santana:** None. **R.A. Abreu-Mendoza:** None. **N. Arias-Trejo:** None. **R. Haro Valencia:** None.

Poster

085. Cognitive Development

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

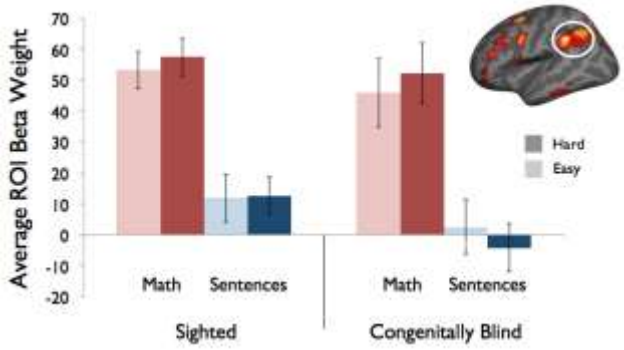
Program#/Poster#: 85.08/NN31

Topic: F.01. Human Cognition and Behavior

Title: Neural substrates of numerical processing develop independent of visual experience

Authors: *S. KANJLIA, C. T. LANE, L. FEIGENSON, M. BEDNY;
Psychological and Brain Sci., Johns Hopkins Univ., Baltimore, MD

Abstract: The ability to perform symbolic math is linked to a primitive number sense. Human adults, infants and other animals rapidly estimate the number of items in a visual array. Adults who are better at estimating numbers of dots score higher on tests of mathematical ability, such as the SAT (Libertus et al, 2012, Acta Psyc). Symbolic and non-symbolic numerical processing depends on the same region in the inferior parietal sulcus (IPS) (Piazza et al., 2007, Neuron). We asked whether visual experience with numerical sets is necessary for the development of cortical circuits involved in numerical processing. Congenitally blind and sighted participants underwent fMRI while solving simple algebra equations. Participants heard pairs of equations (e.g. X-2=5, X-4=3) and judged whether the value of "X" was the same. Half of the equations were simple (single-digit e.g. X-2=5) and half were complex (double-digit e.g. X-12=15). In control task, participants judged whether pairs of sentences had the same meaning. Half of the sentences were syntactically simple (subject-relative clause) and half were syntactically complex (object-relative clauses). fMRI data were motion-corrected, temporally filtered, mapped to the cortical surface, smoothed and fit with a GLM using FSL and Freesurfer. Replicating prior studies, we find that the IPS of sighted adults is 1) more active during a symbolic math task than during sentence comprehension, 2) responds more to complex than simple math equations and 3) is insensitive to syntactic complexity. Critically, we observed the same functional profile in the IPS of congenitally blind adults (group-by-condition ANOVAs main effect of math difficulty $F(1,13)=6.77$, $p=0.02$; group-by-condition interaction $F(1,13)=0.27$, $p=0.61$; main effect of syntactic complexity ROIs ($F(1,13)=0.96$, $p=0.35$; group-by-condition interaction $F(1,13)=1.46$, $p=0.25$). The functional selectivity of the IPS is similar in congenitally blind and sighted individuals. These results suggest that neural structures supporting numerical processing develop independently of visual experience.



Disclosures: **S. Kanjlia:** None. **C.T. Lane:** None. **M. Bedny:** None. **L. Feigenson:** None.

Poster

085. Cognitive Development

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 85.09/NN32

Topic: F.01. Human Cognition and Behavior

Support: Grant-in-Aid for Scientific Research on Innovative Areas

Title: Different strategy for movement imitation in ASD

Authors: *M. KAWASAKI[1,2], H. KOMEDA[3], T. MURAI[3], Y. FUNABIKI[3];
[1]Univ. of Tsukuba, Ibaraki, Japan; [2]RIKEN Brain Sci. Inst., Saitama, Japan; [3]Kyoto Univ., Kyoto, Japan

Abstract: One character of autism spectrum disorder (ASD) includes a problem of overlap between physical and visual representations in movement imitation. It is not clear about what are the differences between the ASD and typical development (TD) subjects in such imitations. To address the issue, we compared the performance, strategy, and neural mechanisms between them, by using a movement imitation task during electroencephalograph (EEG) and near infrared spectroscopy (NIRS) measurements. Eighteen TD and 18 ASD subjects took part in the experiments after they gave written informed consent. The ASD was assessed by the MSPA (Multi-dimensional scale for PDD and ADHD) and ADOS (Autism Diagnostic Observation Schedule). In the movement imitation tasks, both right and left hands were presented from top, right, left or bottom in PC display. Either right or left hand tapped a key, and then subjects were asked to imitate the movement as soon as possible. Each subject completed 3 sessions. In the first session, they performed the task without the instruction of the strategy. After that, we interviewed the strategy with the subjects. In the second and third sessions, they performed the task with the same strategy and the different strategy from the first session, respectively. The results of the interviews showed the different strategy between the ASD and TD subjects in the movement imitation. Most ASD subjects used mental rotation where they rotated the PC's hands and superimposed them with the self-hands in their minds. In contrast, most TD subjects used perspective taking where they superimposed the self-representation to the PC's hands in their minds. The different strategy is correlated with the scores of the MSPA and ADOS. Moreover, the ASD subjects' performance were degraded when they were asked to use the different strategy (i.e. third session), in comparison to the case of using the same strategy (i.e. second session), whereas the differences were not found in the TD subjects. The EEG and NIRS results showed the modulations of the frontal activity in the ASD subjects, which suggested the possibility that the frontal executive systems would be related to the ASD subject's different strategy in the movement imitations.

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Poster

085. Cognitive Development

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 85.10/NN33

Topic: F.01. Human Cognition and Behavior

Title: Developmental changes of short-term special memory of children assessed in the inverted delayed response test

Authors: *N. CHKHIKVISHVILI[1], M. DASHNIANI[1], T. NANEISHVILI[2];
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Abstract: Formation of the mechanisms for evaluation of the object's location in the space and of spatial memory occurs in the human postnatal development in a certain sequence. In our investigation, in order to determine the egocentric and allocentric systems' formation in the spatial short-term memory of the children of various ages, the Inverted Delayed Response test has been implemented, in which location of an object presented prior to the delay, is assessed by the subject following 1800 rotation. Total of 119 children of either sex aged 18-96 months were participating in the experiments. All of these attended a kinder-garden or school. The work complies with the ethical guidelines for human research. According to the data obtained, children aged 18-24 months, localization and retention in the memory of an item perceived, fulfill via the egocentric mechanism of spatial memory. In the landmark-poor environment correct solution of the above task begins in children since 24-36 months of age, which should be attributed to perfection of egocentric spatial memory. At this age the dead reckoning mechanisms, based on the afferent information concomitant to displacement, should be formed. Investigations of regularities of the inverted delayed response task performance, in conditions of functional blockage of sensory afferent systems in the children of various ages, have revealed that the above mechanisms function more perfectly in the children of 60+4 months, than in those of 40+4 months. The children of 7-8 years, unlike the children of 60+4 months, can determine location of an item perceived before the displacement in the inverted delayed response task just according to the surrounding landmarks (distant signals), without the sensory information accompanying the displacement. The data obtained provide for outlining the stages of the spatial short-term memory system's formation in the children.

Disclosures: **N. Chkhikvishvili:** None. **M. Dashniani:** None. **T. Naneishvili:** None.

Poster

085. Cognitive Development

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Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 85.11/NN34

Topic: F.01. Human Cognition and Behavior

Support: NIH Grant R01H047520

Title: Six-year longitudinal growth-curve modeling of functional brain changes during problem solving in children

Authors: ***C. J. BATTISTA**, T. M. EVANS, T. J. NGOON, T. CHEN, V. MENON; Psychiatry, Stanford Cognitive and Systems Neurosci. Lab., Palo Alto, CA

Abstract: Cross-sectional studies of cognitive development and problem solving have shown a general shift from frontal to parietal activity over time. Examination of short-term changes associated with problem solving have found linear increases in parietal and lateral occipital cortex activation between ages 7-8.4, with no evidence for decreases (Rosenberg-Lee et al., 2011). Long-term changes between ages 9-18 have been shown to manifest linear decreases in diffuse prefrontal and temporal lobe activation accompanied by linear increases in focal dorsal parietal and lateral occipital cortex activity (Rivera et al., 2005). Here we use growth curve analysis of functional brain changes associated with arithmetic problem solving in children who were tracked longitudinally from the ages of 7.74 and 13.9 at roughly one-year intervals, with each child contributing at least 2 and at most 4 data points. At each visit participants underwent a 3T fMRI scan, during which they performed single-digit addition, single-digit subtraction, and number identification tasks. Interestingly, linear growth-curve models provided the best fit for the addition task, whereas both and linear and quadratic models fit the subtraction task. During the addition task activity in the left superior parietal lobe (SPL), the anterior intraparietal sulcus (IPS), the posterior right parahippocampal gyrus, and the posterior cingulate cortex (PCC) increased linearly over time. Linear decreases in activity over time were observed in multiple prefrontal areas, including the bilateral insula, superior frontal gyrus, and the anterior cingulate cortex. In the subtraction task, linear increases in right posterior IPS activity were observed, whereas linear decreases were seen in right prefrontal cortex and the supplementary motor area. Notably, a quadratic inverted U-shaped profile was observed in the PCC whereas the bilateral insula showed a U-shaped profile. Our findings provide the first evidence for longitudinal

developmental shifts from prefrontal to parietal cortex during childhood. Importantly, neurodevelopmental profiles depend on the complexity of problems, and are characterized by both linear and nonlinear changes. Critically, more difficult problems showed prominent nonlinear changes in the PCC and insula, pointing to a dynamic role for default mode and salience network hub regions in cognitive skill development during childhood.

Disclosures: **C.J. Battista:** None. **T.M. Evans:** None. **T.J. Ngoon:** None. **T. Chen:** None. **V. Menon:** None.

Poster

085. Cognitive Development

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 85.12/NN35

Topic: F.01. Human Cognition and Behavior

Title: Cognitive function can be affected by the physical properties of dietary lipids in early life

Authors: A. L. SCHIPPER[1], L. M. BROERSEN[2], M. LOOS[3], A. J. W. SCHEURINK[4], G. VAN DIJK[4], *E. M. VAN DER BEEK[5];
[1]Nutricia Res., Danone Nutricia Early Life Nutr., Utrecht, Netherlands; [2]Nutricia Res., Advanced Med. Nutr., Utrecht, Netherlands; [3]Sylics, Amsterdam, Netherlands; [4]Dept Neuroendocrinology, Univ. Groningen, Groningen, Netherlands; [5]Nutricia Reseach - Danone Nutricia Early Life Nutr., Singapore, Singapore

Abstract: Cognitive development in infants can be positively influenced by the mode of feeding in early postnatal life. The duration of breastfeeding as well as differences in dietary lipid quality between Breast Milk (BM) and Infant Milk Formula (IMF) may contribute to this effect. Indeed, alterations in the dietary supply of omega 3 fatty acids (FA) early in life affect adult cognitive function. We studied whether another aspect of lipid quality, i.e. physical properties of lipid globules, may also contribute to cognitive development. We developed an IMF containing a complex lipid matrix (CLM) which more closely resembles the physical properties of lipid globules in BM (i.e. larger lipid droplets coated by phospholipids), without changing omega 3 and 6 FA composition. Healthy male C57Bl/6j mice were subjected to rodent diet containing either CLM or Control lipid composition (CTR) between 16 and 43 days of age. Thereafter regular rodent chow was fed until dissection (day 101). During adolescence and adulthood all mice were subjected to a test battery targeting various cognitive domains including novel object recognition and T maze spontaneous alternation. Biochemical analyses (e.g. lipid profile) were

performed in fresh collected brain material at dissection. In adolescence, CLM fed mice showed a higher preference for novel objects (day 35) and significantly better short term memory performance in the T maze (day 36) compared to CTR. Spatial reference memory in the Barnes maze (day 41) was not affected. In adulthood (day 78), after five weeks on regular rodent chow, preference for novel objects in the CLM fed group was significantly higher compared to CTR, but the improved short term memory performance in T maze test did not persist into adulthood (day 79). Reference and working memory in the 8-arm radial maze (day 101) remained unaffected. In conclusion, dietary exposure between postnatal day 16 and 43 (directly following lactation), to a chow containing a lipid structure closer to BM lipid globules improved attention and working memory in healthy mice without altering their spatial reference memory. These effects were sustained into adulthood. Further analyses of brain material to elucidate possible mechanisms underlying the observed behavioral changes are ongoing.

Disclosures: **A.L. Schipper:** A. Employment/Salary (full or part-time):; Nutricia Research. **L.M. Broersen:** A. Employment/Salary (full or part-time):; Nutricia Research. **M. Loos:** A. Employment/Salary (full or part-time):; Synaptologics BV. **A.J.W. Scheurink:** None. **G. van Dijk:** None. **E.M. Van Der Beek:** A. Employment/Salary (full or part-time):; Nutricia Research.

Poster

085. Cognitive Development

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Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 85.13/NN36

Topic: F.01. Human Cognition and Behavior

Support: UCL Grand Challenge Studentship

Great Ormond Street Hospital Children's Charity

Title: Brain injury and cognitive dysfunction secondary to severe and recurrent hypoglycaemia

Authors: ***G. PITTS**[1], A. KUMARAN[2], J. BULLOCK[4], K. HUSSAIN[2], D. GADIAN[3], F. VARGHA-KHADEM[1];
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Abstract: It is well established that patients who experience severe and recurrent episodes of hypoglycaemia early in life are at risk of brain injury, yet the late effects of such insult are not well documented. Here we report the pattern of brain abnormality associated with hypoglycaemia in relation to cognitive and motor performance. We selected 28 patients with a history of severe and recurrent hypoglycaemia sustained below the age of 5 years, and assessed them along with a group of 28 age- and sex-matched controls (patients mean age= 9.9yrs, sd= 2.15; controls mean age= 10.1yrs, sd=1.9yrs). Analysis of total grey matter (GM) and white matter (WM) volumes indicated a global increase in GM ($p=0.002$) and a reduction in WM ($p=0.025$). These were accompanied by regional abnormalities as indicated by Voxel Based Morphometry (Ashburner & Friston, 2000). Specifically, patients had a regional reduction of GM volume in the left inferior frontal gyrus ($p<0.05$, FWE corrected), and an increased GM volume in bilateral temporal regions ($p<0.05$, FWE corrected). In addition, patients showed reduced WM volume in bilateral occipital and temporal regions, as well as left parietal regions ($p<0.05$, FWE corrected). Manually measured hippocampal volumes were lower on each side in patients relative to controls ($p<0.05$). Neuropsychological test results revealed significantly lower scores relative to controls on working memory, processing speed, academic attainments, attention and motor skills. Although the patient group as a whole was not impaired on measures of long term memory, there was variability in performance with some individuals showing memory deficits. In addition, immediate and delayed recall scores correlated positively with hippocampal volumes ($p<0.01$). Consistent with the frontal GM and posterior WM abnormality, patients were most impaired in the domains of executive function, attention and motor performance. This is the first study documenting the pattern of hypoglycaemia-induced brain abnormality using quantitative whole brain analyses. This abnormality is characterised by a global increase in GM volume and a decrease in WM volume. While damage to WM in posterior areas has been reported previously, the pattern of GM abnormality reported here is novel and warrants further investigation to establish its link with cognitive profiles. The variability in cognitive outcome in the patient group raises the possibility that factors other than hypoglycaemia, such as the timing of brain insult or the presence of hypoglycaemic seizures, may influence long-term outcome.

Disclosures: **G. Pitts:** None. **A. Kumaran:** None. **J. Bullock:** None. **K. Hussain:** None. **D. Gadian:** None. **F. Vargha-Khadem:** None.

Poster

085. Cognitive Development

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 85.14/OO1

Topic: F.01. Human Cognition and Behavior

Support: UPAEP 30108-1030

Title: Development of a pattern recognition System using an artificial neural network for the rehabilitation of children with dysgraphia

Authors: *H. J. PELAYO[1], J. LOPEZ-MARTINEZ[2], J. CASTRO-MANZANO[3], V. REYES-MEZA[4], V. ZANELLA-PALACIOS[2];
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Abstract: A Pattern Recognition System (PRS) is used to identify specific features of an environment or object. We have developed a PRS to detect and identify the patterns specified by the following pairs: (d, b) and (p, q). The system has a high reliability because it is built upon three artificial neural networks with different data inputs based on the same pattern, thus giving three resolutions about such pattern. The PRS discrimination is done because those graphemes (i.e., patterns) are misperceived by children with Dysgraphia, a pathology that increases the risk of failure and children desertion at school. Therefore, new methods to reduce this problem have to be developed, and stimulating tools could be a solution. Our PRS is part of a tool, videogame-inspired, where the player's goal it is to reach a high score by writing down the letters correctly. Additionally, it gives positive or negative feedback strongly indicating if the letter is wrong or well written, stimulating the player by a reward system in order to improve his writing skills and trying to help him with his dysgraphia problem. The preliminary results, from 20 regular students, support previous findings showing that children are highly motivated to work with the system. Financial support from UPAEP 30108-1030.

Disclosures: **H.J. Pelayo:** None. **J. Lopez-Martinez:** None. **J. Castro-Manzano:** None. **V. Reyes-Meza:** None. **V. Zanella-Palacios:** None.

Poster

085. Cognitive Development

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 85.15/OO2

Topic: F.01. Human Cognition and Behavior

Title: Electrophysiological measures of functional connectivity and their relationship with working memory capacity in childhood

Authors: *J. BARNES, D. E. ASTLE;
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Abstract: Functional connectivity enables neurally encoded information to be communicated between different brain areas, and this communication is likely to be highly important for particularly complex aspects of cognition, such as when regions involved in cognitive control need to communicate fluctuating task goals to lower order, functionally specific regions. Some of these functional connections are identifiable even at rest, and are typically observed with fMRI, a method that is limited by its use of indirect measures of neural activity and thereby is insensitive to the rich neurophysiological information conveyed via functional connections. We used magnetoencephalographic (MEG) recordings, projected into source space, to demonstrate that brain networks in childhood have electrophysiological underpinnings that are evident in the spontaneous fluctuations of oscillatory synchronisation and desynchronisation. Using the temporal structure of these oscillatory patterns we were able to identify a number of functional networks, such as resting state networks that have previously been characterised in adults. Importantly, we did not provide any spatial specifications, with the spatial distribution of the networks across cortex being an emergent property of the common underlying temporal pattern of the electrophysiological activity. We also demonstrated that inter-subject variability in these electrophysiological measures of functional connectivity are related to individual differences in cognitive ability: the strength of connectivity between fronto-parietal cortices and lower-level sensory areas at rest corresponded to memory capacity, as measured outside the scanner with educationally-relevant standardised assessments working memory. This study represents an important advance in understanding the electrophysiological mechanisms underpinning functional connectivity at a level of cortex in childhood, and the extent to which characteristics of these functionally connected networks reflect cognitive ability.

Disclosures: **J. Barnes:** None. **D.E. Astle:** None.

Poster

085. Cognitive Development

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 85.16/OO3

Topic: F.01. Human Cognition and Behavior

Title: Domain specific higher-cognitive responses in “visual” cortex of blind adults

Authors: C. LANE, S. KANJLIA, A. OMAKI, *M. BEDNY;
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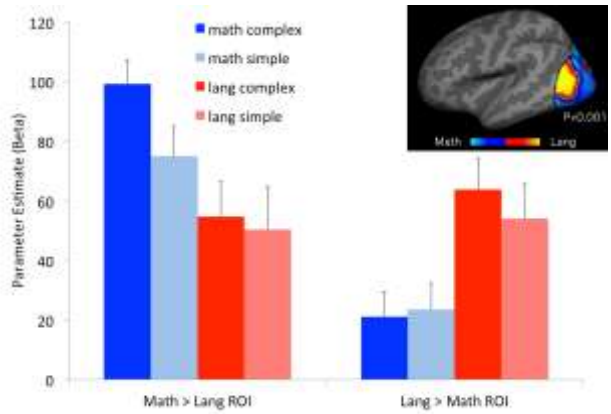
Abstract: Early blind individuals activate "visual" areas of the occipital lobe during a variety of verbal and non-verbal cognitive tasks. Are these plastic responses non-specific or are they selective by cognitive domain? We acquired fMRI data from early blind and sighted adults while they performed three kinds of tasks: sentence comprehension, solving simple algebra equations and remembering sequences of non-words. In Experiment 1, participants listened to sentences of high and low grammatical complexity and answered yes/no questions about them. The high complexity sentences involved a long-distance “movement” dependency. In a control task, participants performed a match to sample task with lists of non-words. In Experiment 2, participants heard pairs of sentences and judged whether they had the same meaning. In a numerical control task, participants heard pairs of spoken equations and had to decide if the value of a variable “X” was the same in both (e.g. X-4=5; X-6=3). Equations were either simple (single digit) or complex (double digit). Prior to fitting a GLM, fMRI data were motion corrected, high pass filtered, mapped to the cortical surface and smoothed using FSL and Freesurfer. Functional ROIs were defined in occipital cortex of blind participants: 1) lateral occipital ROIs responsive to sentences>equations and 2) occipital pole ROIs responsive to equations>sentences. We extracted parameter estimates for orthogonal contrasts. We observed a double dissociation in the functional response of different occipital areas. Regions that responded more to sentences than equations 1) responded more to sentences than sequences of non-words 2) showed greater responses to syntactically complex sentences, and 3) were insensitive to math complexity. By contrast, occipital areas that responded more to math equations than sentences 1) responded more to complex than simple math equations, and 2) were insensitive to syntactic complexity. We conclude that early blindness leads to development of functionally specialized higher-cognitive responses in “visual” cortex.



Disclosures: **C. Lane:** None. **S. Kanjlia:** None. **A. Omaki:** None. **M. Bedny:** None.

Poster

085. Cognitive Development

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 85.17/OO4

Topic: F.01. Human Cognition and Behavior

Support: NINDS 2R37NS021135-27

Nielsen Corporation

Title: Evidence for state-dependent high frequency power changes in human neonatal EEG

Authors: ***M. E. CANO**[1], R. KUPERMAN[3], K. L. ANDERSON[2], R. T. KNIGHT[2,1];
[1]Helen Wills Neurosci. Inst., [2]Psychology, UC Berkeley, Berkeley, CA; [3]Pediatric Epilepsy Program, Children's Hosp. and Res. Ctr. Oakland, Oakland, CA

Abstract: High frequency brain oscillations (high gamma; HG: 70-200 Hz) have been shown to reliably track many cognitive functions and can be used as a measure of neuronal firing. HG activity cannot typically be recorded using scalp EEG due to the 1/f power law and signal attenuation by the skull. Thus, human HG is generally studied using invasive surgical techniques. However, human neonates have thinner skulls than that of adults. Additionally, they have “soft spots”, or fontanelles, where the skull has not yet fused. Accordingly, we hypothesized that it might be possible to detect higher frequency brain oscillations from neonates using noninvasive scalp EEG. High frequency EEG (1000 Hz sampling rate) was continuously recorded from healthy neonates (n=14; 1-4 months) undergoing outpatient EEG monitoring for a possible seizure. Recordings were obtained from 11 electrodes placed according to the 10-20 system. All neonates were developmentally normal, and did not have any epileptic activity in their EEGs. High gamma power (75 - 150 Hz) was extracted from data acquired during both sleep and awake recording periods. We found enhanced high gamma power when the infants were awake compared to when sleeping ($p<0.05$). Additionally, we observed increased beta-high gamma phase-amplitude coupling in the awake periods compared with sleep ($p<0.05$). These findings were evident across almost all electrode sites. To address the possibility that any differences seen between sleep and awake states could be due to muscle artifact, we analyzed event-related activity from three neonates undergoing photic stimulation. We found HG power changes that reflected the stimulation frequency, such that HG power increased following photic stimulation.

The results from both naturalistic sleep state changes as well as event-related visual responses to photic stimulation provide evidence that high frequency activity can be recorded from the scalp of neonates. These findings provided evidence for a potential metric for developmental studies in health and disease in neonates.

Disclosures: **M.E. Cano:** None. **R. Kuperman:** None. **K.L. Anderson:** None. **R.T. Knight:** None.

Poster

085. Cognitive Development

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 85.18/OO5

Topic: F.01. Human Cognition and Behavior

Support: KAKENHI 20578976

Neuro Creative Lab (NPO

Narishige Neuroscience Research Foundation

Title: Dynamic neural network reorganization associated with improvement of prospective metacognition

Authors: *C. HOSODA[1], K. OKANOYA[1], M. HONDA[2], R. OSU[3], T. HANAKAWA[2]; [1]Univ. of Tokyo, Tokyo, Japan; [2]NCNP, Tokyo, Japan; [3]ATR, Kyoto, Japan

Abstract: A number of scientific neuroimaging studies have begun to characterize development of the gray matter (GM) in correlation with various human abilities. Recent studies found that GM volume in the frontal polar cortex (FPC) was correlated with various kinds of retrospective metacognition capacity, i.e., how well one's confidence ratings after a task distinguish between correct judgment and incorrect judgment (Flemming et al, 2010, 2012, McCurdy et al, 2013). However, it remains unclear whether FPC also concerns prospective-type metacognitive capability or not. Additionally, one important question is whether GM volume correlated with those metacognitive capabilities can develop through learning that induces plastic changes of the brain. To address these issues, we implemented 2-month training program for visuo-motor association learning (VML). Forty right-handed participants (31 male and 9 female, aged 21.4 ± 3.2 years) underwent a 10-week computer-based training in which they were required to learn and recall tapping sequences on a trial-and-error basis every weekday. The participants predicted

the time for completing a daily assignment and reported it before learning everyday. The difference between the predicted time and real learning times yielded an index of prospective metacognitive ability. We obtained structural magnetic resonance imaging (MRI) before and after the training intervention. The whole-brain analyses of MRI before the training identified that participant with more accurate prospective metacognition had greater GM volume in the FPC ($p<0.05$, FEW correct). This result indicates that FPC also mediates a metacognitive ability about future. Moreover, we found improvement of the prospective metacognitive ability after the training, and this change was correlated with plastic development of FPC GM volume. No correlation was found between the learning time and the change of FPC GM volume. These findings provide compelling evidence for the improvement of prospect metacognition associated with experience-dependent plastic changes of GM in FPC.

Disclosures: **C. Hosoda:** None. **K. Okanoya:** None. **M. Honda:** None. **R. Osu:** None. **T. Hanakawa:** None.

Poster

085. Cognitive Development

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Topic: F.01. Human Cognition and Behavior

Support: NIH Grant 2R01MH06470806A1

NIH Grant R01MH064580

NIH Grant MH061696

Autism Speaks Grant 2099

Title: Early brain structure and later behavioral outcomes in fragile X syndrome

Authors: ***J. BRUNO**[1], A. STARK[1], A. A. LIGHTBODY[1], H. C. HAZLETT[2], J. PIVEN[2], A. L. REISS[1];
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Abstract: Fragile X syndrome (FXS) is associated with behavioral, cognitive and neurological deficits attributed to limited or lack of the fragile X mental retardation protein (FMRP). One

particularly maladaptive behavioral feature is the presence of restricted and repetitive behaviors also associated with autism spectrum disorder (ASD). Neuroimaging indicates regional alterations in caudate morphology are significantly correlated with these behaviors in early life. The relationship between early brain measures and behavioral outcomes in later development may help us understand the mechanism by which FMRP influences behavior and could aid in the design of target treatments. We sought to elucidate the predictive power of early brain structure on later behavioral outcomes among boys with FXS and a developmentally matched comparison group. Using T1-weighted MRI, we scanned 116 boys aged 1-3 years (52 FXS, 64 ASD/developmental delay). Bilateral caudate volumes, extracted using FreeSurfer, were adjusted for total brain volume. We assessed behavioral outcomes (age 8-14) via the Autism Diagnostic Observation Schedule (ADOS-2), a structured behavioral assessment including a restricted/repetitive behavior subscale. Behavioral outcomes were collected for 25 boys thus far in this ongoing longitudinal study. We performed within group correlations to determine if caudate volume in early life predicted later ADOS scores. Within the FXS group, bilateral caudate volumes were significantly positively related to ADOS restricted/repetitive behavior subscale scores in later childhood. In the comparison group only left caudate volumes were significantly positively related to behavior. Total brain volume was not a significant predictor of behavioral outcome in either group. These results indicate a positive predictive relationship between caudate volume measured early in life and restricted repetitive behaviors measured in later childhood. Relationships were present, in at least one hemisphere, for both groups. The relationships were positive; larger volumes were associated with more restricted/repetitive behaviors. Predictive relationships with the caudate were significant after adjusting for total brain volume, suggesting the caudate may play a unique role in the development of restricted/repetitive behavior. Caudate volumes measured early in development could be a useful biomarker in FXS and, potentially, in idiopathic neurodevelopmental disorders. These relationships further elucidate the neurobiological basis of restricted/repetitive behavior and may aid in development of more targeted interventions.

Disclosures: **J. Bruno:** None. **A. Stark:** None. **A.A. Lightbody:** None. **H.C. Hazlett:** None. **J. Piven:** None. **A.L. Reiss:** None.

Poster

085. Cognitive Development

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 85.20/OO7

Topic: A.10. Adolescent Development

Title: Visuospatial working memory assessed by Corsi Block Tapping Task: changes with aging

Authors: *J. C. HEVIA, M. GUEVARA, M. HERNÁNDEZ GONZÁLEZ, L. RIZO MARTÍNEZ, M. ALMANZA SEPÚLVEDA;
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Abstract: It has been postulated that the execution of working memory is in direct correspondence with the maturation of the prefrontal cortex (PFC) which reaches maturity around the third decade of life. This work was developed in order to explore changes in the performance of working memory in relation to some critical stages of maturation of the CPF through the implementation of the backward Corsi Block Tapping task. Participants were 36 men divided into 3 groups (n = 12), group 1 (G1): 11-13 years, group 2 (G2): 18 -20 years and group 3 (G3): 26 to 30 years old. The G2 and G3 had a greater number of correct trials and more elements retained in memory with respect to G1. However, only the G3 showed a lower total runtime compared to G1. These results provide further evidence that the proper execution of cubes Corsi is reached early adulthood, while efficiency in visuospatial working memory is obtained until the end of the third decade of life. These findings are in agreement with the theory of development executive functions in relation to the maturity of the CPF.

Disclosures: **J.C. Hevia:** None. **M. Guevara:** None. **M. Hernández gonzález:** None. **L. Rizo martínez:** None. **M. Almanza sepúlveda:** None.

Poster

086. Aging Brain

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 86.01/OO8

Topic: F.01. Human Cognition and Behavior

Support: NIH Grant R01 AG15019

Title: Increased daily cortisol secretion and aging are associated with smaller amygdala volume

Authors: *E.-M. QUINTIN[1], G. E. ENNIS[1], K. M. KENNEDY[2], J. SHIN[1], C. K. HERTZOG[1], S. D. MOFFAT[1];
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Abstract: Many studies have reported that high levels of cortisol, a stress hormone, are associated with measures of cognitive aging but the relationship between cortisol and structural brain aging has not been studied extensively. In animals, glucocorticoid receptors are abundant in the hippocampus, amygdala, and frontal and cingulate cortex (Kolber et al., 2009) and chronic exposure to high levels of corticosteroids is associated with decreased hippocampal volume (Landfield et al., 1978). In humans, there is evidence that higher cortisol levels are associated with thinner frontal and anterior cingulate cortex (Kremen et al., 2010). Atrophy of the frontal cortex, hippocampus, and amygdala is also associated with normal aging. In the present study, we investigated the association between cortisol secretion and age and volume of neuroanatomical regions of interest. Participants were healthy community-dwelling volunteers (N = 36; Mean age = 64, range 42-83) who had both cortisol and MRI data available. Salivary cortisol was collected 7 times per day over 10 days. Cortisol measures have been previously described (Scott al., 2013) and included cortisol awakening response, total daily secretion (area under the curve [AUC]), and daily slope, all averaged over the 10 days. MRI scans (3T Siemens; MPRAGE) were conducted approximately two years following cortisol data collection. ROIs, as computed from Freesurfer and expert operators, were used as outcome measures (ROIs: hippocampus, amygdala, and the frontal and anterior cingulate cortices). All ROIs were adjusted for total intracranial volume. Older age was significantly associated with smaller volumes for all ROIs. Higher daily secretion of cortisol AUC and older age were significantly associated with smaller amygdala volume. Cortisol AUC was not significantly related to the volume of the other ROIs. Thus, cortisol levels may have a specific effect on amygdala integrity which appears to be independent of brain aging. Results will be discussed within the framework of the amygdala response to threatening stimuli as well as how cortisol may impact circuits including the amygdala that play a role in human behavior and cognition.

Disclosures: **E. Quintin:** None. **G.E. Ennis:** None. **K.M. Kennedy:** None. **J. Shin:** None. **C.K. Hertzog:** None. **S.D. Moffat:** None.

Poster

086. Aging Brain

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 86.02/OO9

Topic: F.01. Human Cognition and Behavior

Support: Intramural Research Program of the NIH, National Institute on Aging

Title: Age differences and longitudinal changes on diffusion tensor imaging measures

Authors: V. K. VENKATRAMAN[1], C. E. GONZALEZ[1], *B. A. LANDMAN[2], J. O. GOH[3], S. M. RESNICK[1];
[1]Natl. Inst. of Aging, Natl. Inst. of Hlth., Baltimore, MD; [2]Vanderbilt Univ., Brentwood, TN; [3]Grad. Inst. of Brain and Mind Sci., Natl. Taiwan Univ. Col. of Med., Taipei, Taiwan

Abstract: Diffusion tensor imaging (DTI) has been used extensively to investigate age effects on white matter tracks and tissue microstructure. Studies have shown age differences in white and gray matter integrity in normal aging (Bennett et al 2010) and in relation to behavioral measures (Madden et al 2009). Previous studies have also shown that these measures can detect age-changes over a short follow-up period (Charlton et al 2010). In this study we use a large longitudinal sample with regional gray and white matter diffusion measures to investigate the regions that are most sensitive to estimates of cross-sectional age differences and longitudinal age changes over time, after controlling for scanner field strength. DTI MRI scans from the Baltimore Longitudinal Study of Aging were analyzed for this study (545 subjects; 800 visits; mean baseline age = 68.54 ± 12.66 years; mean follow-up interval = 0.86 years). All participants included in this study were cognitively normal and the data was acquired on 1.5T Intera and 3T Achieva scanners. DTI processing followed standard practice for tensor fitting and quality assessment (Lauzon et al 2013). The regional DTI outputs, mean diffusivity (MD) and fractional anisotropy (FA), were obtained from the BrainColor and EVE labels for gray and white matter regions, respectively. The MD and FA from gray and white matter regions were modeled as dependent variables in a mixed-model approach with subject-specific intercept and slope as random effects. Baseline age, follow-up interval, scanner field strength and longitudinal age effect (age*interval) were independent variables. The results indicate greater cross-sectional age is associated with significantly ($p < .05$) greater MD across gray and white matter regions and significantly lower FA in most white matter regions. FA showed significant decreases over time across gray and white matter regions, except medial superior frontal gyrus, superior and posterior corona radiata. MD increased over time across gray and white matter regions such as bilateral hippocampus and corpus callosum. Baseline age affected rates of change in several regions. FA in left superior longitudinal fasciculus, posterior and superior corona radiata, showed significant longitudinal decline which decelerates with increasing baseline age. Higher baseline age was associated with accelerated longitudinal increases in MD in gray (bilateral hippocampus, precuneus, amygdala) and white matter (bilateral superior longitudinal fasciculus, posterior corona radiata, left anterior and superior corona radiata). Overall, the cross-sectional and longitudinal age effects seem to be detectable in a consistent manner.

Disclosures: V.K. Venkatraman: None. **C.E. Gonzalez:** None. **B.A. Landman:** None. **J.O. Goh:** None. **S.M. Resnick:** None.

Poster

086. Aging Brain

Location: Halls A-C

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Program#/Poster#: 86.03/OO10

Topic: F.01. Human Cognition and Behavior

Support: This work was supported by the University Research Priority Program "Dynamics of Healthy Aging" of the University of Zurich

This work was supported by the Velux Stiftung (project No. 369)

Title: Predicting age from surface-based morphometry data via support vector regression

Authors: *F. LIEM, S. MÉRILLAT, L. BEZZOLA, S. HIRSIGER, L. JANCKE;
Univ. of Zurich, Zurich, Switzerland

Abstract: Introduction Recently published work by Franke et al. demonstrated how a subject's age can be predicted solely based on brain imaging data (Franke et al., 2010; 2012). Via voxel-based morphometry (VBM) and machine learning algorithms, these authors made brain-based age predictions on a single subject level. The present work is based on the work of Franke et al. but further develops these authors' approach by extending it to data resulting from surface-based morphometry. As compared to voxel-based morphometry, surface-based morphometric analyzes allow for a more elaborate description of cortical anatomy (Winkler et al., 2010). As, for instance, cortical thickness and surface area differentially change with age (Hogstrom et al., 2013), using those metrics separately should give more accurate age-predictions. Methods T1w images (1x1x1mm) were obtained from 220 healthy subjects (age between 64 and 87 years; M = 70.4, SD = 4.9). Measures of cortical thickness, surface area and volume were computed via Freesurfer (Fischl, 2012). To predict age, these data were fed into a support vector regression (SVR) (Ben-Hur et al., 2008), in a 10-fold cross validation approach. The SVR implementation of scikit-lean was used (Abraham et al., 2014) with a linear kernel. The mean average error (MAE) and the correlation between the real age and the predicted age were taken as indicators of prediction success. Results MAE for cortical thickness was 3.09 years (correlation between predicted an real age: $r = 0.64$, $p < 0.001$). Similar results were found for cortical surface area (MAE = 3.48; $r = 0.50$, $p < 0.001$), and cortical volume (MAE = 3.55; $r = 0.43$, $p < 0.001$). Conclusion We demonstrated that surface-based morphometry data allow for a good brain-based prediction of a subject's chronological age. Furthermore, brain-based predictions are not only restricted to age. They also might aid, for instance, in predicting future cognitive abilities of children (Ullman et al., 2014), or in predicting conversion rates from mild cognitive impairment

to Alzheimer's disease (Gaser et al., 2013). References Abraham, A. et al. (2014) Front Neuroinform 8. Ben-Hur, A. et al. (2008) PLoS Comput Biol 4. Fischl, B. (2012) NeuroImage 62. Franke, K. et al. (2012) NeuroImage 63. Franke, K. et al. (2010) NeuroImage 50. Gaser, C. et al. (2013) PLoS ONE 8. Hogstrom, L.J. et al. (2013) Cerebral Cortex 23. Ullman, H. et al. (2014) J Neurosci 34. Winkler, A.M. et al. (2010) NeuroImage 53.

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Poster

086. Aging Brain

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Program#/Poster#: 86.04/OO11

Topic: F.01. Human Cognition and Behavior

Support: CNPq

CAPES

FAPERJ

FAPESP

INNT

Title: Absolute cellular composition of the human cerebral cortex is preserved for neurons but not for non-neuronal cells during aging

Authors: *E. CASTRO-FONSECA[1], C. GOMES DA SILVA[1], C. H. ANDRADE-MORAES[1], V. CARDOSO DE OLIVEIRA[1], A. T. ALHO[2], R. E. LEITE[2], R. E. FERRETTI-REBUSTINI[2,3], J. M. FARFEL[2], C. K. SUEMOTO[2], C. A. PASQUALUCCI[2], W. JACOB-FILHO[2], L. T. GRINBERG[2,4], R. LENT[1,5];
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Abstract: In the nervous system, the different cognitive functions of the cerebral cortex (CC) such as memory, attention and executive control can be altered during aging process. Since brain cells (and synapses) are the processing units underlying these functions, we studied the quantitative cell composition of the CC in post-mortem brains of people without cognitive impairment. Four regions of interest (frontal, parietal, temporal and occipital) were dissected using principal anatomic landmarks: central, lateral, parieto-occipital sulci and pre-occipital notch. We used the isotropic fractionator for cell quantification, based on nuclei identification by immunocytochemistry in representative aliquots of an isotropic nuclei suspension obtained from the original region. To date, we have studied five female subjects split into two groups: from 20 to 40 years and 70 to 90 years. We found an average of 10.4 ± 0.6 billion neurons in the whole cortex, of which approximately 38% are in the frontal lobe, 19% in the temporal, 24% in the parietal and 19% in the occipital. We observed that neuronal density has an inverse profile: greater in the posterior lobes than in the anterior ones; 25.1 ± 7.3 million/g in the frontal lobe and 31.8 ± 5.2 million/g in the occipital. Comparing the two age groups, we found no significant difference ($p < 0.05$) in the number of neurons or in the neuronal density neither for any particular lobe nor for the entire cortex. However, a lower number of non-neuronal cells in the entire cortex was found for the older group ($p= 0.0243$). Our results suggest that during normal aging (NA) there is a preservation of the number of neurons in female CC, which may explain the absence of cognitive decline in this population. In a previous study of our group, neuronal loss and an increase in the number of non-neuronal cells were found in cases with diagnosed Alzheimer´s Disease with dementia. This is probably associated with inflammatory reactions as shown in other works. Since these cells represent mainly glia, our hypothesis is that in NA the decrease in non-neuronal cells may exert a protective role to avoid inflammatory pathways and the consequent degeneration of neuronal cells, a possible cause associated to dementia. The absence of brain inflammation in the course of NA must be explored. This hypothesis can justify the preservation of neurons, the decrease in non-neuronal cells and the absence of cognitive decline found in NA. Despite the currently low n in our sample, these results serve as a baseline for quantitative composition of cerebral normal aging and can be useful to compare them with different neurological entities such as other neurodegenerative diseases.

Disclosures: **E. Castro-Fonseca:** None. **C. Gomes da silva:** None. **C.H. Andrade-moraes:** None. **V. Cardoso de oliveira:** None. **A.T. Alho:** None. **R.E. Leite:** None. **R.E. Ferretti-rebustini:** None. **J.M. Farfel:** None. **C.K. Suemoto:** None. **C.A. Pasqualucci:** None. **W. Jacob-filho:** None. **L.T. Grinberg:** None. **R. Lent:** None.

Poster

086. Aging Brain

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 86.05/OO12

Topic: F.01. Human Cognition and Behavior

Support: Wellcome Trust Senior Research Fellowship (WT090961MA)

Title: Profiles of age-related structural decline and stability in neuroanatomical systems supporting vocal emotion processing

Authors: *C. F. LIMA[1,2], N. LAVAN[3], S. EVANS[1], Z. AGNEW[4], P. SHANMUGALINGAM[1], J. WARREN[5], S. CASTRO[2], S. SCOTT[1];
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Abstract: Humans use several cues to infer others' emotional states, such as facial expressions, body postures, or vocal information. Being effective at perceiving these cues is crucial for interpersonal functioning. Although a number of studies report age-related decrements in emotion recognition accuracy, most of these studies resort to facial expressions. Much less is known about the auditory expression of emotions and, crucially, it remains to be determined whether and how behavioural decrements in emotion recognition result from age-related grey matter loss. In this study, we focussed on nonverbal vocalizations, such as laughs, screams and sighs, and examined how the processing of these emotion signals change across the adult life span at behavioural and neuroanatomical levels. A cross-sectional sample of 61 healthy adults aged between 20 and 81 years was tested. Participants were also assessed for hearing thresholds and general cognitive functioning. The emotional stimuli consisted of a set of vocalizations previously validated to communicate two positive emotions, amusement and pleasure, and two negative ones, disgust and fear. A multidimensional rating procedure was implemented in which participants rated how much each vocalization expressed the intended emotion as well as all the other emotions. As expected, increasing age was associated with decrements in ratings and in a derived measure of accuracy. These decrements were similar across positive and negative emotions, and they were not mediated by hearing and cognitive losses. Participants also underwent a magnetic resonance imaging (MRI) scan; T1-weighted volumetric images were acquired in a Siemens Avanto 1.5 Tesla system, including 32-channel head coil. Voxel-based morphometry was performed and multiple regressions were conducted to assess the relationships between individual differences in grey matter volume, ageing, and individual differences in emotion recognition (statistical maps were thresholded at $p < .005$, cluster corrected with Family Wise Error correction of $p < .05$). Age-related grey matter decrements in the right middle temporal gyrus and in the left middle orbital gyrus were found to mediate behavioural decrements in emotion recognition. On the other hand, clusters in the left and right inferior frontal gyri, right hippocampus, right amygdala, and left inferior parietal lobe correlated

negatively with behavioural performance in emotion recognition similarly in younger and in older adults. These results are discussed in relation to theoretical perspectives on emotional ageing, particularly positivity effects, general cognitive decline, and neurocognitive decline.

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Poster

086. Aging Brain

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Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 86.06/OO13

Topic: F.01. Human Cognition and Behavior

Support: NIA P01-AG03991

Title: Age effects on self-reported navigation ability are mediated by regional brain volume

Authors: ***N. LUCENA**[1], T. L. S. BENZINGER[2], D. HEAD[3];
[1]Psychology, Washington Univ., St Louis, MO; [2]Neuroradiology, Washington Univ. Sch. of Med., St. Louis, MO; [3]Psychology, Radiology, Washington Univ. in St. Louis, St. Louis, MO

Abstract: Existing research indicates age-related differences in experimental tasks of spatial navigation ability, and these deficits have been associated with regional brain volumes. Although a substantial proportion of older adults do also report difficulty finding their way in unfamiliar places, there has been minimal research devoted to specifying the navigational skills and strategies that older adults report using in their daily lives. Furthermore, no studies have examined the neural correlates of self-reported navigational skills in older adults. The goals of the current study were to: a) assess the reliability of self-report measures of navigation skills in older adults, b) assess whether brain volumes of regions known to be involved in navigation are related to self-report measures of navigation, and c) assess whether regional brain volumes mediate age effects on self-reported real world navigation skill in older adults. Three self-report measures of navigation skill and previously collected structural imaging data were obtained from 70 cognitively normal older adults (Mage=71 (range: 66-83 yrs, 35 females) enrolled in studies at the Knight Alzheimer's Disease Research Center. Self-report scales of navigation skill generally evidenced adequate reliability (Cronbach's α range=0.51-0.86). Age effects on self-reported navigation were characterized by increased reliance on navigational aides and dependence on landmark use. General sense of direction showed the strongest brain-navigation

associations, and was positively correlated with prefrontal, parietal, and medial temporal gray matter volumes. Further, dorsolateral prefrontal and supramarginal gray matter volumes mediated the association between age and landmark use. These regions are associated with spatial working memory, body-centered coding of spatial layouts, and disambiguating previously learned routes from new routes. The current results provide initial evidence that quickly administered, self-report surveys could serve as valid measures of navigation skill in older adults. These easily obtained measures of navigation skill can be successfully used in conjunction with biomarker data (e.g., structural brain data) to investigate mechanisms leading to navigation difficulties in older adults.

Disclosures: **N. Lucena:** None. **T.L.S. Benzinger:** None. **D. Head:** None.

Poster

086. Aging Brain

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Topic: F.01. Human Cognition and Behavior

Support: NIH 1 RC1 AG035927A ARRA to M. Fabiani

NSF IGERT Fellowship 0903622 to B. Zimmerman

Title: Cardiorespiratory fitness mediates the effect of age on mean cerebral blood flow in the gray matter of the visual cortex

Authors: *B. ZIMMERMAN[1], B. P. SUTTON[2], K. A. LOW[2], C. TAN[2], M. A. FLETCHER[2], N. SCHNEIDER-GARCES[2], E. L. MACLIN[2], G. GRATTON[2], M. FABIANI[2];
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Abstract: Cognitive declines especially in fluid, online processing, are associated with normal aging, and are likely to be linked to age-related changes in cerebrovascular health. In order to gain a better understanding of the relationships between age, cardiorespiratory fitness (CRF), and cerebral blood flow, arterial spin labelling (ASL), a functional magnetic resonance technique, was used in a study of mean cerebral blood flow in healthy older adults ranging in age from 56-88. Previous research in our lab using the same method found that estimated CRF fully mediated the age effects on mean cerebral blood flow over frontal and parietal cortices. Here we present an

extension of this analysis to the visual cortex, using data collected on a subset of the participants in the first study one year later. We found that estimated CRF was positively correlated with gray matter mean flow in the visual cortex. Additionally, we found that CRF fully mediated the effects of age on mean cerebral blood flow in the gray matter of the visual cortex. These results indicate that the impact of CRF on age-related declines in blood flow is relevant across the brain, even in areas that are thought to be less affected by normal aging, such as the visual cortex. In the future, we will integrate these findings with additional aspects of cerebrovascular health to build a stronger understanding of the complex relationship between age, fitness, cerebrovascular health, and cognitive decline.

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Poster

086. Aging Brain

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Program#/Poster#: 86.08/OO15

Topic: F.01. Human Cognition and Behavior

Support: Robert Bosch Foundation

Bundesverband Seniorentanz

Title: Differential effects of cardiovascular and motor fitness on grey matter volume

Authors: *C. VOELCKER-REHAGE, C. NIEMANN, B. GODDE;
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Abstract: It has been demonstrated that cardiovascular fitness may counteract age-related brain volume loss in several cortical and sub-cortical regions. Less is known whether also motor fitness (i.e., fitness components based on information-processing like balance, speed, and coordination, see below) benefit brain volume and whether fitness effects differ with regard to the type of fitness (cf.[1]). In the current study we were interested in the association of fitness with volume of cortical brain regions. We obtained data from 79 healthy older adults between 65 and 82 years of age. cardiovascular fitness was measured and a heterogeneous motor test battery consisting of nine tasks measuring balance performance, action speed, reaction speed, and fine

coordination was applied to calculate a motor fitness index. Separately for both types of fitness, a median split was used to split the sample into low and high performing older adults. T1-weighted anatomical brain scans were collected within a 3-Tesla MR Scanner. Voxel-based morphometry (VBM8) with fitness levels as between factors was conducted to calculate brain volume differences separately for low vs. high cardiovascular fitness (analysis 1) and low vs. high motor fitness (analysis 2). Age was included as a covariate in the analyses. Analysis 1 revealed that cardiovascular high fit participants had larger brain volumes in the anterior cingulate gyrus as well as the lingual gyrus of the left hemisphere. Analysis 2 revealed that highly motor fit participants showed larger brain volumes in the right cingulate gyrus and right fusiform gyrus as well as the left postcentral gyrus and the left posterior cingulate. Findings suggest that both, higher cardiovascular fitness and higher motor fitness are associated with larger brain volumes in the cingulum as well as other distributed brain regions. Both types of fitness might prevent brain volume loss that goes in line with aging. Whether larger brain volumes in these areas go also in line with better cognitive performance in high fit older adults as compared to their low fit counterparts needs to be tested. [1] Voelcker-Rehage C & Niemann C (2013). Neurosci Biobehav Rev, 37, 2268-95.

Disclosures: **C. Voelcker-Rehage:** None. **C. Niemann:** None. **B. Godde:** None.

Poster

086. Aging Brain

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

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Topic: F.01. Human Cognition and Behavior

Support: AMOREPACIFIC Corp. R&D Center

Title: Brain responses reflecting age-related differences in perceptual experience of cosmetic cream

Authors: R. LEE[1], M.-J. KANG[1], M.-J. CHOI[2], J. BAE[2], J. NAM[1], S. KIM[1], Y. KIM[1], M. LEE[1], S.-A. LEE[1], D. CHOI[2], S. KIM[2], *C.-Y. KIM[1];
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Abstract: Background: Perceptual sensitivity tends to decline with age (Cain & Gent, 1991; Sekuler et al., 1980), which seems to be accompanied by changed neural responses in relevant

brain areas (Moscovitch, 1982; Wang et al., 2005). For the tactile modality, this tendency has been shown by exploiting vibrotactile (Verrillo, 1980), thermal (Kenshalo, 1986), and grating stimuli (Tremblay et al., 2003). However, little is known about age-related differences associated with experience of cosmetic product on skin. In the present study using fMRI, we investigated whether brain activity reflects age-related differences in perceptual experience of cosmetic cream. Methods: Behavioral and fMRI data were collected from twelve young adult females in their 20's and twelve elderly females in their 50's-60's. We manipulated absorption rate (A_fast vs. A_slow) and oil content (O_high vs. O_low) of a basic mosturizing cream prescription, which yielded a total of four stimulus conditions. The stimulus formulations were designed by using Cetyl Ethylhexanoate, coconut oil, Caprylic/Capric Triglyceride, Cyclopentasiloxane, and Cyclohexasiloxane to manipulate oil content, and silica or cellulose gum to control absorption rate. The fMRI scanning session consisted of five functional runs each of which repeated four stimuli five times in a pseudo-randomized order. In each trial, the stimulus was applied to the back of the participant's left hand for 4 seconds. After 4 seconds of stimulus removal, there was a 2-second interval prior to the next trial. Participants underwent a bahavioral testing including preference rating outside the scanner. Results: According to stimulus conditions, significant differences in BOLD signal were observed between age groups in several brain regions. In the absorption rate condition, the left angular gyrus showed such difference; greater BOLD signal was associated with A_fast compared to A_slow stimulus only in the elderly group. In the oil content condition, those regions included the precentral and supramarginal gyri and the insula in the right hemisphere. Specifically, BOLD signal was greater in response to O_high than to O_low stimulus in the supramarginal gyrus of the elderly group, whereas in the precentral gyrus and the insula of the young group, BOLD signal was greater in response to O_low than to O_high stimulus. Behavioral preference response buttressed the fMRI results; the elderly group tended to prefer A_fast to A_slow and O_high to O_low stimulus. Conclusion: These results imply that elderly females differ from young adult females in their experience of cosmetic cream, which was reflected in differential brain activation patterns.

Disclosures: **R. Lee:** None. **M. Kang:** None. **M. Choi:** A. Employment/Salary (full or part-time):; AMOREPACIFIC Corp. **J. Bae:** A. Employment/Salary (full or part-time):; AMOREPACIFIC Corp.. **J. Nam:** None. **S. Kim:** None. **Y. Kim:** None. **M. Lee:** None. **S. Lee:** None. **D. Choi:** A. Employment/Salary (full or part-time):; AMOREPACIFIC Corp. **S. Kim:** A. Employment/Salary (full or part-time):; AMOREPACIFIC Corp. **C. Kim:** 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.; AMOREPACIFIC Corp..

Poster

086. Aging Brain

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 86.10/OO17

Topic: F.01. Human Cognition and Behavior

Support: NIH U01 HL54434

NIH R01 NS41558

Title: Hippocampal volume is associated with verbal memory performance in older, but not younger, adults

Authors: *M. F. SCHMIDT[1], M. E. GRISWOLD[2], K. B. FREEMAN[3], T. H. MOSLEY[4]; [1]Program in Neurosci., [2]Biostatistics, [3]Psychiatry and Human Behavior, [4]Med. (Geriatrics), Univ. of Mississippi Med. Ctr., Jackson, MS

Abstract: The hippocampus plays a key role in memory storage and retrieval. Over the adult lifespan, hippocampal volume (HV) declines, accelerating in dementia. Relationships between HV and memory have been widely reported in the elderly and in neuropsychiatric disorders, eg. dementia, major depressive disorder, and schizophrenia. This relationship remains poorly understood in normal populations, particularly African Americans. We utilized the Genetic Epidemiology Network of Arteriopathy (GENOA) study, whose participants are members of sibships in which at least one sibling had essential hypertension diagnosed prior to age 60 to determine associations between HV, measured by Freesurfer 5.3 segmentation of 1.5T T1 SPGR images, and verbal memory (VM), measured by the Rey Auditory Verbal Learning Test (RAVLT). We included participants who underwent MRI and cognitive testing between 2001 and 2006, resulting in a sample of 634 African Americans (AA) (68% Female, aged 40 to 91) in 342 sibships and 667 non-Hispanic Whites (NHW) (61% Female, aged 37 to 83) in 305 sibships. Those with possible dementia (MMSE < 24; n = 94) were excluded. We used linear regression with Generalized Estimating Equations (GEE) to estimate slopes (β) while accounting for sibship clustering and adjusting for age, intracranial volume, education, gender, and race; robust Huber-White variances were employed. The mean HV (left + right) was 7.9cm3 (SD=0.9) and the mean RAVLT was 8.0 words recalled (SD=3.6). Overall, a 1cm3 increase in HV was associated with an additional β=0.23 words recalled (p=0.102, n=1200). Assessing left and right hippocampi individually, a 1cm3 increase in HV was associated with β=0.40 (p=0.121) on the left, and β=0.41 (p=0.109) on the right. Splitting the cohort into subsamples, a stronger relationship between HV and VM was found in females (β=0.51, p=0.001, n=776) compared to males (β=-0.18, p=0.366, n=424), in AA (β=0.40, p=0.020, n=545) compared to NHW (β=0.04, p=0.834, n=655), and in older participants (≥70 years: β=0.86, p=0.000, n=225) compared to younger (<70 years: β=0.02, p=0.901, n=975). These data support the hypothesis that hippocampal

atrophy contributes to memory loss in advanced age, but relationships between HV and VM are weak or nonexistent in younger populations.

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Poster

086. Aging Brain

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Topic: F.01. Human Cognition and Behavior

Support: NIH Grant AG021133

Boston University Grant TG-PHYS100030

Title: Model for aging and cognitive decline

Authors: *M. P. HENDERSON;
Physics, Drexel Univ., Philadelphia, PA

Abstract: A population of neurons in the cerebral cortex of humans and other mammals organize themselves into vertical microcolumns perpendicular to the pial surface. Anatomical changes to these microcolumns have been correlated with neurological diseases and normal aging, and in particular in area 46 of the rhesus monkey brain the strength of microcolumns was shown to decrease with age. We have previously developed a model to simulate aging brains by constructing a microcolumnar network of neurons and allowing the neurons to undergo Brownian motion while being constrained by a harmonic force that weakens as a function of age. Now, we expand on this model by constructing and simulating the generated neural networks. By generating a young neural network from strong restorative forces, one can create an initial distant dependent connectivity. Then, we age these networks and presume that connectivity between neurons either weakens or severs as a function of neural displacement from initial neuronal positions. We aim to show that older networks are unable to efficiently shift between different firing regimes, providing a potential mechanism for loss of information processing in relation to microcolumnar structure.

Disclosures: **M.P. Henderson:** None.

Poster

086. Aging Brain

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 86.12/OO19

Topic: F.01. Human Cognition and Behavior

Support: Intramural Research Program of the NIH, National Institute on Aging

Title: Hippocampal activation in the older brain

Authors: *L. L. BEASON-HELD[1], J. O. GOH[2], J. A. ASH[1], S. M. RESNICK[1];
[1]NIA/NIH, BALTIMORE, MD; [2]Natl. Taiwan Univ. Col. of Med., Taipei, Taiwan

Abstract: The hippocampus is a critical component of the medial temporal lobe memory system, and is a site of early pathologic change in aging and age-related disease. Because patterns of memory decline may be related to the progression of hippocampal dysfunction with increasing age and disease, the goal of this study was to determine a robust functional MRI (fMRI) task for assessing functional integrity of the entire hippocampus. We performed 3 pilot studies that tested several tasks and scan designs to assess the extent of hippocampal activation elicited by each task. 17 volunteers participated in the pilot studies (7F, 10M; mean age= 57). In each pilot study, participants underwent fMRI scanning while performing 2 tasks. The first pilot study assessed Indoor/Outdoor scene and Scrambled Images encoding tasks (Binder et al., 2005), the second pilot compared a Water/No Water scene encoding task (Liu et al., 2013) with the Indoor/Outdoor scene task, and the third pilot compared a Face/Name encoding task (Putcha et al., 2011) with our own Face/Place encoding task. Scanning was performed on a Philips 3T scanner. The tasks were administered using blocked, event-related, or mixed block/event scan paradigms. Brain activation during task performance was compared to a fixation condition. Hippocampal activation of anterior, middle and posterior regions was assessed for each task. We found that anterior to posterior activation can be achieved with a scene encoding task. In the first two pilots, the Indoor/Outdoor scene task evoked more extensive hippocampal activation compared to either the Scrambled Images task or the Water/No Water task, especially when using a blocked scan paradigm. For pilot 3, the Face/Place task evoked more robust hippocampal responses than the Face/Name task when using a mixed block/event design. Overall, the most extensive pattern of hippocampal activation was seen with the Face/Place task using a mixed event/block design. These results show that variations in the type of episodic encoding stimuli and scan paradigms used are critical considerations for assessing hippocampal functional integrity.

Acknowledgements: This research was supported by the Intramural Research Program of the NIH, National Institute on Aging. We thank the NIA 3T staff for their support and assistance in this study.

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Poster

087. Alzheimer's Disease: Novel Therapeutics

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 87.01/OO20

Topic: F.01. Human Cognition and Behavior

Title: Meditation for improving cognitive functions in age related cognitive decline-a systematic review

Authors: *G. B. PATRUDU;
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Abstract: Many previous studies showed that meditation practice improves cognitive functions in a wide range of age groups .To assess the current evidence on the effect of Meditation on age-related cognitive decline, a search was performed in PubMed, Embase, &Google Scholar. A total of nine studies were found and all of them showed a better performance on attentional , executive & memory tasks in elderly subjects who practiced meditation compared to elderly subjects with no meditation practice. Gard etal (2014), showed that Fluid intelligence declined slower in yoga practitioners and meditators combined than in controls. Prakash etal (2012),found that elderly regular meditators performed better than elderly controls on tests of short term memory, executive skills , perceptual speed & attention . Nguyen & Kruse (2012), found that Elderly subjects when given Tai Chi (with meditation component) practice, showed better performance on Trail making test than elderly controls . Newberg etal (2010),found that Elderly subjects with age-related memory loss showed improved cortical blood flow & performance on tests of verbal fluency, Trails B & logical memory after 8 weeks of meditation . Kampanaros etal (2010), found that Elderly subjects when given body-oriented meditation showed improvement on fluid & crystalline intelligence. Van leeuwen etal (2009),found that ,Elderly mindfulness practitioners performed better than elderly controls on attentional blink task . Pagnoni & Cekic (2007),found that Elderly, Zen meditators performed better on a sustained attention task than elderly controls and didn’t show age-related decline in grey matter volume .Lazar etal (2005),

showed that Elderly regular Insight meditation practitioners showed less age-related cortical thinning and increased thickness in areas sub serving attention , interoception & sensory processing . Alexander etal (1989),found that Elderly subjects when given Transcendental Meditation or Mindfulness practice performed better on paired associate learning, tests of cognitive flexibility and word fluency than elderly controls. Overall, the current evidence shows that meditation has a beneficial effect on age-related cognitive decline ,however more studies involving standardized meditation techniques are needed as the current studies on meditation vary widely on the type of meditation studied.

Disclosures: G.B. Patrudu: None.

Poster

087. Alzheimer's Disease: Novel Therapeutics

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 87.02/OO21

Topic: F.01. Human Cognition and Behavior

Support: NIH R01HL084178-01

Title: Cerebrovascular reactivity and cardiovascular disease in older adults: Results from an fMRI breath-hold hypercapnia task

Authors: *N. F. SCHWARZ[1], J. D. LUKEMIRE[1], U. S. CLARK[2], H. H. RISKIN-JONES[3], X. XU[4], D. LABBE[5], B. E. HAWKSHEAD[1], S. W. LIEBEL[1], L. H. SWEET[1];
[1]Psychology, The Univ. of Georgia, Athens, GA; [2]Mount Sinai Sch. of Med., New York, NY; [3]VA Greater Los Angeles Healthcare, Los Angeles, CA; [4]Idaho State Univ., Pocatello, ID; [5]Warren Alpert Sch. of Med. of Brown Univ., Providence, RI

Abstract: Functional magnetic resonance imaging (fMRI) is frequently used to investigate the neurophysiological changes that underlie cognitive decline in older adults (OA). Decline in cognitive performance is not only associated with advanced age but also with cardiovascular disease (CVD). FMRI blood-oxygenation-level-dependent (BOLD) signal is sensitive to changes in cerebral hemodynamics, which are modulated by neural activity. Relatively little is known about how changes in cerebrovascular reactivity resulting from normal aging and CVD affect the BOLD response. Dissociating cerebrovascular from neural contributions to the BOLD fMRI signal is critical in determining whether the BOLD signal is providing valid information with regard to underlying cognitive performance-related neurophysiology. We examined

cerebrovascular components of the BOLD signal by acquiring echoplanar fMRI data during a blocked breath-hold hypercapnia paradigm in a group of ten healthy OA (age: mean=67 years, SD=8; 6 females) and ten OA with CVD (age: mean=69 years, SD=8; 2 females). Based on previous findings in healthy OA, we hypothesized that the BOLD signal in OA with CVD would exhibit greater delay and variability in comparison to the control group. A conventional GLM was used to determine hypercapnia effects versus normal breathing. Replicating previous results, we observed that the BOLD response was delayed in both populations. Four prefrontal regions (left anterior cingulate, bilateral medial frontal and right middle frontal gyri) showed significant group differences in the magnitude of percent signal change observed during breath-hold blocks compared to baseline resting states ($p<.001$, corrected), where the control OA group showed less negative percent signal change during breath-hold than did the OA group with CVD. The percent signal change was expected to be negative as the BOLD response was delayed with respect to a standard response model convolved with our boxcar design. Furthermore, the BOLD response was significantly more variable in three out of the four regions in the CVD group. These results indicate that cerebrovascular reactivity differs in frontal regions between healthy OA and those with CVD. Accounting for cerebrovascular reactivity may provide a more sensitive characterization of the BOLD response in fMRI studies of brain function in OA and may be especially useful in those with CVD.

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Poster

087. Alzheimer's Disease: Novel Therapeutics

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Program#/Poster#: 87.03/OO22

Topic: F.01. Human Cognition and Behavior

Title: The influence of demographic and disease risk factors on paired associates learning in an internet recruited cohort of over 29,000 individuals

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Abstract: There is significant interest in understanding the health and disease risk factors that influence cognition. However, most studies performed to date are underpowered to detect all but the factors that exert the largest effect. To address sample size concerns and to power our study to examine even those risk factors with minor influences on cognition, we developed a web-based task that examines paired associates learning (PAL). At www.mindcrowd.org we have studied the performance of over 29,000 individuals on the PAL task. This large data set and the nature of the recruited cohort, which spans the age spectrum from 18 to 85 years, uniquely powers us to identify even subtle factors that may influence PAL across the lifespan. The MindCrowd PAL task consists of 12 word pairs that are presented sequentially to the test taker. From all completed tests we applied several demographic filters to select a final cohort for analysis, including filters to remove duplicate test takers and those subjects who didn't understand the rules of the task. This yielded a final cohort size of 19,202 subjects between the ages of 18-85. A multiple regression model was fitted with the PAL results as the dependent value and all of the demographic, health/disease risk factor, and reaction time main effects as independent values. Of all the demographics tested, we found that Handedness ($p = 5.9 \times 10\text{-}4$, Right Handedness; $r = 1.66\%$) Marital Status ($p = 1.71 \times 10\text{-}4$, Single vs Married $r = 1.32\%$) and Race ($p = 2.71 \times 10\text{-}9$) significantly influence PAL performance. High Blood Pressure significantly affects PAL performance but only when present in individuals under the age of 35 (Age:HighBloodPressure interaction $p = 0.003$; <35 years of age, $p = 0.002$, $r = -4.37\%$). Smoking affects PAL performance in females only (Smoking:Gender $p = 0.004$; Females $p = 0.01$; $r = -2.00\%$; Males $p = 0.626$). There was no effect of Drug/Alcohol abuse, Diabetes, Dizziness, Heart Disease, Loss of Consciousness, Seizures and Stroke on PAL performance after correcting for all other demographics. We identified novel demographic and health risk factors associated with PAL performance in humans using a large web-recruited cohort. Previous studies examining hypertension status and cognitive task performance have mainly focused on older age groups and our data demonstrates that the largest effect is found in young adults. Limitations of our study include the fact that it is a cross sectional study and the potential for biased recruiting and/or PAL test taking through the use of the internet. Web-based recruitment and testing of a large and demographically diverse sample is both feasible and can uncover novel associations with cognitive task performance.

Disclosures: **A.L. Siniard:** None. **I. Schrauwen:** None. **J.J. Corneveaux:** None. **J. Peden:** None. **M.N. Turk:** None. **M.D. De Both:** None. **R.F. Richholt:** None. **M. Mueller:** None. **J. Langbaum:** None. **E. Reiman:** None. **R. Caselli:** None. **P. Coleman:** None. **C. Barnes:** None. **E. Glisky:** None. **L. Ryan:** None. **M.J. Huentelman:** None.

Poster

087. Alzheimer's Disease: Novel Therapeutics

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 87.04/OO23

Topic: F.01. Human Cognition and Behavior

Title: MindCrowd: Web-Based paired associates testing of 19,202 individuals demonstrates significant main effects of chronological age, gender, education, and Alzheimer's disease family history on performance

Authors: *M. J. HUENTELMAN[1,2,3], I. SCHRAUWEN[1,2,3], J. CORNEVEAUX[1,2,3], A. SINIARD[1,2,3], J. PEDEN[1], J. LANGBAUM[2,4], E. REIMAN[2,4], R. CASELLI[5], E. GLISKY[2,3,6], L. RYAN[2,3,6];
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Abstract: It is well documented that many aspects of human cognitive performance are influenced by both a heritable and non-heritable component; however, most studies of these components are largely underpowered to detect all but those that exert large effects on the cognitive task of interest. To address these sampling and cohort diversity concerns, we created a web-based (at mindcrowd.org) paired associate learning task (PAL) in an attempt to interrogate a large cohort of individuals that span a more complete range of genetic, demographic, and health risk factors. The MindCrowd PAL task consists of 12 word pairs that are presented sequentially to the test taker. We applied several demographic filters to select a final cohort for analysis, including approaches to remove duplicate test takers and those subjects who didn't understand the rules of the task. This yielded a final cohort size of 19,202 subjects between the ages of 18-85. Regression analysis demonstrated significant PAL performance differences, after correction for all other factors, for chronological age ($p<2.2 \times 10\text{-}16$, r=4.4% performance decline every decade), gender ($p<2.2 \times 10\text{-}16$, r=5.4% lower scores in males), education level ($p<2.2 \times 10\text{-}16$, r=4.4/7.8/11.2% increased performance versus 12 years or less of education for 14/16/20 years of education respectively), marital status (p=0.0002, r=1.3% higher performance in single individuals), handedness (p=0.0006, r=1.7% higher scores in right handed subjects), and the presence of a first-degree relative diagnosed with Alzheimer's disease (AD, p=0.002, r=1.3% higher performance in individuals without a first degree relative with AD). Chronological age, gender, and education have the most significant statistical associations with PAL performance. In

this study we extend the gender differences on PAL to even the youngest participants. The age:gender interaction for PAL performance becomes most noticeable near the average age of menopause onset suggestive of a potential link between sex hormone levels (either natural decline and/or hormone replacement therapy decisions) and PAL performance. AD first degree relative family history also has a significant effect on PAL performance. This effect is enhanced when individuals under the age of 42 are analyzed separately from those over 42. This finding suggests that heritable factors associated with first degree AD risk exert an effect on cognitive performance even in healthy individuals at young adult ages (note the significant effect of AD first degree relative status is evident in the 20s). Our results demonstrate the effectiveness of web-based recruitment for the study of cognition across a diverse cohort.

Disclosures: **M.J. Huentelman:** None. **I. Schrauwen:** None. **J. Corneveaux:** None. **A. Siniard:** None. **J. Peden:** None. **J. Langbaum:** None. **E. Reiman:** None. **R. Caselli:** None. **E. Glisky:** None. **L. Ryan:** None.

Poster

087. Alzheimer's Disease: Novel Therapeutics

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Topic: F.01. Human Cognition and Behavior

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UCLA Academic Senate grant

Title: Age differences in brain response to cues that signal the value of to-be-remembered information

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Abstract: People often need to selectively remember important information. This skill is particularly important for older adults who must be efficient at directing limited encoding resources to important items. We tested 20 young adults and 17 older adults in a value-directed remembering paradigm (Castel et al., 2002) adapted for fMRI. Participants were presented with 5 lists of 24 words each. Each word was preceded by a numeric value cue (half high-value and half low-value) indicating how many points could be earned if that item was later recalled. Here, we examined how BOLD signal during the cue period preceding each word differed for high vs. low-value cues. We defined sets of ROIs based on automated meta-analyses obtained from the Neurosynth database. In young adults, activity was greater for high-value cues in a number of regions, including the "semantic" and "reward" networks defined from Neurosynth, but greater value-related increases in brain activity during the cue period were not associated with better subsequent memory or selectivity. In these subjects, memory selectivity was strongly related to value-related differences in activation while the words were being encoded, particularly in brain regions associated with semantic processing (see Cohen et al., 2014, CABN). Older adults showed a similar pattern of value-related activation as young adults while the to-be-remembered words were present, with value-related differences in activity in semantic processing regions during word encoding correlating with selectivity. However, during the cue period, we find a main effect of age on the degree of value-related modulation of brain activity. Unlike young adults, older adults did not show a significant overall increase in activity during high-value cues in semantic processing regions, in reward-sensitive regions, nor in other prefrontal regions sensitive to value cues in young adults. Individual differences in the degree to which value modulated older adults' cue period activity were, however, positively associated with later recall of valuable items. These data suggest that it may be beneficial to proactively engage brain areas that can strengthen memory encoding. Young adults seem to generally respond proactively to value, selectively engaging these regions prior to encoding regardless of their eventual performance, while many older adults do so only reactively, after the word appears. When older adults do engage these regions selectively prior to encoding, however, it was beneficial, suggesting a possible means by which older adults can learn to improve their cognitive performance.

Disclosures: **M.S. Cohen:** None. **J. Rissman:** None. **N.A. Suthana:** None. **A.D. Castel:** None. **B.J. Knowlton:** None.

Poster

087. Alzheimer's Disease: Novel Therapeutics

Location: Halls A-C

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Program#/Poster#: 87.06/OO25

Topic: F.01. Human Cognition and Behavior

Support: NIA/NIH grant # R01 AG028466

Intramural Research Program, NIA, NIH

Title: Long-term cortisol variability predicts Alzheimer's Disease risk

Authors: *S. D. MOFFAT[1], G. E. ENNIS[1], Y. AN[2], S. M. RESNICK[2], L. FERRUCCI[2], R. J. O' BRIEN[3];
[1]Psychology, Georgia Inst. of Technol., Atlanta, GA; [2]Natl. Inst. on Aging, Baltimore, MD; [3]Johns Hopkins Univ., Baltimore, MD

Abstract: Elevations in central and peripheral measures of cortisol and hypothalamic-pituitary-adrenal (HPA) axis dysregulation are recognized features of Alzheimer's Disease (AD) (e.g., Armanini et al., 2003; Giubilei et al., 2001; Näsman et al., 1995, Peskind et al., 2001). Whether cortisol dysregulation is a cause or consequence (or both) of AD, however, is not known. We used data from the Baltimore Longitudinal Study of Aging (BLSA) to examine whether longitudinal measures of urinary free cortisol: creatinine ratio (UFC/Cr), collected from 24 hour urine samples over an average interval of 8.17 years, was related to risk for AD. We also investigated apolipoprotein E, ε4 allele, a known genetic risk factor for AD, as a moderator of the relationship between UFC/Cr and AD risk. When examining continuous and trichotomized measures of UFC/Cr level, slope, and variability (i.e., within-person standard deviation) as predictors of AD risk, we found that UFC/Cr level and variability were significantly related to AD risk rate. Specifically, moderate UFC/Cr level and variability were related to a reduced AD risk compared to high level and high variability. The relationship between UFC/Cr moderate variability and AD risk was further moderated by APOE-e4 carrier status. APOE-e4 non-carriers with low and high UFC/Cr variability had an increased AD risk relative to those with moderate variability. UFC/Cr variability did not influence risk in APOE-e4 carriers. Cortisol dysregulation as manifested by low and high UFC/Cr variability may be a causative factor or early prodromal marker of AD in APOE-e4 non-carriers.

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Poster

087. Alzheimer's Disease: Novel Therapeutics

Location: Halls A-C

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Program#/Poster#: 87.07/OO26

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: ICMR Delhi India

CUShah College of Pharmacy India

Title: The polyphenolic and f1 fractions isolated from butea frondosa ameliorates learning memory disruption in the laboratory animal models

Authors: *G. A. DESHPANDE[1], S. MENGI[2];
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Abstract: Background: Butea frondosa is commonly used herb in Ayurvedic medicine. In the present study a systematic pharmacological and phytochemical investigation is performed. we examined *in vivo* nootropic activity of isolated polyphenolic (15, 30 and 60 mg/kg p.o) & F1 fraction (20, 40 and 80 mg/kg p.o) of the plant leaves. Methods: *In vivo* nootropic activity was investigated using exteroceptive, interoceptive and chronic stress induced memory models. The effects of both the fractions were measured during the acquisition/retention trials of exteroceptive models like elevated plus maze, shuttle box, cooks pole model. Fractions were also tested for diazepam-, scopolamine- and chronic stress-induced amnesia models. The putative biochemical markers of amnesia like cholesterol, glucose, total protein, corticosterone and hydrolyzed levels of acetylcholine were estimated spectrophotometrically. Statistical analysis was performed by using one way ANOVA. Result: In the exteroceptive models, the F1 fraction showed significant (*P<0.05) acquisition and retention ability. Chronic electroshock-induced retrograde- and scopolamine-induced amnesia was significantly reversed in a dose dependent manner by polyphenolic than F1 fraction during the acquisition/retention trials. Stress induced biochemical perturbations were significantly attenuated by both the fractions. Acetylcholine is reported to be a putative marker of cognition. Interestingly, we observed significant (*P<0.05) decrease in hydrolyzed levels of acetylcholine by both the fractions in a dose dependent manner. Conclusion: Both fractions show nootropic activity that may be applicable in treating cognitive and neurodegenerative conditions. Further studies will be required to elucidate the molecular targets of such conditions.

Disclosures: **G.A. Deshpande:** None. **S. Mengi:** None.

Poster

087. Alzheimer's Disease: Novel Therapeutics

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 87.08/OO27

Topic: F.01. Human Cognition and Behavior

Support: Australian Research Council Discovery Grant

Co-funding received from Flordis, Horphag and Blackmores

Title: A randomized, controlled trial investigating the psychopharmacological and cognitive enhancing effects of the herbal medicines Pycnogenol and Bacopa: The Australian Research Council Longevity Intervention (ARCLI)

Authors: M. P. PASE[1], A. SCHOLEY[1], K. SAVAGE[2], K. NOLIDIN[2], *C. STOUGH[2]; [1]Ctr. for Human Psychopharmacology, Swinburne Univ. of Technol., Hawthorn, Australia; [2]Ctr. for Human Psychopharmacology, Swinburne Univ., Melbourne, Australia

Abstract: Ageing is associated with cognitive decline, particularly in the domains of memory and processing speed. With the population ageing rapidly, it is important to elucidate ways to help the elderly remain as mentally healthy as possible. Certain dietary factors and even herbal medicine have been shown to have beneficial effects on cognitive performance and such interventions could be useful for ameliorating age-associated cognitive decline. For example, short term trials have shown that a special standardized extract of the Indian Herb Bacopa can improve verbal memory. The French Maritime Pine Bark extract Pycnogenol has also been shown to improve memory after three months of supplementation. The Australian Research Council Longevity Intervention (ARCLI) was developed to examine the effects of pharmacologically active supplements on cognitive performance. ARCLI is a randomized, placebo-controlled, double-blind, 4-arm clinical trial in which 465 participants are randomized to receive an extract of Bacopa monnieri (CDRI08 300 mg/day), Pycnogenol (150 mg/day), a micronutrient combination formula or a placebo daily for 12 months. Following baseline assessment, participants are tested after 3, 6 and 12 months of supplementation across an extensive battery of cognitive, neuropsychological and mood measures, cardiovascular (blood pressures and aortic stiffness), biochemical (inflammation, oxidative stress and safety) as well as genetic assessments (telomere length and several Single Nucleotide Polymorphisms). Neuroimaging is also performed in a subset of the sample before and after treatment. The primary aim is to investigate the effects of the supplements on cognitive performance. ARCLI represents one of the largest and most definitive clinical trials in which psychopharmacological

supplements are administered to elderly participants. To date, approximately 150 participants have been enrolled into ARCLI with just under 100 participants having completed the 12 month intervention. The study remains ongoing. This presentation will outline some of the preliminary results generated from ARCLI. The presentation will focus on the cognitive enhancing effects of both Pycnogenol and Bacopa.

Disclosures: **M.P. Pase:** None. **A. Scholey:** None. **C. Stough:** 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.; Flordis, Horphag, Blackmores. **K. Savage:** None. **K. Nolidin:** None.

Poster

087. Alzheimer's Disease: Novel Therapeutics

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 87.09/OO28

Topic: F.01. Human Cognition and Behavior

Title: Mechanisms of music-induced neurophysiology - a proposed model for prescriptive listening applications in dementia patients

Authors: *L. E. MAGUIRE;
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Abstract: Studies in music-based interventions for Alzheimer's suggest physiological mediators as primary mechanisms to explain music's power in obtaining desired Mood, Cognitive and Behavioral (MCB) outcomes in Alzheimer's patients. Music reliably promotes healthy physiological outcomes including improved heart rate variability, blood pressure, oxygen saturation, lower cortisol levels and increases in melatonin, dopamine, immunoglobins and hormones (Fancourt et al, 2014) leading to positive MCB outcomes in most music-dementia studies (Guetin et al, 2013). Music appreciation is remarkably well preserved in Alzheimer's patients. Therefore, targeted, individualized therapeutic listening programs, created through development of computer-generated Prescription iPods, can ease both patient and caregiver burden in the medical field. The listening programs are based on clients' clinical baseline demographics, psychological profiles, cultural background, musical preferences, co-morbidities, best-case scenario prognostics and patient schedule of activities. The proposed medical music model produces computer-generated, individualized playlists using internal algorithms that draw

from an archive of refined, psychological and physiology-matched music recordings. Scheduled listening programs carry patients through longitudinal, multi-staged progressive musical programming levels that effectively escort and mediate improved MCB outcomes through physiologic mediation. The prototype model focuses on difficult afternoon hours (Alzheimer's "Sundowning Syndrome" between 1-5 pm) and uses music selections to escort function and redirect specific dysfunctional behaviors, cognitive distortions and emotional lability. Multiple weeks of varying, 20-50 minute, progressive music programming stages are created contingent on progressive clinical presentations, patient's response to music, scheduled activities and clinician/physicians' best-case prognosis. Unlike traditional music programming, prescriptive music programs introduce up to 90% novel (previously unheard but appealing) music programming to achieve intended outcomes. Personalized programs can automatically change, for example, every 10-14 days or adapt contingent on new clinical presentations. Ultimately, a field of expert musicians, performers, composers and musicologists who are also medically educated can potentially create, perform and deliver transformative, prescriptive medical music and new compositional designs intended for purposeful health entrainment in listeners of all kinds.

Disclosures: L.E. Maguire: None.

Poster

087. Alzheimer's Disease: Novel Therapeutics

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 87.10/OO29

Topic: F.01. Human Cognition and Behavior

Title: Novel ROCK inhibitors developed for both cognitive enhancement and blockade of pathological tau phosphorylation

Authors: *M. TURK[1,2,4,5], M. D. ADAMS[2,4,6], T. WANG[3], T. DUNCKLEY[2], M. J. HUENTELMAN[2,4,5];
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Abstract: Rho-associated protein kinase (ROCK) is an enzyme that plays important roles in neuronal cells including mediating actin organization, critical for cell migration and axon path-finding, as well as dendritic spine morphogenesis. The ROCK inhibitor (ROCK-i) Fasudil has

been shown to increase learning and working memory in aged rats, but another ROCK-i, Y27632, was shown to impair learning and memory in rats. These observations suggest different mechanisms of action for these two albeit structurally different inhibitors. Thirteen different ROCK-i, five of which are commercially available and eight of which were newly designed and synthesized for this study, were used to treat human neuroglioma cells overexpressing 4-repeat tau (H4-tau) across a 96-hour time course. The IC-10 dosage, at which 10% of H4-tau cells are no longer viable after 120 hours, was used and fresh drug-containing media was applied every 24 hours. The ratio of Serine 396 phosphorylated tau (p-tau) to total tau was measured using ELISA at each of 8 time points. All drug treatments were compared against the corresponding time point for vehicle-treated cells. Fasudil was the only commercial drug to decrease the p-tau total tau ratio ($p=0.0004$). Of note, Y27632 did not decrease this ratio ($p=0.218$). Several of the novel ROCK-i significantly decreased p-tau:total tau; of these, T343 had the greatest significance ($p=0.003$). T299, another newly designed ROCK-i, displayed no change in the p-tau:total tau despite its similarity to T343 in ROCK-I and ROCK-II inhibition. The results of these four drugs were replicated in H4-tau cells using the higher IC-50 dosage in order to ensure that the dose alone was not playing a significant role in the observed effect. While results of treatment with Fasudil, Y27632, and T343 at IC-50 were significant in the same direction of effect ($p=0.01$; 0.08; 0.003), T299 at the IC-50 dosage in contrast significantly increased the p-tau total tau ratio ($p=0.0009$). These findings detail several drugs with differential effects on tau. This presents a unique opportunity to utilize these molecules to dissect the changes associated with each, and perhaps further fine-tune the drugs to more effectively target p-tau. Of note, phosphorylation of tau at Serine 396 decreases tau mobility and the ability of tau to bind microtubules, contributing to the tauopathy of Alzheimer's disease. Further research is necessary to parse out whether the effects of Fasudil on learning and memory are mediated through changes in p-tau to total tau expression, or through other on- or off-target effects.

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Poster

087. Alzheimer's Disease: Novel Therapeutics

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 87.11/OO30

Topic: F.01. Human Cognition and Behavior

Title: Gene expression profiling of human astrocytes treated with bexarotene and related compounds shows increase in neuroprotective cytokine GMCSF

Authors: *R. RICHHOLT[1,2,3], I. PIRAS[4], A. M. PERSICO[4], M. J. HUENTELMAN[1,2,3];
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Abstract: Characteristic neuropathology of Alzheimer's disease (AD) includes the accumulation of extracellular amyloid plaques in the brain. These plaques are thought to be formed through an imbalance between beta-amyloid (Aβ) production and clearance. Recent studies in multiple AD mouse models show that treatment with the RXR agonist bexarotene (BEX) restores cognitive functions and in some models results in reduced soluble and oligomeric Aβ. These observations position BEX as a potential agent for AD prevention therapy. RXR and LXR activation has been shown to increase expression of the cholesterol transporters ABCA1 and ABCG1, as well as APOE. These increases in expression were attributed to the benefits of the BEX treatment on Aβ, but they also caused concern regarding its potential use in patients carrying the epsilon 4 allele variant of APOE. How these molecules facilitate Aβ clearance is not fully understood; therefore we utilized gene expression profiling to investigate BEX and related RXR/LXR agonists in human cells. Human primary astrocytes (Lonza) were treated for 48 hours with 100nM concentrations of the following RXR/LXR agonists - BEX, honokiol, and 9-cis retinoic acid (RA). Gene expression analysis was conducted with Illumina HumanHT-12 v4 BeadChips and differential expression was performed with the R package Limma. Hierarchical clustering and gene ontology analysis was performed. BEX and RA significantly upregulated ABCA1 and ABCG1 (P value < 0.01, validated by qRT-PCR), but APOE was unaffected by any of the three drug treatments. Cluster analysis identified a group of immune response genes that were upregulated at three hours by all drugs. Among these genes, BEX increased granulocyte-macrophage colony stimulating factor (GMCSF) (Log2 fold change 1.64, pvalue < 0.01), a cytokine that is known to be neuroprotective. Additionally, treatment of cultured human microglia with BEX demonstrated a significant increase in GMCSF across a similar time course. This study is the first to examine the molecular effects of BEX in human cells. Our results suggest that BEX treatment does not upregulate APOE expression and therefore should remain a strong candidate for anti-amyloid therapy in humans. Additionally, our results demonstrate that BEX may act at least in part via upregulation of GMCSF. Several studies demonstrate the importance of GMCSF in cognition and show that upregulation of GMCSF can reverse both cognitive impairment and amyloidosis. BEX likely represents a novel approach to upregulate GMCSF in the central nervous system and therefore warrants further investigation as a potential anti-amyloid agent in humans.

Disclosures: **R. Richholt:** None. **I. Piras:** None. **A.M. Persico:** None. **M.J. Huentelman:** None.

Poster

087. Alzheimer's Disease: Novel Therapeutics

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 87.12/OO31

Topic: F.01. Human Cognition and Behavior

Title: Noncoding and micro RNAs associated with KCl-induced neuronal depolarization

Authors: *M. DE BOTH[1,2,3], A. SINIARD[1,2,3], J. CORNEVEAUX[1,2,3], H. ZHANG[4], J. COLEMAN[4], M. HUENTELMAN[1,2,3];
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Abstract: During new memory formation, the cellular constituents of the activated circuit undergo rounds of de novo transcription and translation. Characterizing and understanding these molecular changes are of great interest, because they may represent some of the most basic initial responses to activation. Because of that, they likely embody key points of intervention where memories could be enhanced or inhibited. Next-generation RNA sequencing (RNA-seq) provides the ability to digitally quantify transcript levels, construct an unbiased whole transcriptome map, and identify key transcriptional changes that are key to cell function and dysfunction. Here, we identify transcriptional changes following potassium chloride (KCl)-induced neuron depolarization *in vitro* in an attempt to characterize those transcriptional changes associated with neuronal cell activation. Cell cultures were initiated from the neocortex of E15 C57BL/6 mouse embryos. Neocortices were pooled, dispersed, and seeded on poly-L-lysine+laminin coated 6-well plates. After 20 days *in vitro* (DIV20), cells were silenced in 1 uM TTX + 100 uM AP5 for 24 hours. Depolarization was induced with 55 uM KCl for 0, 0.25, 0.5, 1, 6 and 24 hours, with RNA and protein collected at each time point. RNA was isolated using the RNeasy kit (Qiagen) and double stranded cDNA was synthesized using the Ovation RNA-Seq System (Nugen). NGS Libraries were prepped with the Encore Rapid Library Prep Kit (Nugen) and sequenced on the HiSeq 2500 instrument using paired-end 100bp chemistry (Illumina). RNA-reads were trimmed (AlienTrimmer), aligned (STAR), and quantified (HTSeq-count). The resulting raw counts were compared and analyzed in R for expression changes through the timecourse. We utilized custom pattern analysis to identify genes with interesting expression profiles, such as increasing or decreasing over various periods. Of the 174 transcripts that consistently increase expression over 24 hours, 37% are non-coding or miRNAs.

Additionally, of the 99 transcripts that consistently decrease over 24 hours, 41% are non-coding or miRNAs. Non-coding and miRNAs are a key class of transcripts that don't encode proteins directly but can significantly alter the expression of their targets and therefore, cellular physiology. Our results highlight that sustained transcriptional differences following neuronal depolarization are largely characterized by regulatory RNA molecules, suggesting that the action of this class of RNAs may play a key role in regulating the molecular process of learning and memory. Additional work is needed to explore this finding *in vivo*.

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Poster

087. Alzheimer's Disease: Novel Therapeutics

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Program#/Poster#: 87.13/OO32

Topic: F.01. Human Cognition and Behavior

Support: NIH grant P50AG005136

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Title: Integrated approaches for analyzing u1-70k cleavage in alzheimer's disease

Authors: ***B. BAI**[1], P.-C. CHEN[1], C. M. HALES[2], Z. WU[1], V. PAGALA[1], A. A. HIGH[1], A. I. LEVEY[2], J. J. LAH[2], J. PENG[1];
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Abstract: Protein cleavage is commonly discovered in the brain of neurodegenerative disease, leading to the accumulation of neurotoxic fragments. We have recently found in Alzheimer's disease (AD) the aggregation of U1 small nuclear ribonucleoprotein complex (snRNP) and abnormal RNA processing. The U1 snRNP subunit U1-70K (~70 KDa) is also cleaved into an N-terminal truncation (N40K, ~40 KDa). Here we present evidence to indicate that U1-70K cleavage frequently occurs in AD, and the N40K abundance is inversely proportional to the U1-70K level, implying that this cleavage event contributes to the loss of U1-70K function. To map the cleavage site(s) in U1-70K, we compared tryptic peptides of N40K and stable isotope labeled U1-70K by liquid chromatography-tandem mass spectrometry (LC-MS/MS). When equally mixed, shared peptides display approximately 1:1 ratio, but protein-specific peptides have large

ratio distortion, revealing that the proteolysis is located in a highly repetitive and hydrophilic domain of U1-70K, which is essentially not compatible with current LC-MS/MS settings. We then adapted high-resolution Western blotting to map the cleavage site(s) in multiple steps: (i) U1-70K and N40K proteins were demonstrated to share the same N-termini by MS; (ii) our high coverage MS analysis identified no major modification in N40K, otherwise the modification might alter protein migration; (iii) we matched N40K with a series of 8 recombinant U1-70K truncated proteins, determining the cleavage site(s) within a small region (Arg300 ± 6 residues). Since most proteases exhibit residue specificity, we considered all possible unique residues in the region (Ser295, Ala299, Arg300, Glu302, and Lys306) to make related recombinant proteins. Finally, these N40K proteins caused substantial degeneration of rat primary hippocampal neurons, suggesting that they were functionally indistinguishable. Our analysis combined multiple approaches for probing proteolytic site(s) in a difficult protein, and the results support that U1-70K cleavage and the N40K fragment may contribute to neuronal toxicity in Alzheimer's disease.

Disclosures: **B. Bai:** None. **P. Chen:** None. **C.M. Hales:** None. **Z. Wu:** None. **V. Pagala:** None. **A.A. High:** None. **A.I. Levey:** None. **J.J. Lah:** None. **J. Peng:** None.

Poster

088. Healthy Aging

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 88.01/PP1

Topic: F.01. Human Cognition and Behavior

Support: NSF GRFP

Title: Chronic life stress predicts decreased white matter volume in the prefrontal cortex among healthy older adults

Authors: ***G. L. MORENO**, J. BRUSS, N. L. DENBURG;
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Abstract: Previous animal and human research has indicated that stress may have deleterious effects on the brain (Gianaros et al., 2007; Lupien et al., 2009). Surprisingly, several of the brain regions vulnerable to increased levels of stress, such as the hippocampus and prefrontal cortex, are also known to undergo disproportionate decline during normal aging. To gauge the integrity of brain regions involved in the stress response, we investigated whether chronic stress was

predictive of frontal and temporal lobe region volumes in healthy older adults. We hypothesized that older adults who evidenced more chronic stress would have decreased volumes in those brain regions involved in the stress response, namely prefrontal and temporal lobe regions. Structural MRI data and two measures of chronic stress, the UCLA Life Stress Interview (LSI; Hammen et al., 1985) and the Perceived Stress Scale (PSS; Cohen, Kamarck, & Mermelstein, 1983), were collected in 30 healthy, cognitively intact older adults (i.e., persons without overt neurological and psychiatric disease) between the ages of 65 and 90 years. As hypothesized, elevated chronic stress, as measured by both subjective (PSS) and objective (LSI) measures, was predictive of brain volume in temporal and frontal lobe regions, particularly in white matter. Notably, PSS predicted less white matter in the ventromedial prefrontal cortex bilaterally, the orbital gyri bilaterally, the dorsal lateral prefrontal cortex bilaterally, and the left ventrolateral prefrontal cortex. Our results suggest that elevated chronic stress is associated with a decrease in volume of specific brain regions; especially in those that are involved in the stress response and that are thought to be most vulnerable to age-related decline. These reductions in volume in relation to neuropsychological and cognitive measures, specifically in cognitive domains that are mediated by prefrontal and temporal lobe regions, will also be discussed.

Disclosures: **G.L. Moreno:** None. **N.L. Denburg:** None. **J. Bruss:** None.

Poster

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Topic: F.01. Human Cognition and Behavior

Support: Grant-in-Aid for JSPS Fellows 24・1926

Title: Elderly with high aerobic fitness maintain executive function predominantly by recruiting the primary hemisphere in the prefrontal cortex: A neuroimaging study with fNIRS

Authors: *K. HYODO[1], I. DAN[2], K. SUWABE[1], K. BYUN[1], G. OCHI[1], H. SOYA[1];
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Abstract: Elderly adults with high aerobic fitness are known to have high executive function associated with the prefrontal cortex (PFC). Despite various research, the underlying neural mechanism for this remains unclear. With aging, pre-frontal activation during executive tasks shifts from predominant activation in the primary hemisphere to bilateral activation (i.e.,

hemispheric asymmetry reduction). Our previous studies have shown left-lateralized prefrontal activation in young adults compared to the bilateral prefrontal activation in elderly adults during color-word matching Stroop tasks (CWST) as observed using functional near infrared spectroscopy (fNIRS) (Hyodo et al., Neurobiol Aging, 2012; Yanagisawa et al., Neuroimage, 2010). Thus, In this study, we aimed to clarify whether the association between higher aerobic fitness and executive function would be mediated by lateralized PFC activity by using mediation analysis.To address this issue, 27 healthy elderly men (mean age 70.3 ± 3.5 years) participated in a graded exercise test using a recumbent ergometer, followed by a computer-based CWST. During the exercise test, respiratory gas was measured and ventilatory threshold (VT) analyzed to measure submaximal aerobic fitness. The CWST consisted of incongruent and neutral conditions, and the difference in reaction times between conditions (incongruent-neutral) was calculated as Stroop interference to determine executive control. Multichannel fNIRS was attached to the forehead during the CWST to measure task-related prefrontal activation. As the dorsolateral prefrontal cortex (DLPFC) is mainly associated with the CWST, this region was focused upon, and left-lateralized DLPFC activity (Left DLPFC - Right DLPFC) was calculated to evaluate activation laterality. Partial correlation analyses revealed that there was a significant relationship among higher VT, shorter Stroop interference time and greater left-lateralized DLPFC activity, with controlling age and years of education as covariates. Hierarchical linear regression and non-parametrical mediation analyses showed that the left-lateralized activity in the DLPFC significantly mediated the relationship between VT and task performance. CONCLUSION:Our results provide evidence validating the hypothesis that the elderly with high aerobic fitness maintain executive function predominantly by recruiting the primary hemisphere. Thus, lateralized brain activation during cognitive test could be a physiological indication for hyper executive functions.

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Poster

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Topic: F.01. Human Cognition and Behavior

Support: NIH Grant 1R56MH097973-01

Title: The effects of fitness on subcortical brain anatomy and cognition across the life span

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Abstract: Recent evidence suggests that fitness leads to cognitive and anatomical preservation, although the majority of these studies have examined these effects in adults over the age sixty (Voss et al., 2013). Here we examined the associations between age and fitness on the subcortical anatomy and cognitive performance of healthy middle-aged adults (N=31, ages 18-62). Participants underwent structural Magnetic Resonance Imaging and a battery of neuropsychological tests. Subcortical anatomy (normalized to estimated intracranial volume) was quantified using FreeSurfer©. Subjects were classified as highly active if they had a physical activity score (PAS) of four or five indicating that they participated in aerobic exercise for more than an hour each week (Jurca et al., 2005). Our results indicate that age has a significant impact on both brain anatomy and cognition. Specifically, age was associated with significant loss in most of the subcortical volumes studied. The largest volumetric losses with age were found in the nucleus accumbens, and in the mid-anterior and central corpus callosum. Running averages were suggestive of non-linear volumetric losses for the amygdala and nucleus accumbens, with the largest declines occurring after age 38 in these regions. Age-related volumetric losses were also examined separately for subjects reporting varying levels of weekly physical activity. Individuals with high and low PAS scores showed similar rates of atrophy in the hippocampus and basal ganglia. Subjects reporting higher weekly physical activity showed larger volumes in the anterior corpus callosum across the age range investigated in our study. The effects of age and physical activity on cognition were also examined. With age, participants experienced a large drop in performance on the operation span task and Raven's Progressive Matrices, and also committed more perseverative errors on the Wisconsin Card Sorting Task. In line with other studies, Shipley's Vocabulary scores increased with age. A two sided t-test (*$p<.05$) demonstrated that subjects with higher PAS had significantly higher scores on the CFL test, indicative of higher verbal fluency. These data supports the claim that fitness effects are already evident at a young age, and that fitness may provide a reserve against age-related volumetric and cognitive losses.

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Poster

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Topic: F.01. Human Cognition and Behavior

Support: NIH Grant 1R03AG044610-01A1

Title: Adult age differences in "online" learning from positive and negative probabilistic feedback

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Abstract: Learning from probabilistic feedback requires using unreliable feedback (both positive and negative) from past experiences to inform future decisions. In general, studies show that older adults can learn from probabilistic feedback, but to a lesser degree than younger adults. However, previous conclusions may be limited for two main reasons. First, most task structures have not separated positive from negative feedback learning and therefore, it is not clear whether performance deficits are specific to one type of feedback. Second, other tasks rely on delayed tests to measure learning, and as a consequence, it is difficult to know whether the ability to learn declines with age or the ability to apply that learned information. To address these limitations, this study tested younger and older adults on a probabilistic category-learning task that dissociates positive (reward) from negative (punishment) feedback learning. Stimuli in the positive feedback condition gave either positive feedback or no feedback and stimuli in the negative feedback condition gave either no feedback or negative feedback; each type of feedback was reliable on 90% of trials. We examined "online" learning, as it was occurring, and found that older adults did learn from probabilistic feedback in both conditions, but not as well as younger adults. In addition, both age groups performed significantly better in the negative compared to the positive feedback condition. These results challenge the view that learning from negative feedback is preserved in healthy aging. Moreover, while previous work has shown that younger adults *prefer* positive feedback and older adults negative feedback, here we show that both age groups are better at *learning* from negative than from positive feedback.

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Poster

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Topic: F.01. Human Cognition and Behavior

Support: PAPIIT Grant IN225414

CONACYT Scholarship 210873

Title: Cognitive performance in healthy elderly subjects with excess of electroencephalographic theta activity

Authors: *S. A. CASTRO-CHAVIRA[1], T. FERNÁNDEZ[1], C. ALATORRE[1], S. SÁNCHEZ MOGUEL[1], M. RINCÓN[2], T. HARMONY[1], J. SANDOVAL[1], M. ESPINO[3];
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Abstract: Cognitive decline in elderly subjects can be predicted by the excess of electroencephalographic theta activity 7-10 years before its presentation (Prichep et al, 2006). NEUROPSI is a neuropsychological test normalized for the Mexican population that thoroughly explores cognitive functions for neuropsychological deficits. Twenty eight right-handed healthy subjects older than 60 years were selected. They had no neurological or psychiatric disorders. Their blood count, cholesterol test, triglycerides, glucose and TSH levels were normal, and their IQ was superior to 85. Their EEGs were recorded in the 19 leads of the 10-20 International System, considering the short-circuited earlobes as reference. Z values of Absolute Power corrected by Geometric Power were computed to separate the subjects into two groups: excess of theta (ET; n = 16) or theta between normal limits (NT; n = 12). Differences between groups in the NEUROPSI scores were analyzed using multivariate non-parametric permutation analyses (Galán et al., 1998). There were no differences between groups in the sepatared cognitive processes (attention, memory coding or retrieval, language, reading comprehension, writing, or executive functions). However, the ET group showed a significantly lower performance in the total score of the NEUROPSI test ($p = 0.05$). There were no significant differences between subjects with EEG risk to develop cognitive decline (ET) and without EEG risk (NT) for any of the individual cognitive processes evaluated. Furthermore, the subjects in both groups showed no cognitive deterioration. Even though the subjects with excessive EEG theta activity did not

express cognitive deterioration, their performance was poorer than the performance of the subjects with normal EEG.

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Poster

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Topic: F.01. Human Cognition and Behavior

Support: Ellison Medical Foundation New Scholar in Aging Award

NIH Grant 1R01MH098861

Title: Impaired learning in multidimensional environments in healthy human aging

Authors: *R. DANIEL[1], A. RADULESCU[2], Y. NIV[2];
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Abstract: To flexibly and efficiently behave in rich multidimensional environments, we need to learn to focus only on the dimensions that are currently predictive of reward. This ability is relies on two core mechanisms: learning from trial and error, and selective attention. Unfortunately, both of these mechanisms are impaired in healthy human aging. We present an experiment in which we aimed to develop a precise understanding of the computational and neural mechanisms underlying the interaction between learning and attention in older adults, and how these differ from the mechanisms employed by younger adults. To this end, we acquired behavioral and fMRI data from 23 healthy older adults (M = 70.0; range = 61-80), and an equal number of young controls (M = 22.7; range = 18-35). Participants performed a bandit task in which they chose one of three stimuli that differed on three dimensions. Only one of those dimensions was predictive of reward within a given game. The task was presented in two variants: in the "simple learning" variant participants were informed about the relevant dimension. In the "attention learning" variant they had to learn this information from trial and error. Older adults showed worse performance on both tasks, with a trend towards an increased impairment in the attention learning variant. Neurally, in the attention learning task younger adults showed a correlation

between the width of the attentional filter as predicted by our reinforcement learning model, and activation in the right temporo-parietal junction (TPJ)/angular gyrus, an area that has been implicated in filtering task-irrelevant information. Older adults failed to show this correlation. We propose that overall lower task performance in older adults might be due to a failure to selectively engage TPJ during the learning process, resulting either in an impaired ability to search for the relevant dimension early in learning, or an impairment in suppressing task-irrelevant information later on, or both.

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Poster

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VA Merit Award

Title: Emotional recognition memory for faces and words in healthy young and older adults

Authors: ***C. SUMIDA**[1], L. J. ROTBLATT[1], S. N. GLUHM[1], D. M. SCHIEHSER[2,3], J. V. FILOTEO[2,3], P. E. GILBERT[1,4];
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Abstract: Emotional enhancement of memory is a phenomenon whereby emotional information is remembered better than neutral information. Young adults have been reported to demonstrate a "negativity effect", such that negative stimuli are remembered better than positive stimuli. Older adults have been reported to show a "positivity effect", in which positive stimuli are remembered better than negative or neutral stimuli. These age-related differences in the enhancement of emotional memory comprise the Socioemotional Selectivity Theory. Age-related differences in emotional enhancement of memory may stem from functional and structural changes in the amygdala. However, some studies debate this theory and have shown that the pattern of memory

for emotional stimuli is similar in young and older adults. As a result, additional studies are needed to better understand the effect of emotion on recognition memory. The present study measured recognition memory for neutral, positive, and negative words and facial expressions in healthy young and older adults using d' as a sensitivity/discriminability index. The hit rate and false alarm rate are used to calculate d', whereby larger d' values indicate a better ability to discriminate "new" and "old" stimuli presented in a recognition memory test. During the study phase of the present task, participants were shown a series of either 24 target faces or 24 target words one at a time on a computer screen and were asked to rate the intensity of the stimuli on a seven-point Likert scale. For the recognition phase of each task, the participant was presented with the target faces or words amongst distractors and was asked to indicate if they had seen the faces or words during the study phase. Target and distractor faces were matched for valence, gender, approximate age, ethnicity, and facial hair. Target and distractor words were matched for valence, emotionality, imagery, concreteness, and frequency. Each participant completed each task, and the presentation of the faces task and words task was counterbalanced across participants. We found that d' scores were significantly higher ($p < .05$) in young adults compared to older adults for faces and words. However, no emotional enhancement was found in recognition memory for faces or words in either group. In contrast to the Socioemotional Selectivity Theory, we did not find a "negativity effect" in young adults or a "positivity effect" in older adults. However, the present findings are in accordance with other studies reporting that memory for emotional stimuli is similar in young and older adults.

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Poster

088. Healthy Aging

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KI stiftelser

Gun och Bertil Stohnes Stiftelse

Stiftelsen för Gamla Tjänarinnor

Title: Effects of brain iron concentration on neural activity and memory performance in normal aging

Authors: *G. KALPOUZOS[1], B. GARZÓN[1], R. SITNIKOV[2], C. HEILAND[1], J. PERSSON[1], L. BÄCKMAN[1];
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Abstract: In the brain, non heme iron, stored in ferritin protein, is a fundamental mineral for cellular metabolism. However, free iron is highly toxic, inducing oxidative stress, cellular dysfunction, and cell death. Increased iron concentration has been shown in normal aging, notably in basal ganglia, and linked to lower cognitive performance. In the ongoing IRON project, we investigate potential associations between iron loading, brain activity measured with functional MRI, and cognition in 40 healthy adults aged 30 to 80 years old. The major aim is to examine whether higher iron loading can account for age-related differences in brain activity and cognition. Brain data are collected on a 3.0T GE MR scanner. A multiecho Gradient Recalled Echo (GRE) sequence with 8 echo times is used to calculate the mean transverse relaxation rate R2*, which has been shown to correlate strongly with iron concentration in brain tissue. In preliminary analyses including 12 men and women (age range = 34-72 years), we related age, R2* in basal ganglia, activity during a motor-imagery memory task where participants imagine and encode actions (e.g., playing tennis), and offline recall performance of the actions. Mean R2* values for caudate, putamen, and pallidum were determined. Regarding the functional MRI data, imagery blocks were contrasted against control blocks. R2* (reflecting increased iron concentration) increased with increasing age in right caudate and bilateral putamen ($.63 < r < .73$, $p \leq .01$), the relationship being marginal in left caudate ($r = .43$, $p = .08$). During the task, brain activity decreased with advancing age in caudate and putamen bilaterally ($r < -.71$, $p \leq .005$). Activity in these regions was correlated with the number of recalled actions ($r > .52$, $p \leq .04$) and with R2*, where higher values were related to lower activity, notably in bilateral putamen ($-.75 < r < -.62$, $p < .01$), and marginally in left caudate ($r = -.47$, $p = .06$). Interestingly, when controlling for age, the correlation in right putamen did not completely disappear ($r = -.47$, $p = .07$). Older subjects recalled fewer actions ($r = -.50$, $p = .05$), and R2* in left caudate and right putamen was negatively linked to recall performance ($r < -.50$, $p \leq .05$); when controlling for age, the correlation in left caudate was marginally significant ($r = -.47$, $p = .07$). These preliminary findings suggest an effect of increased age-related iron concentration in basal ganglia on local brain activity during cognitive task performance and on memory, and also a possible age-independent iron-activity-cognition relationship.

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Poster

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Support: NIHR Grant Oxford Biomedical Research Centre A93182

Title: Long-term memory guided attention in healthy ageing

Authors: *G. SALVATO[1], E. Z. PATAI[2], A. C. NOBRE[2];
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Abstract: Recent progress in the study of cognitive decline in ageing has shown that the profile of the elderly is not consistent. Regarding implicit memory, contradicting accounts of the ability to utilize learned associations in ageing have been reported, with some groups showing intact cueing of attention through implicitly learned spatial configurations (contextual cueing - CC) (Howard, Howard, Dennis, Yankovich, & Vaidya, 2004), while others report deficits in CC with spared sequence learning (Smyth & Shanks, 2011). Additionally in the field of attention research, it has been shown that the elderly have an inability to prioritize information through endogenous (top-down) control and consequently to suppress distracting information (Bollinger, Rubens, Masangkay, Kalkstein, & Gazzaley, 2011; Gazzaley & D'Esposito, 2007; Gazzaley et al., 2008; Gazzaley, Cooney, Rissman, & D'Esposito, 2005; Zanto et al., 2011). Using a paradigm building on long-term memory guided attentional orienting, we investigated the ability of elderly participants to utilize encoded spatial locations of objects within complex scenes to deploy attention in order to optimize behavioural performance in a perceptual detection task. Our results show that over learning, older participants become faster at finding relevant targets, indicating a sparing of item-context association learning (linear contrast $F(1,17)= 156.6$; $p < .001$). Additionally, their memory for target locations and target identity was significantly above chance on a subsequent memory test ($p < .001$), and their self-rated confidence in their response is a valid reflection on their performance, indicating that these memories may be explicit in nature (linear contrast $F(1,17)= 403.2$; $p < .001$). Crucially, we found a significant effect of memory validity in the attention task: items that appeared in previously learned locations had faster RTs than items appearing in a novel location ($F(1,17)= 21.1$; $p < .001$). These results suggest that older adults are able to a) form robust memories of item-context associations, and b) exploit this information as top-down attentional cues. Our findings contribute to the understanding of memory and attention processes during healthy ageing, as well as the

interaction between these two systems, reflecting a non-uniform ability to apply top-down attentional cues depending on the source of the bias. Further research is needed to disentangle the contribution of implicit as opposed to explicit memory in this effect, as well as potential automaticity with which endogenous, long-term memory cues may incur a benefit on attentional guidance over explicitly directed top-down instructions.

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Poster

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Topic: F.01. Human Cognition and Behavior

Support: CIHR Grant MOP126105

Title: Investigating the neural correlates of spatial and temporal context memory across the adult lifespan

Authors: *E. ANKUDOWICH[1], D. KWON[1], D. MAILLET[1], S. PASVANIS[2], A. SWIERKOT[1], L. WALLACE[1], M. N. RAJAH[1];
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Abstract: Healthy aging is associated with greater reductions in memory for context, or source, information compared to item memory. Previous functional neuroimaging studies have focused primarily on context memory in older compared to young adults, and have reported group differences in prefrontal and posterior visual areas during both encoding and retrieval. Although volumetric studies have reported decreases in prefrontal volume as early as midlife, little is known about the functional changes of prefrontal regions supporting context memory in middle-aged compared to young and older adults. The current study aimed to investigate group differences and similarities in the neural correlates of context memory across the adult lifespan. Using functional magnetic resonance imaging (fMRI), we compared young (20-35; n=27), middle-aged (40-56; n=22), and older (60-76; n=31) adults on context memory tests for the spatial and temporal details of face stimuli at both encoding and retrieval. Participants completed two versions of the task (easy and difficult) in order to characterize performance effects vs. age effects. Behaviorally, a Task (spatial, temporal) x Difficulty (easy, difficult) x Group (young,

middle, older) ANOVA yielded a significant Task x Group interaction on retrieval accuracy ($p<.05$). Although young adults outperformed both middle-aged and older adults on the temporal task, they performed no differently from the middle-aged adults on the spatial task. In addition, middle-aged adults outperformed older adults on the spatial task but not on the temporal task. We used multivariate partial least squares (PLS) analysis to identify whole-brain patterns of fMRI activity associated with successful context encoding and retrieval across age groups. We found age-related differences in lateral prefrontal recruitment during successful context encoding and retrieval, regardless of task type. We also found age-related changes in posterior cortical regions during encoding and retrieval. Our results suggest that age-related changes in the neural correlates of episodic memory occur as early as midlife.

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Poster

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Support: JO and JR Wicking Trust

Australian NHMRC Project Grant

Title: Effects of cognitive reserve and the BDNF Val66Met polymorphism on episodic memory, working memory, executive function and language processing in healthy older adults

Authors: *J. C. VICKERS[1], D. WARD[2], K. STUART[2], N. SAUNDERS[2], M. SUMMERS[2]; [1]Sch. of Med., [2]Wicking Dementia Res. and Educ. Ctr., Univ. of Tasmania, Hobart, Australia

Abstract: Objective. The Tasmanian Healthy Brain Project (THBP) is a long-term, longitudinal investigation into whether later-life tertiary education reduces ageing-related cognitive alterations and/or provides protection from dementia. Cognitive reserve (CR) has also been proposed to be protective for dementia, and brain derived neurotrophic factor (BDNF) has been implicated as having a significant role in mediating the effects of environmental enrichments in experimental studies. Method. We have investigated the THBP cohort at baseline to determine whether common allelic variation in BDNF Val66Met and APOE interact with CR to produce

cognitive outcomes. This study involved 433 participants (66.7% female), aged 50-79 years (M = 62.16, SD = 6.81). A measure of CR was established through a principal components analysis (PCA) of THBP study variables (WTAR IQ, prior education and the Lifetime of Experiences Questionnaire) previously shown to contribute to the construct. Composite variables of episodic memory, working memory, executive function and language processing were also derived through PCA, with the component accounting for the largest proportion of variance retained to represent that domain. Multivariate regression analyses then assessed the main and interaction effects for predictors: age; CR; APOE; and BDNF, for each cognitive domain separately. Results. This analysis demonstrated that: (1) CR was positively associated with each cognitive domain ($p < .05$) while age was negatively associated with each cognitive domain ($p < .05$), excluding language processing, (2) individual APOE and BDNF predictors were not associated with performance in any cognitive domain, (3) BDNF moderated the association between CR and executive function performance, in that BDNF Met carriers had a significantly reduced association when compared to Val homozygotes ($p < .05$). Conclusions. This study has shown that CR is associated with cognitive function in healthy older adults. In addition, BDNF Met carriers demonstrated a reduced ability to generate or access CR resources when compared to BDNF Met non-carriers with respect to executive function.

Disclosures: **J.C. Vickers:** None. **D. Ward:** None. **K. Stuart:** None. **N. Saunders:** None. **M. Summers:** F. Consulting Fees (e.g., advisory boards); Eli Lily (Australia) Pty Ltd, Novotech Pty Ltd.

Poster

088. Healthy Aging

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 88.12/PP12

Topic: F.02. Animal Cognition and Behavior

Support: CIHR

NSERC

Title: Correlates of brain anatomy and cardiac function in healthy aging and atherosclerosis in mice

Authors: ***P. POULIOT**[1,2], P. AVTI[2], C. BOWEN[3], A. CASTONGUAY[1], M. TABATABEI[1], M. MOEINI[1], É. THORIN[4], F. LESAGE[1];

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Abstract: Healthy aging is accompanied by broad and subtle anatomical and functional decline. Few MRI studies have correlated brain health measures with cardiovascular health measures in the same animals. 3 cohorts of mice (n=10 per group, age 4, 12 and 24 months) on a reduced-fat diet from the same C57/B6 lineage were imaged at 7T. Two LDLr−/−; hApoB+/+ (ATX) mice (aged 12 and 14 months), a mouse model that naturally develops atherosclerosis, were also scanned. Blood pressures were measured on 5 consecutive days and averaged. MRI scanning session durations were kept under about an hour with heart and brain scans done on different days. Brain scans consisted of 3D balanced TFISP with TR/TE=4.4/2.2 ms with 140 um isotropic resolution giving T2/T1 contrast, lasting 40 minutes. The cardiac scans were long axis (1 slice) and short axis (10 slices) CINEs with 16 frames, 156 um in plane resolution and 800 um thickness, lasting 35-40 minutes. Heart CINE scans were quantified with Segment (Medviso) to obtain ejection fraction and cardiac output. Brain scans were analyzed with Advanced Normalization Tools (ANTs). For each mouse, a brain mask was created; 16 TFISP scans were sum-of-square reconstructed and realigned, then N4 corrected and Otsu segmented into 3 tissue types. The cortical thickness was then estimated at each voxel. Its average value above the genu corpus callosum was extracted and was also measured graphically directly on anatomical images. Mean cortical thickness was 1.88 ± 0.09 mm, with no significant difference between groups while cortical thickness of the ATX mice was 2.0 and 3.6 sigma smaller. No significant correlation was found between cortical thickness and blood pressure.

Disclosures: **P. Pouliot:** None. **P. Avti:** None. **C. Bowen:** None. **A. Castonguay:** None. **M. Tabatabei:** None. **M. Moeini:** None. **É. Thorin:** None. **F. Lesage:** None.

Poster

088. Healthy Aging

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 88.13/PP13

Topic: F.01. Human Cognition and Behavior

Support: NIH R01 AG033036

Title: Relationships between longitudinal changes in functional brain activation, white matter integrity and cognitive performance in healthy older adults

Authors: *J. G. HAKUN, Z. ZHU, N. JOHNSON, B. T. GOLD;
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Abstract: Three major neurocognitive variables affected by aging are prefrontal cortex (PFC) functional brain activation, white matter (WM) microstructural integrity, and cognitive task performance. However, our understanding of the inter-relationships between these neurocognitive factors has been inferred almost entirely from cross-sectional comparisons between young and older adults. Here we used a multimodal imaging approach to explore longitudinal change in PFC activation, PFC WM integrity, and task switching performance in eighteen healthy older adults. In support of a neural efficiency model of neurocognitive aging, we found that longitudinal increases in PFC activation were associated with longitudinal decreases in commissural microstructural integrity and increased response latencies during task switching. These findings provide the first evidence of correlated change in brain microstructural integrity and task-related PFC functional recruitment. In addition, the observed correlation between increased functional recruitment and increased response latencies serves as a challenge to compensation accounts of PFC over-recruitment in older adults. Overall, our study reveals a tight connection between brain structural integrity and functional activation that holds significant implications for brain imaging studies of cognition.

Disclosures: **J.G. Hakun:** None. **Z. Zhu:** None. **N. Johnson:** None. **B.T. Gold:** None.

Poster

088. Healthy Aging

Location: Halls A-C

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Support: The U.S. Department of Veterans Affairs

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National Institute for Neurological Disorders and Stroke (R01 NS052585-01)

National Institutes of Health through the NIH Roadmap for Medical Research, Grant U54 EB005149

Title: White-matter integrity and cognitive flexibility in normal aging

Authors: *D. RINKER[1,2], C. FENNEMA-NOTESTINE[3], M. S. PANIZZON[3], D. J. HAGLER[4], C. FRANZ[3], P. M. THOMPSON[2], A. M. DALE[4], W. S. KREMEN[3];
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Abstract: The ability to switch between different attentional states is known as cognitive flexibility, and is a fundamental indicator of neurological health. As such, it is ubiquitously found in neuropsychological test batteries. Poor performance is associated with cognitive impairments, learning problems and can be an indicator of various diseases including Alzheimer's. Functional imaging has allowed researchers to study set-shifting *in vivo*, building on an existing body of lesion studies (Stuss & Levine, 2002). Most of the literature implicates the dorsolateral prefrontal cortex specifically superior and middle frontal gyri as essential in set-shifting (Zakzanis, et al., 2005). Many of these regions are important for sub-components of set-shifting, such as executive processing speed, motor tasks and visual scanning. Still, the neural recruitment of these diverse regions would suggest that a higher-level neural circuit contributes to set-shifting function. Association tracts in white matter (WM) facilitate neural communication between regions, forming functional neural circuits. Individual differences in WM integrity may explain some of the variance in corresponding cognitive tasks (Perry et al., 2009). Here we examined (1) set shifting, and (2) its role in cognitive and neurobiological aging. DTI and T1 data were obtained at 1.5T from 342 men (average age = 55.8 SD +/- 2.2), who underwent neuropsychological testing as part of the Vietnam Era Twin Study of Aging (Kremen, 2006). Images were processed with FreeSurfer and probabilistic tractography to define WM tracts. Time to completion on the shifting component of the Delis-Kaplan Executive Function System Trail-Making Test adjusted for sequencing components was used to assess cognitive flexibility, controlling for overall processing speed. FA for several tracts was significantly correlated with task performance ($P<0.05$), including: left fornix, bilateral inferior longitudinal fasciculus (ILF), and bilateral inferior occipitofrontal fasciculus (IFOF). MD was also was correlated significantly with task performance ($P<0.05$), in the bilateral cingulum, left uncinate, bilateral ILF, bilateral IFOF and left superior longitudinal fasciculus. These results mostly fit our a priori circuit hypothesis and were in the expected direction. While cognitive flexibility is often thought of as a frontal lobe function, our results suggest widespread networks are involved, and also give may indicate key tracts mediating the task.

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Poster

088. Healthy Aging

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Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 88.15/PP15

Topic: F.01. Human Cognition and Behavior

Support: German Aerospace Center (DLR) on the behalf of the German Ministry for Research and Technology (grant 50WB1224)

Title: The operation of control devices in old age: A new approach to assess motor and cognitive performance during realistic working scenarios

Authors: *M. DALECKI[1], F. STEINBERG[2], M. KALICINSKI[3], O. BOCK[3];
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Abstract: Cognitive and motor functions are known to degrade with advancing age, but it is unclear how these deficits - revealed by standardized laboratory tests - affect seniors' performance in everyday life. Unlike laboratory tests, realistic tasks include complex responses to complex environmental inputs, executed under varying levels of stress. The present study therefore compares the performance of young and older subjects in a complex process-control task.
We hypothesized that the elderly perform less well, and that their impairment is associated with cognitive and motor decline.
12 younger (mean age: 25 years) and 12 elderly subjects (mean age: 65 years) participated. They sat in front of a screen with multiple displays next to a working panel with multiple knobs and levers. Their task was to operate a simulated power plant by adjusting parameters such as temperature, fuel and power, for which they used the knobs and levers. Their earnings (proceeds minus costs) were presented continuously on the screen. Twenty-six work episodes of twenty seconds each alternated with short rest breaks. Gaze time on the screen and on the panel was recorded by an eye tracker (Tobii T60), and maximum force production on the knobs by force sensors (ATI Nano). Stress level was assessed by established questionnaires (NASA TLX), and

cognitive abilities (concentration, attention) by PC-based tests.
If compared to young subjects, elderly participants generated earnings at a lower rate (-73%), directed their gaze longer to the panel (+75%) and - accordingly - shorter at the screen, and applied higher grip forces to the knobs. Perceived stress didn't differ between age groups, but cognitive ability scores were significantly smaller in the elderly (-45%). There was a significant correlation between cognitive scores and magnitude of earnings.
We conclude that seniors' performance on a realistic process-control task is substantially degraded, possibly because elderly persons must focus more on their responses, thus missing important information presented on the screen. The deficit is not paralleled by an increased subjective stress level.

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Poster

088. Healthy Aging

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Topic: F.01. Human Cognition and Behavior

Support: NIA Grant P50 AG05146

NIA Grant R01 AG034613

NIA Grant T32 AG027668

Title: Effects of aging on mnemonic discrimination of emotional information

Authors: *S. L. LEAL[1], M. A. YASSA[2];
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Abstract: Episodic memory loss is one of the hallmarks of age-related cognitive decline and a major symptom of Alzheimer's disease. The persistence and strength of memories is determined by modulatory factors such as emotional arousal. Whether emotional memories are preserved with age or if these memories are just as susceptible to loss and forgetting is not well understood. We have recently shown that emotion alters how similar memories are stored using non-overlapping representations (i.e. pattern separation), in an emotional mnemonic discrimination task. Here, we extend this work to testing young and older adults at two time-points

(immediately after encoding and 24 hours later). Overall, older adults performed worse than young adults, a memory deficit that was not secondary to perceptual or attentional deficits. When tested immediately after the encoding session, older adults were impaired on neutral target recognition but intact on emotional target recognition. We also found that a pattern we previously reported in young adults (reduced emotional compared to neutral discrimination of similar items) was reversed in older adults. When tested after 24 hours, young adults exhibited less forgetting of emotional targets compared to neutral, while older adults exhibited more forgetting of emotional targets. Finally, discrimination of highly similar positive items was preserved in older adults. These results suggest that emotional modulation of memory interacts with age in a complex manner such that the emotion-induced memory trade-off reported in young adults is reversed in older adults. These findings shed light on how emotion and memory interact in the aging brain.

Disclosures: **S.L. Leal:** None. **M.A. Yassa:** None.

Poster

088. Healthy Aging

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 88.17/PP17

Topic: F.01. Human Cognition and Behavior

Title: Risk and pathological factors associated with aging in a sample of Panamanians over 64 years old

Authors: *A. E. VILLARREAL[1,2], S. GRAJALES[1], G. B. BRITTON[1], .-. PANAMA AGING RESEARCH INITIATIVE[3];
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Abstract: More than 5.4 million of Americans suffer from AD. In Panama the geriatric population is gradually increasing as in the rest of the world. According to the census (2010), 7.4 % of Panamanian population is over 64 years of age. Dementia is among the diseases that primarily affect the elderly population. It is estimated that the rate of dementia increases with the increase in the rate of aging. Alzheimer's disease (AD) is the most common form of dementia. Among the primary risk factors for development of AD in Hispanics/Latinos are (1) age, life expectancy will be around 87 years by 2050, (2) low educational level and (3) vascular diseases together with high incidence of diabetes mellitus and other chronic diseases. The peculiar

characteristic about the Hispanics/Latinos is that they represent a wide mix of national origins, and the genetic complexity is greater compared with other ethnicities. We are conducting a biomarker based study to identify the risk and protective factors associated with cognitive function during aging in a sample of Panamanians over 64 years of age. To date, there are no published epidemiological surveys of aged individuals in Panama, placing our country among the least prepared to handle the boom in aging that is taking place. We report the results of a cross-sectional analysis of aged individuals (N=154). The following measures were obtained: (1) a medical history to identify diabetes mellitus, atherosclerosis, hypertension, and obesity, (2) blood samples for a biomarker profile (serum, plasma, and DNA), (3) cognitive function (Mini Mental State Examination, TMT A and B, and clock test), (4) muscle strength (dynamometer), (5) functional status (basic activities of daily living), (6) depression, and (7) telomere length measures. Preliminary results indicate that 63% of the sample shows no sign of dementia, 12% meet criteria for AD, 8% for mild cognitive impairment, 9% for vascular dementia, and 9% in other classifications. Also, 36% of the sample evidenced moderate to severe deficits in cognitive function according to the MMSE. Cognitive function (MMSE) and functionality (ABVD) showed a negative correlation. Lastly, 16% of the sample met the criteria for depression. This study is a first attempt to assess the factors associated with aging in Panama. Our aim is to establish the basis for future prospective studies.

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Poster

088. Healthy Aging

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Topic: F.01. Human Cognition and Behavior

Support: NSC102-2410-H002-004-MY3

Title: Dietary macronutrient composition is differentially associated with gray matter volumes of cortical and limbic regions in young and older adults

Authors: *S.-Y. YUAN[1], Y.-Z. TU[2], P.-Y. WANG[1], J. O. S. GOH[1,3,4];
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Center, Natl. Taiwan Univ., Taipei, Taiwan; [4]Dept. of Psychology, Natl. Taiwan Univ., Taipei, Taiwan

Abstract: Energy and molecular substrates from dietary intake are important factors for neuronal structural development and function over the lifespan. There is limited information, however, on how human dietary macronutrient levels are associated with brain structure and function, particularly for those not affected by obesity or other dietary dysfunctions. We hypothesized that macronutrient intake levels in humans should have measurable associations with gray matter volume and cognitive ability even in individuals maintaining healthy dietary habits. Importantly, dietary-brain associations may differ across age due to differential nutritional requirements. 32 young (mean age (SD) = 23.3 (2.1) yrs) and 21 older (mean age (SD) = 70.2 (4.8) yrs) Taiwanese adults participated in (a) systematic dietary monitoring over 3 to 30 days, (b) neuropsychological tests of cognitive abilities, and (c) T1-weighted magnetic resonance imaging (MRI) of gray matter volumes (256*256 mm in-plane field of view, 1*1*1 mm3 voxels, TR = 2 s, inversion time = 0.9 s, flip angle = 9°). Participants were physically healthy at time of testing with no dietary dysfunction or neurocognitive counter-indications. Individual intake levels of calorie, carbohydrates, lipids, and proteins were scored based on the local food and drug administration database. T1 images were analyzed using Freesurfer software ver. 5.2.0 to parcellate regional gray matter volumes across the whole brain. We found no age differences in dietary macronutrient levels in this relatively healthy sample. Higher calorie and carbohydrate intake correlated with smaller orbitofrontal volumes in younger adults but larger volumes in older adults. By contrast, higher carbohydrate, protein, and lipid intake correlated with larger volumes in medial temporal and striatal regions in young adults, but smaller volumes in older adults. Adjusting for calorie intake revealed that higher percentages of carbohydrate levels correlated with smaller medial temporal and striatal volumes in young adults but either with larger or no difference in volumes in older adults. Higher percentages of protein correlated with larger hippocampal volumes in young adults but smaller volumes in older adults. Across these diet-sensitive regions, there were mixed correlations between brain volume and cognitive performances in older adults and minimal associations with young adult performances. Our findings demonstrate age-related differences in the association between dietary macronutrient intake and gray matter regional volumes. Age-related changes in nutritional requirements may modulate the effect of diet on brain and cognition.

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Poster

089. Appetitive and Incentive Learning and Memory I

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Topic: F.02. Animal Cognition and Behavior

Support: DA027535

5T32DA015040-10

5T32MH087977-05

Title: Locus coeruleus optoICSS: A role for selective noradrenergic activation in reinforcement

Authors: *K. SCHMIDT[1], C. KOLLER[1], E. VAZEY[2], I. WITTEN[3], C. BASS[4], G. ASTON-JONES[2], K. DEISSEROTH[5], D. WEINSHENKER[1];
[1]Emory Univ., Atlanta, GA; [2]Med. Univ. of South Carolina, Charleston, SC; [3]Princeton, Princeton, NJ; [4]Univ. at Buffalo, Buffaolo, NY; [5]Stanford, Stanford, CA

Abstract: It is generally accepted that dopamine is the primary neurotransmitter of the brain's reward system. While accumulating data indicates that norepinephrine (NE) may also be important for some forms of reward and reinforcement, there are conflicting reports from the literature. For example, some groups found that electrical stimulation of the noradrenergic locus coeruleus (LC) could maintain operant behaviors, while others obtained the opposite result. Because these intracranial self-stimulation (ICSS) studies were limited by issues of electrode placement, could not discriminate between stimulation of LC cell bodies or fibers of passage, and likely resulted in collateral stimulation of non-noradrenergic cells in the area, a clear interpretation of these studies is elusive. We are taking an optogenetic approach to overcome these technical obstacles and conclusively determine how the LC fits into the brain's reward circuitry and whether noradrenergic activity can function as a reinforcer. We set out to use optogenetic techniques to selectively express channelrhodopsin (ChR2) in noradrenergic cell groups and assess the effects of their activation as a consequence of lever pressing behavior. We have tested the selective expression of ChR2 in the LC using two different viral systems: a Cre-dependent AAV-DIO in TH:Cre transgenic rats and a lentivirus driven by an LC-specific phox2b-dependent PRSx8 promoter. Because the PRSx8 had stronger expression in the LC, we have been using this system for our behavioral experiments. Preliminary results suggest that optogenetic stimulation of the LC can serve as a reinforcer to maintain lever-pressing behavior, implicating this noradrenergic nucleus as a component of the brain's reward system.

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Poster

089. Appetitive and Incentive Learning and Memory I

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Topic: F.02. Animal Cognition and Behavior

Support: TNDA 5T32DA024635

NIH Grant DA035443

Hellman Foundation Fellowship

UCLA Faculty Career Development award

Title: The role of BLA rapid glutamate signaling in value-based decision-making

Authors: *M. MALVAEZ[1], A. M. YORITA[2], L. FENG[2], H. G. MONBOUQUETTE[2], K. M. WASSUM[1,3];
[1]Psychology, [2]Chem. Engin., UCLA, Los Angeles, CA; [3]Brain Res. Institute, UCLA, Los Angeles, CA

Abstract: Reward-seeking decisions are heavily controlled by the incentive value (i.e., desirability) of the reward they attain. This incentive value is acquired and continually updated through the process of instrumental incentive learning such that it tends to reflect the emotional response elicited during the most recent reward experience. Although the basolateral amygdala (BLA) has been implicated in the encoding and use of reward value information to guide decisions, the neurotransmitter processes within this structure mediating value-guided reward-seeking decisions are not well characterized. Recently, using a glutamate biosensor technology, we demonstrated a relationship between rapid BLA glutamate signaling and reward seeking; the frequency of rapid glutamate release events increases when rats are engaged in reward-seeking actions and the occurrence of these events positively predicts reward-seeking behavior. However, it remains unknown how these signals relate to the decision-making process and with which specific aspects of reward seeking they are associated. Therefore, we evaluated the hypothesis that transient glutamate release in the BLA tracks changes in reward value important for value-guided decisions. To examine the role of BLA rapid glutamate signaling in value-guided reward seeking, we used electroenzymatic biosensors to make near-real time measurements of BLA glutamate concentration changes during an incentive learning experience

and during the use of this updated information to guide reward-seeking decisions. We have found that BLA glutamate transient frequency is elevated during the experience of the reward in a novel, hungry state, but not during reward experience in the sated, control state. Moreover, the BLA glutamate transient frequency tracked reward seeking following instrumental incentive learning. These preliminary data suggest rapid BLA glutamate signaling may relate to the reward evaluation process that drive value-based reward seeking decisions.

Disclosures: **M. Malvaez:** None. **A.M. Yorita:** None. **L. Feng:** None. **H.G. Monbouquette:** None. **K.M. Wassum:** None.

Poster

089. Appetitive and Incentive Learning and Memory I

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Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant DA11717

Kavli Fellowship

PEO Scholar Award

Title: NMDA receptor regulation of prediction error in mice: Relevance for psychosis

Authors: *C. A. GIANESSI[1], S. L. QUICK[2], P. R. CORLETT[2], J. R. TAYLOR[2];
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Abstract: One hypothesis about the underlying mechanism of psychosis is that psychosis is caused by abnormal prediction error signals in the brain (Corlett et al., 2010). Prediction error is the difference between actual and expected events, and this calculation is used to update expectations to more accurately reflect the environment. Inappropriate prediction error signals would drive noticing coincident but random events in the environment, with delusions forming to explain the salience. Inappropriate prediction error related activity has been observed in the brains of first episode psychotic patients and psychiatrically healthy volunteers when administered sub-anesthetic doses of ketamine (Corlett et al., 2006, 2007). Results in human imaging studies are correlational, so the causal neural circuitry underlying anomalous prediction error related activity is not known. Here we used a Kamin blocking task and ketamine

administration in male C57/Bl6J mice to model the neurobiological underpinnings of prediction error deficits in schizophrenia. Blocking is a normal learning phenomenon, in which no learning occurs to redundant information. Animals learn to associate an initially neutral light with delivery of food. The second phase of learning consists of pairings of a novel tone together with the previously learned light with the same food delivery. This results in no learning about the tone. Learning to the tone is blocked because the light is already fully predictive of food, and there is no prediction error. We hypothesized that if ketamine produces altered prediction error signals then aberrant learning would result in an increased Pavlovian approach response to the tone. Preliminary data suggest that blocking is reduced after acute administration of 6 mg/kg (ip) ketamine given before each training session in the second phase of learning. The mice treated with ketamine mice show an increased Pavlovian approach response to the tone compared to animals treated with saline. Blocking is a behavioral and neural biomarker for delusions in human subjects that can be modeled in mice. This model can take full advantage of powerful neuroscience tools like transgenic mice to better understand what might underlie psychosis. This is a prelude to rationally developing novel therapies for psychosis that target the underlying pathophysiology based on empirical data rather than serendipity. These studies were supported by public health service grants: DA11717 (JRT), Kavli Fellowship (CAG), and PEO Scholar Award (CAG).

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Poster

089. Appetitive and Incentive Learning and Memory I

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Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

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Topic: F.02. Animal Cognition and Behavior

Support: NIDA Grant SCIDA034995

Title: Neural correlates of contingency in appetitive Pavlovian conditioning

Authors: *H. NASSER[1], J. AVILA[2], K. GILROY[3], P. SERRANO[2], A. R. DELAMATER[4]; [1]Sch. of Psychology, City Univ. of New York, Brooklyn, NY; [2]Hunter College-CUNY, Manhattan, NY; [4]Psychology, [3]Brooklyn College-CUNY, Brooklyn, NY

Abstract: The contingent presentation of cues via Pavlovian conditioning can affect synaptic plasticity and alter behavior. What neural mechanisms are reflected as a result of contingency are somewhat unknown. It is believed that the formation of associations is correlated with neural changes in the amygdala and nucleus accumbens. We were interested in measuring expression of postsynaptic density of the AMPA receptor subunit GluA2 in addition to nuclear expression of the immediate early gene c-Fos as a result of contingency manipulations. In this experiment, rats received appetitive Pavlovian conditioning with a tone stimulus and sucrose. Half of the rats received positive contingency training such that delivery of the sucrose was 100% contingent upon the presentation of the tone. The rest received zero contingency training, such that the delivery of sucrose was equally likely during each second of the session also known as a truly random control. Responding to the tone was then assessed in the absence of sucrose, at which point rats were sacrificed to measure expression of GluA2 and c-Fos. Results showed increased colocalized expression of GluA2 and post-synaptic density marker (PSD-95) in the nucleus accumbens core and a marginal increase in shell as a result of positive contingency training. These data are consistent with increases in GluA2/PSD-95 clustering observed after spatial learning and memory and now support a role for a similar mechanism in the nucleus accumbens during Pavlovian learning.

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Poster

089. Appetitive and Incentive Learning and Memory I

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Topic: F.02. Animal Cognition and Behavior

Support: ARC Australian Laureate Fellowship to BWB FL0992409

Title: Effects of delta-opioid receptor accumulation and internalisation in the nucleus accumbens shell on cue-guided choice

Authors: *A. K. MORSE[1], V. LAURENT[1], J. BERTRAN-GONZALEZ[2], B. BALLEINE[1]; [1]Brain and Mind Res. Inst., Univ. of Sydney, Camperdown, Australia; [2]Clem Jones Ctr. for Ageing Dementia Research, Queensland Brain Institute, The Univ. of Queensland, Brisbane, Australia

Abstract: Animals can use information extracted from their environment to guide their behaviour towards a desired outcome. In the laboratory, this critical ability can be studied through outcome-specific Pavlovian-instrumental transfer (PIT), in which a stimulus associated with a particular outcome biases response choice towards an instrumental action that earned that same outcome. Recent evidence from our laboratory indicates that expression of this bias requires the translocation of delta-opioid receptors (DOR) to the plasma membrane of cholinergic interneurons (mCINs) within the nucleus accumbens (NAc) shell. Interestingly, this plastic change in receptor expression occurs during Pavlovian training, in which specific and contingent stimulus-outcome associations are established. The present experiments examined behavioural and pharmacological manipulations of DOR expression, and their subsequent effects on specific PIT. Consistent with the literature, only manipulations of Pavlovian contingencies, degradation and non-contingent stimulus/outcome presentations, abolished specific PIT. Surprisingly, these procedures failed to reverse the DOR accumulation present after contingent Pavlovian training. DOR accumulation on mCINs within the NAc shell during Pavlovian learning therefore appears to be necessary but not sufficient for specific PIT. The effect of pharmacological DOR internalisation was also investigated to determine whether DOR accumulation triggered by Pavlovian learning changed receptor expression recovery following this internalisation. The specific DOR agonist SNC80, known to produce strong receptor internalisation, was administered to both trained and naïve mice. Although trained mice showed higher overall DOR expression, both trained and naïve mice given SNC80 has less DOR expression on mCIN than vehicle mice 24 hours after injection, consistent with previous findings. SNC80 administration 24 hours prior to test can therefore be used to investigate the effect of DOR internalisation on specific PIT. Disruption of DOR accumulation following Pavlovian learning may also disrupt specific PIT if animals are tested before this accumulation recovers.

Disclosures: **A.K. Morse:** None. **V. Laurent:** None. **J. Bertran-Gonzalez:** None. **B. Balleine:** None.

Poster

089. Appetitive and Incentive Learning and Memory I

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 89.06/PP24

Topic: F.02. Animal Cognition and Behavior

Support: NIH P50 DA05312

K99 DA 033373

Title: The role of d1 and d2 receptors in incentive value attribution

Authors: *J. J. CHOW[1], M. DARNA[2], J. S. BECKMANN[1];
[1]Psychology, [2]Pharmaceut. Sci., Univ. of Kentucky, Lexington, KY

Abstract: Reward-predictive stimuli compel some individuals to approach and interact with the stimulus (sign-tracking), while others approach the location of forthcoming reinforcement delivery (goal-tracking). Furthermore, rats that have a propensity to attribute incentive value to reward-related cues and display sign-tracking behavior are more susceptible to abuse-related behavior. It has been hypothesized that these different response types are mediated by different valuation systems. However, very little is known about the neural mechanisms that drive these proposed systems. Here we initially examined the role of the dopamine system in these different valuation systems with a single-stimulus, between-subjects Pavlovian conditioned approach (PCA) task using a D1 antagonist, SCH-23390 (0.01 and 0.03 mg/kg) and a D2 antagonist, eticlopride (0.01 and 0.03 mg/kg). The results indicated that at the 0.01 mg/kg dose, sign-tracking was suppressed by D1 antagonism, while D2 antagonism had no effect. The 0.03 mg/kg dose of both the D1 and D2 antagonists had non-specific effects. Next, we took advantage of the fact that different stimuli can specifically elicit different response types (lever for sign-tracking; tone for goal-tracking) to investigate the role of the D1 and D2 receptors on the relative valuation of a stimulus within-subjects. Sprague Dawley rats were trained on a 2-CS PCA task for fourteen consecutive days where a lever or a tone was presented for 8s and a food reward was delivered non-contingently. Rats were pretreated with either saline, SCH-23390 + eticlopride (0.01 mg/kg), SCH-23390 (0.01 mg/kg), or eticlopride (0.01 mg/kg) 15 minutes prior to start of each 2-CS PCA session. Following 2-CS PCA training, the valuation of the different stimuli was examined through extinction, conditioned reinforcement, and a novel choice procedure. Results from the 2-CS PCA task revealed that sign-tracking behavior was eliminated by D1+D2 antagonism, while goal-tracking behavior was mildly attenuated. However, when D1 and D2 receptors were individually examined, D1 antagonism eliminated sign- and goal-tracking behavior while D2 antagonism had no effect on sign-tracking behavior but did attenuate goal-tracking behavior. Furthermore, D1+D2 antagonism and D1 antagonism following the 2-CS PCA task also increased sign-tracking extinction rates, decreased conditioned reinforcement by the lever CS, and increased discounting of incentive value attributed to the lever CS. Overall, D1 and D2 receptor signaling seem to have differential roles in the attribution of incentive value to reward-predictive stimuli.

Disclosures: **J.J. Chow:** None. **J.S. Beckmann:** None. **M. Darna:** None.

Poster

089. Appetitive and Incentive Learning and Memory I

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Topic: F.02. Animal Cognition and Behavior

Support: Hellman Fellowship Fund

UCLA Faculty Career Development Award

Title: Differential regulation of cue-induced incentive motivation by muscarinic and nicotinic acetylcholine receptors within the nucleus accumbens core

Authors: *A. L. COLLINS[1], I. XU[1], S. B. OSTLUND[2], K. M. WASSUM[1];
[1]UCLA, Los Angeles, CA; [2]UCI, Irvine, CA

Abstract: Reward-associated cues elicit excitation/arousal through association with an appetitive outcome and this excitation can act to enhance a non-selective range of reward-seeking actions: a process known as general Pavlovian-to-instrumental transfer (PIT). Evidence has implicated the nucleus accumbens core (NAc) in this cue-induced incentive motivation. Additionally, within the NAc, the two types of acetylcholine (ACh) receptors, muscarinic and nicotinic, have been demonstrated to distinctly modulate ACh and dopamine signaling, and reward-related behaviors generally. However, what remain unclear are the precise psychological processes modulated by NAc ACh signaling. Here we evaluate the hypothesis that muscarinic and nicotinic ACh receptors differentially modulate cue-induced incentive motivation. We utilized the PIT task in order to assess the role of NAc ACh receptors on Pavlovian cue-induced incentive motivation. In Experiment 1, we bilaterally infused a nicotinic antagonist, mecamylamine (10μg/0.5μl), a muscarinic antagonist, scopolamine (10μg/0.5μl), or equivalent volumes of ACSF in the NAc immediately prior to a PIT test. Under intra-NAc ACSF control conditions when the reward-paired cue was present rats invigorated their responding relative to the pre-cue periods and neutral cue presentations. This incentive motivation effect was blocked by intra-NAc antagonism of muscarinic receptors, while intra-NAc antagonism of nicotinic receptors enhanced the expression of PIT compared to ACSF-controls. In Experiment 2, we bilaterally infused either a nicotinic receptor agonist, nicotine (20μg/0.5μl), a muscarinic receptor agonist, pilocarpine (80μg/0.5μl), or equivalent volumes of ACSF within the NAc immediately prior to a PIT test. Either infusions of pilocarpine or nicotine attenuated the expression PIT relative to ACSF controls. In all groups, head entries into the food port were significantly elevated during the

reward-paired cue presentation relative to the pre-cue periods and neutral cue presentations indicating that these effects were unlikely due to general motivational or motor effects. These data indicate that alterations in NAc nicotinic receptor activity produces bidirectional effects on PIT, with enhanced signaling attenuating the impact of reward-paired cues on invigorating reward-seeking actions. Conversely, in either direction, modulating muscarinic receptors impaired PIT. Collectively, these data indicate distinct roles for muscarinic and nicotinic ACh receptors with the NAc in mediating the ability of reward-paired cues to generally invigorate reward-seeking actions.

Disclosures: **A.L. Collins:** None. **I. Xu:** None. **S.B. Ostlund:** None. **K.M. Wassum:** None.

Poster

089. Appetitive and Incentive Learning and Memory I

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Program#/Poster#: 89.08/QQ2

Topic: F.02. Animal Cognition and Behavior

Title: The effects of increased dopaminergic transmission on cognitive bias in mice

Authors: *R. A. CLIBURN, K. M. LOHR, T. S. GUILLOT, G. W. MILLER;
Emory Univ., Atlanta, GA

Abstract: Monoamines play a key role in regulating affective behavior. We and others have shown that mice with decreased vesicular monoamine transporter 2 (VMAT2) display decreased dopaminergic signaling and express a depressive-like phenotype (Taylor et al., J Neurosci, 29(25), 8103-8113, 2009). Furthermore, we show that mice containing a BAC-mediated overexpression of VMAT2 (Slc18a2) display enhanced levels of basal dopamine and dopaminergic transmission and exhibit significantly improved outcomes in tests of depressive- and anxiety-like behavior. This resilience to depressive-like behavior is gene-dose dependent: fewer copies of the VMAT2 gene result in a depressive affective state while multiple copies of the VMAT2 gene result in a positive affective state. This continuum of phenotypes seen in VMAT2 under- and over- expressing mice reflects the variation in human emotional states from depression to optimism. In order to further characterize the effects of VMAT2 levels in mice, we designed a novel cognitive bias test. Cognitive bias tests measure the tendency to interpret ambiguous stimuli more ‘positively’ or more ‘negatively.’ Our novel test for mice utilizes a modified t-maze protocol. Mice were trained with two distinct auditory cues predicting either food availability in one arm (positive reinforcement) or an aversive stimulus from which there

was shelter in the opposite arm (negative reinforcement). Cognitive bias is determined by playing intermediate tones and investigating whether the mouse interprets the ambiguous tone as a negative or positive cue. In this way, the reduced depressive- and anxiety-like behavior in VMAT-overexpressing mice, as evidenced by reduced immobility time in a forced swim test (21.7%) and fewer marbles buried in a marble burying assay (38.7%), could be further elucidated using a novel cognitive bias test.

Disclosures: **R.A. Cliburn:** None. **K.M. Lohr:** None. **T.S. Guillot:** None. **G.W. Miller:** None.

Poster

089. Appetitive and Incentive Learning and Memory I

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Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 89.09/QQ3

Topic: F.02. Animal Cognition and Behavior

Support: CNPq

FAPESP

Title: The reinforcement magnitude of flavored stimulus interferes with omission effects in rats

Authors: ***J. O. BUENO**, D. M. JUDICE-DAHER, H. G. DELIBERATO;
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Abstract: Reinforcement omission effects (ROEs) have been interpreted as behavioral transient facilitation after nonreinforcement induced by primary frustration, and/or behavioral transient inhibition after reinforcement induced by demotivation or temporal control. According to frustration theory, the size of the ROEs depend directly on the reinforcement magnitude: the behavioral facilitation after the reinforcement omission of larger magnitude should be greater than that observed after the reinforcement omission of smaller magnitude. However, studies involving operant paradigms have presented difficulty to demonstrate this relationship. Thus, the present study aimed to clarify the relationship between reinforcement magnitude and ROEs manipulating the magnitude linked to discriminative stimuli in a partial reinforcement fixed interval schedule. Rats were trained on a fixed-interval (FI) 12 s with limited hold 6 s signaled schedule in which correct responses were always followed by one of two reinforcement magnitudes (0.5 and 0.05 ml of a 0.15% saccharin solution). After acquisition of stable

performance, the training was changed from 100% to 50% reinforcement schedules. Data from acquisition training showed that there was a discriminative control during the signal, producing different response distributions depending on the reinforcement magnitude anticipated. The performance during the FI signaled by the Lm stimulus was more effective than that observed during the FI signaled by Sm stimulus. The response during FI Lm schedule was higher than during FI Sm schedule when recorded in the last seconds of FI, but smaller in the first seconds. Comparing the performance in the trials after nonreinforcement and after reinforcement, the results showed that there were ROEs: the response rates were higher after omission than after reinforcement delivery for both reinforcement magnitudes. Besides, data in the trials after nonreinforcement showed that the responding was higher after the larger reinforcement omission than the smaller one. Thus, the manipulation of reinforcement quality using the flavor of saccharin corroborates with the hypothesis that the reinforcement magnitude can operate in temporal discrimination and omission effect. But, it was not obtained any increase in the responding during trials after nonreinforcement compared with those immediately preceding ones, which did not support the behavioral facilitation approach of ROEs.

Disclosures: **J.O. Bueno:** None. **D.M. Judice-Daher:** None. **H.G. Deliberato:** None.

Poster

089. Appetitive and Incentive Learning and Memory I

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Topic: F.02. Animal Cognition and Behavior

Support: DGAPA-PAPIIT Grant IN209911

CONACyT Grant 152208

Title: Differential involvement of dopaminergic and beta-adrenergic receptors in the medial prefrontal cortex in latent inhibition of conditioned taste aversion after chronic consumption of sugar

Authors: ***S. CAYNAS**, G. RODRÍGUEZ-GARCÍA, M. I. MIRANDA;
Univ. Nacional Autonoma de Mexico - Inst. de Neurobiologia, Querétaro, México, Mexico

Abstract: Evidence suggests that the medial prefrontal cortex (mPFC) is a structure through which the taste system interacts with the reward and feeding system. For example, lesions,

catecholamine denervation and c-Fos studies have clearly established a role of the mPFC during conditioned taste aversion (CTA) acquisition, retrieval and extinction, and that acute sucrose intake increases extracellular levels of dopamine and noradrenaline in the mPFC. Recently, we have demonstrated significant changes in latent inhibition of CTA and a differential new aversive learning after chronic exposure to sugar. Accordingly, we evaluated if consumption of sugar solution during 14 days alters dopamine and noradrenaline systems in the mPFC, and if dopamine and noradrenaline activity may account for the increase in latent inhibition of CTA. Therefore, male rats were exposed during 14 days to 10% sucrose solution as the only liquid consumed ad libitum. After this chronic exposition, taste preference was measured and mPFC bilateral infusions of dopaminergic and beta-adrenergic receptors agonist and antagonist were made before CTA acquisition. Blockade of dopaminergic receptors and activation of beta-adrenergic receptors in the mPFC cortex prevented appetitive re-learning after chronic consumption of sugar, while the opposite treatment increases latent inhibition of CTA after acute consumption of sugar. These results suggest that, after chronic sugar exposure, the dopaminergic and beta-adrenergic receptors in the mPFC have a differential involvement during new aversive learning and re-learning of appetitive taste learning.

Disclosures: **S. Caynas:** None. **G. Rodríguez-García:** None. **M.I. Miranda:** None.

Poster

089. Appetitive and Incentive Learning and Memory I

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 89.11/QQ5

Topic: F.02. Animal Cognition and Behavior

Title: Sex differences in conditioned orienting behavior and related phenotypes

Authors: S. M. LEWIS[1], M. E. OLSHAVSKY[1], E. S. SMITH[1], *H. J. LEE[2];
[1]Univ. of Texas at Austin, Austin, TX; [2]Psychology Dept, Univ. Texas, Austin, AUSTIN, TX

Abstract: Repeated pairings of light cues (Conditioned Stimulus, CS) followed by food pellets (unconditioned stimulus, US) in appetitive training provokes high conditioned orienting/rearing responses to the light in some male rats (Orienters) and less so in others (Non-Orienters). Our lab has shown that male Orienters tend to make more risky and impulsive choices in relation to male Non-Orienters, be more distracted in an attentional task, and to display more 50 kHz ultrasonic vocalization (USV) in response to amphetamine. Since this line of research has not yet been expanded to females, we investigated conditioned orienting behavior and related phenotypes in

female rats. We used intact females and ovariectomized females, either with estradiol replacements (OVX-E) or cholesterol control (OVX-C), alongside intact male rats. The rats received Pavlovian appetitive conditioning in which light presentation was followed by a grain pellet delivery. Then, they were tested in a dark-light open field (to measure baseline activity and preference for a novel/risky area), in an attentional shifting task, and for 50 kHz USV response to amphetamine. In appetitive training, all the female rats showed higher conditioned orienting compared to the male rats, but OVX-E females showed the highest levels of conditioned orienting behavior. During the dark-light open field test, the female rats were generally quicker to leave the dark and familiar area to enter the brightly lit and novel area compared to the male rats with OVX-E group being the quickest to enter the bright and novel area. In the attentional shifting task, all the groups showed comparable performance except OVX-E group that showed significant impairment. When the rats were injected with 2 mg/kg amphetamine, intact male and female rats displayed comparable 50 kHz USV while OVX-C and OVX-E groups showed lower levels of USV with OVX-E group showing the lowest level. Our study shows sex differences in conditioned orienting behavior and suggests related phenotypes might be partly influenced by different ovarian hormones.

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Poster

089. Appetitive and Incentive Learning and Memory I

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Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant R01DA027688

NSF Grant IOS0922075

Title: Inhibiting ventral pallidum with DREADDs impairs sign-tracking in rats

Authors: *S. E. CHANG, T. P. TODD, D. J. BUCCI, K. S. SMITH;
Dartmouth Col., Hanover, NH

Abstract: An environmental cue that predicts a rewarding event can capture attention and trigger pursuit of the cue itself due to the cue acquiring incentive salience, a process in which the cue acquires incentive value due to its association with the rewarding event (Berridge, 2004). The

phenomenon of autoshaping has been used to study the neural circuitry underlying the attribution of incentive salience, in which rats will approach, contact, and attempt to "consume" a lever conditioned stimulus (CS) that is paired with the delivery of a food unconditioned stimulus (US) upon retraction (sign-tracking). Previous studies have shown that the nucleus accumbens and its dopaminergic inputs are critical for the acquisition and expression of sign-tracking (e.g., Flagel et al., 2011; Saunders & Robinson, 2012). A region with bidirectional connections with accumbens is the ventral pallidum (VP), which has been shown to mediate reward processes including taste hedonics and salt appetite (Smith et al., 2009). The present study investigated the effects of inhibiting VP on sign-tracking using Designer Receptors Exclusively Activated by Designer Drugs (DREADDs), a technology that allows for repeated activation of engineered receptors by systemic injection of the otherwise inert ligand clozapine N-oxide (CNO). DREADDs are a particularly useful technology for repeated temporary inactivation of VP because lesions of VP abolish the motivation to pursue pleasant rewards (Cromwell & Berridge, 1993), and repeated inactivation using intracranial injections cause unwanted physical damage. Rats received surgery in which the hM4Di receptor was inserted into the VP through viral vectors. Rats underwent 12 days of training in which each session consisted of 25 CS+ and 25 CS- trials. CS+ trials consisted of insertion of one lever for 10 s that resulted in the delivery of a food US (2 grain pellets) upon retraction, while CS- trials consisted of insertion of another lever that was followed by nothing. In each session, the VP was inactivated in half of the rats with systemic injections of CNO, while the other half of rats received injections of distilled water (Control). Initially, both CNO and Control rats acquired sign-tracking at comparable rates, pressing more to the CS+ than the CS-. However, Control rats showed approximately double the levels of sign-tracking compared to CNO rats as training progressed, particularly over the last 4 days. In contrast, CNO and Control rats showed no differences in food cup behavior. These results demonstrate the utility of chemogenetics technology for studying mechanisms of incentive value and provide evidence that the VP is a critical region mediating this process.

Disclosures: **S.E. Chang:** None. **T.P. Todd:** None. **D.J. Bucci:** None. **K.S. Smith:** None.

Poster

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Topic: F.02. Animal Cognition and Behavior

Support: NIH 5R01DA034178-02

NSF CBET-1263785

2013 Alfred P. Sloan Research Fellowship

2013 Harvey L. Karp Discover Award

Title: Decoding frontostriatal network activity during occasion setting

Authors: *J. L. SHOBE[1], L. D. CLAAR[2], K. I. BAKHURIN[2], S. C. MASMANIDIS[2]; [1]UCLA, IRVINE, CA; [2]UCLA, Los Angeles, CA

Abstract: The frontostriatal circuit is important for learning specific cue-reward associations in order to select among behaviorally appropriate responses. One prominent hypothesis suggests that neuronal activity within cortical and striatal structures make distinct contributions with respect to action selection and action value. However, a network-level understanding of how this contributes to these distinct components of learning remains unclear. As a general approach to address these questions, our laboratory has pioneered the development of large-scale multi-unit recording systems to sample spiking data from 512 channels simultaneously in multiple brain regions in awake, behaving mice. Animals are trained in a head-fixed preparation and perform appetitive Pavlovian olfactory discrimination tasks. Following a common two-odor discrimination task with an odor1-reward pairing (CS+) and an odor2 alone presentation (CS-), we observe distinct task modulated striatal activity during (i) CS onset, (ii) CS+ vs CS- epochs, and (iii) reward delivery, suggesting a robust encoding of these trial features (Bakhurin et al SFN 2013). However, these findings do not capture a critical hallmark of cognitive learning which requires that an animal understands the relationship between different cues (stimulus-stimulus interactions; S-S) to successfully perform the task. As an entry point to investigate this, we have utilized a feature negative discrimination task (occasion blocking) where a mouse learns that the presentation of odor1 alone is rewarded (CS+) but on a trial where odor1 (target) is preceded by odor2 (blocker) it is unrewarded (blocker-->target). Thus the mouse must perceive the relationship between the cues to resolve stimulus ambiguity (ie. odor1 only predicts reward 50% of the time). After several days of training, we found that mice are able to successfully discriminate between these trial types with near 100% accuracy (ie correctly respond on CS+ trials and correctly withhold on blocker-->target trials). We have also made a number of interesting preliminary physiological observations that fall outside the scope of the earlier hypothesis (i) many cells in both structures responded to both CS+ and the blocker cue suggesting that they encode for salience rather than action value and/or action selection and (ii) the blocker dramatically blunts neuronal activity during target presentations suggesting that it may cause a global downshift in task-modulated activity. We are currently analyzing the circuit and systems level connectivity between these brain regions to determine if this in combination with their spiking pattern is predictive of trial type.

Disclosures: **J.L. Shobe:** None. **L.D. Claar:** None. **K.I. Bakhurin:** None. **S.C. Masmanidis:** None.

Poster

089. Appetitive and Incentive Learning and Memory I

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Topic: F.02. Animal Cognition and Behavior

Support: NIDA-IRP

Title: Signaling prediction for size versus value of rewards in rodent orbitofrontal cortex during Pavlovian unblocking

Authors: *N. LOPATINA[1], M. A. MCDANNALD[2], B. F. SADACCA[2], G. SCHOENBAUM[2]; [1]Dept. of Anat. and Neurobio., Univ. of Maryland Sch. of Med., Baltimore, MD; [2]Natl. Inst. on Drug Abuse, Intramural Res. Program, Baltimore, MD

Abstract: Modern reinforcement learning models and learning theories distinguish at least two different forms of reward prediction: specific features or properties of rewards, and value or general utility of rewards. Formation of specific goals requires intact prediction of reward features. Maximizing the value of these goals requires intact prediction of reward value. While reward size and reward value are inextricably linked, the changes of neural activity of individual units in response to rewards of different sizes can shed light on whether individual neurons' encoding reflects reward size or value. The current study examined changes in orbitofrontal cortex (OFC) neural activity using single-unit electrophysiological recording, measuring activity during a novel Pavlovian unblocking procedure that assesses excitatory and inhibitory cue learning driven by upshifts or downshifts in expected reward size. We have recorded hundreds of OFC neurons during the task. Preliminary analyses show that cue-related activity is regulated by the unblocking paradigm used, with findings of differential firing to blocked, size-downshift and size-upshift cues in individual neurons. A comprehensive analysis of these neural data will be presented.

Disclosures: **N. Lopatina:** None. **M.A. McDannald:** None. **B.F. Sadacca:** None. **G. Schoenbaum:** None.

Poster

089. Appetitive and Incentive Learning and Memory I

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Topic: F.02. Animal Cognition and Behavior

Support: CNPq

CAPES

Fundação Araucária

Title: Selective loss of the rat nigral DA neurons needed for approach response to appetitive, but not aversive, stimuli and those needed for aversive, but not appetitive associative learning

Authors: *C. DA CUNHA[1], B. F. C. DE LIMA[1], A. GÓMEZ-A[1], S. L. BOSCHEN[1], J. K. BARBIERO[1], A. M. FIORENZA[1], D. L. ROBINSON[2], C. D. BLAHA[3];
[1]Univ. Federal do Parana, Curitiba, Brazil; [2]Dept. of Psychiatry and Ctr. for Alcohol Studies, Univ. of North Carolina, Univ. of North Carolina, Chapel Hill, NC; [3]Univ. of Memphis, Memphis, TN

Abstract: It is controversial whether different populations of midbrain dopamine (DA) neurons play a role in appetitive and aversive associative learning. Here, we show that partial bilateral lesions of DA neurons in the rat substantia nigra pars compacta (SNc) induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP, 200 ug/side) or 6-hydroxydopamine (6-OHDA, 3 ug/side) impaired conditioned place avoidance (CPA) for an environment paired with quinine pellets without affecting conditioned place preference (CPP) for an environment paired with sucrose pellets. In addition, approach and consummatory responses to sucrose, but not to quinine, pellets were affected by the 6-OHDA, but not MPTP, treatment. Importantly, after tasting sucrose or quinine the MPTP and 6-OHDA rats presented facial expressions that were not different compared to the controls. Furthermore, no motor or emotional alteration was observed when the lesioned rats were tested in an open field. Reductions in issue content of DA in the striatum were similar in the MPTP and 6-OHDA lesioned rats, as shown by high performance liquid chromatography with electrochemical detection. Immunohistochemistry for tyrosine hydroxylase showed partial loss of DA neurons in the SNc, but not in the ventral tegmental area; no significant difference between MPTP and 6-OHDA rats or between anterior and posterior areas of the SNc was observed. Overall, these data suggest that: (i) there are different populations of neurons distributed in the SNc that encode incentive salience value and hedonic value for appetitive and aversive stimuli; (ii) such neurons differently impact associative learning involving rewarding and aversive stimuli; (iii) these different populations of DA neurons have different sensitivity to the toxic effects of MPTP and 6-OHDA, which makes them good tools to tease the DA populations apart. This finding has important implications in basic and applied

research of Parkinson´s disease, addiction, learning and memory, and other functions and diseases where striatal DA is implicated.

Disclosures: **C. Da Cunha:** None. **B.F.C. de Lima:** None. **A. Gómez-A:** None. **S.L. Boschen:** None. **J.K. Barbiero:** None. **A.M. Fiorenza:** None. **D.L. Robinson:** None. **C.D. Blaha:** None.

Poster

089. Appetitive and Incentive Learning and Memory I

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Program#/Poster#: 89.16/QQ10

Topic: F.02. Animal Cognition and Behavior

Support: University of Missouri Research Board

Title: Effects of phenotype and exercise on diet preference in a rat model

Authors: ***H. JOHNS**[1], K. PARKER[2], M. MCCABE[3], F. BOOTH[1], M. J. WILL[1];
[1]Univ. of Missouri, Columbia, MO; [2]Univ. of Minnesota, Minneapolis, MN; [3]Univ. of Vermont, Burlington, VT

Abstract: The obesity epidemic and associated diseases are a direct result of both diet choice and inactivity, as over-consumption of palatable foods and a sedentary lifestyle invariably precede the onset of weight gain. Our laboratory uses opioid activation of the nucleus accumbens to model hedonically-driven feeding of energy-dense palatable food in the sated condition, a behavior that typically precedes the onset of obesity. However, it is not clear how dietary choice and physical inactivity interact, and to what extent. The current study used two novel rat phenotypes, developed by selectively breeding for either high- or low-levels of voluntary running (HVR and LVR, respectively). As of the 10th generation, HVRs ran approximately 10-fold greater daily distances compared to the LVR rats, thus providing a unique model to examine cross-generational influence of inactivity on diet preference and overall feeding behavior. The current study sought to investigate the influence of voluntary exercise or forced sedentary conditions in HVR and LVR rats using an opioid feeding model choice task between either a low-fat/high-carb or high-fat/low carb diet. LVRs demonstrated a strong preference for the low-fat/high carb diet at baseline; this preference became further pronounced in a dose-dependent fashion following intra-Acb infusions of the mu opioid agonist DAMGO. HVR rats did not demonstrate a clear preference for either diet at baseline; however, their consumption of the high-fat/low carb diet increased dose-dependently following DAMGO infusions. Analysis of

low-fat/high-carb consumption revealed interactions between phenotype and baseline preference, as well as between phenotype, exercise condition, and DAMGO dose, suggesting differential opioid signaling across phenotype-by-exercise conditions.

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Poster

089. Appetitive and Incentive Learning and Memory I

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Program#/Poster#: 89.17/QQ11

Topic: F.02. Animal Cognition and Behavior

Title: Effects of thiamine-deficiency on flavor consumption after flavor-thiamine pairings

Authors: D. KLAKOTSKAIA[1], R. RICHARDSON[1], M. MCCABE[1], E. WOODALL[1], C. WEINSTEIN[1], *T. SCHACHTMAN[2];
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Abstract: Research has demonstrated that a specific flavor can come to be preferred if it is associated with a needed nutrient (e.g., thiamine). This study sought to demonstrate such a preference and show the dependence of this effect on the timing of the nutritional need. Flavor-thiamine pairings were administered to Sprague-Dawley rats, and then they were tested for flavor consumption. Flavor preference has been shown to be independent of nutritional need at the time of testing.

Disclosures: **D. Klakotskaia:** None. **T. Schachtman:** None. **R. Richardson:** None. **M. McCabe:** None. **E. Woodall:** None. **C. Weinstein:** None.

Poster

089. Appetitive and Incentive Learning and Memory I

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 89.18/QQ12

Topic: F.02. Animal Cognition and Behavior

Title: Chronic ketamine pretreatment enhances the effects of pramipexole on contrafreeloading for water in rats

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Abstract: INTRODUCTION Chronic treatment with the D3 agonist pramipexole (PPX) potentiates contrafreeloading for water (CFL), consisting in a biased selection of a contingent over a noncontingent access to water. CFL arises from an impairment of choice shifting between different options in conditions where behavioral cost is unbalanced. We provided preliminary evidence that the inability to switch to a more economical strategy depends on a loss of hippocampal control over behavior, and thus it could be sensitive to glutamatergic manipulation. **OBJECTIVES** We tested the effects of the full NMDA antagonist ketamine and of D-cycloserine (DCS), a partial agonist at the glycine binding site on NMDA receptor, on PPX induced CFL. **MATHERIALS AND METHODS** Rats were trained under a fixed ratio 3 (FR3) schedule of reinforcement for water. On days 1-6 (operant phase), water was only available through lever pressing while on days 7 to 14 (choice phase), choice between operant and free access was provided. In EXP 1, 15 mg/kg of ketamine or saline where intraperitoneally injected 30 minutes before PPX treatment (0.5 mg/kg). In EXP 2, DCS (15 mg/kg) or saline where administered in combination with PPX. **RESULTS** During the operant phase, PPX did not affect responding whereas it decreased drinking, resulting in a significant decreased in percentage of water consumed over that gained. Neither ketamine nor DCS modify the effects of PPX on contingent drinking. In condition of choice, PPX produced a significant preference for the contingent over the non-contingent access to water. Ketamine further boosted PPX effect on CFL by increasing the amount of water consumed through lever pressing. DCS was ineffective against PPX. Ketamine or DCS given alone did not induce any significant effect during both the operant and the choice phase. **DISCUSSION** The NMDA receptor antagonist ketamine worsens the effects of D3 stimulation on CFL. This finding suggests that blocking the NMDA component of glutamatergic transmission adds a further layer of dysfunction in the expression of PPX-driven inflexible behavior. However, facilitating NMDA transmission through DCS fails to restore adaptive drinking during PPX treatment. In conclusion, although NMDA inhibition is not necessary nor sufficient to elicit inflexible drinking, glutamatergic transmission is likely to be a target of PPX effects on compulsive behavior in CFL.

Disclosures: **C. Schepisi:** None. **S. Doronzio:** None. **P. Nencini:** None.

Poster

089. Appetitive and Incentive Learning and Memory I

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Topic: F.02. Animal Cognition and Behavior

Support: R01DA031852

HL28481

F31DA027309

Title: Inbred mouse genetics approach yields novel genetic and genomic determinants of variation in appetitive reward-driven instrumental learning and motivation

Authors: *A. S. JAMES[1], B. M. SAFAIE[1], A. BAUTISTA[1], A. J. LUSIS[2], J. D. JENTSCH[3]; [1]Psychology, [2]Medicine, Cardiology, Human Genetics, Microbiology, Immunol. & Mol. Genet., [3]Psychology, Psychiatry & Biobehavioral Sci., UCLA, Los Angeles, CA

Abstract: Learning to exploit the environment in the pursuit of desired outcomes is a central to a wide array of behaviors that humans and other animals exhibit to achieve internal goals. In its extreme forms, variation in rates of learning about rewards, and motivation to seek them, may underlie psychiatric disorders (e.g., substance dependence), while in its normative forms, could influence aspects human behaviors that and mold our individual temperament and traits. However, little is known about the genetic markers that modulate these individual differences in reward-related behaviors. Therefore, we sought to ascertain novel genetic loci in control of variation in learning to perform an instrumental response to produce an appetitive outcome, and in motivation to perform this reward-seeking behavior. We performed a genome-wide association study of instrumental learning using the Hybrid Mouse Diversity Panel (HMDP) in concert with FaST-LMM (Lippert et al. 2011), which together offer substantial improvements in the domains of power, resolution, and type I error rate relative to traditional genetics approaches. We assessed spontaneous acquisition and performance of instrumental behavior in 70 strains (n = 2- 10 per strain) during three 8-hour daily sessions in which lever responses produced sucrose rewards under a schedule of reinforcement that gradually advanced from FR1 to VR5. Quantitative trait loci (QTL) for lever pressing behavior were identified on chromosomes 3, 11, and 19. We then used expression-QTL data and polymorphism effect prediction algorithms positional candidates. In conjunction, we applied weighted correlational techniques to gene

expression in the striatum of HMDP mice (Park et al. 2011) - a brain region critical for instrumental performance. In doing so, we were able to identify networks of highly co-expressed genes that associate with strain-level variation in instrumental reward-seeking. Ontology analyses of these gene networks and their highly intra-connected 'hub' genes, and analysis of the position of QTL candidates within these networks, further refined our QTL gene list. Importantly, because networks segregate a coherent set of signaling pathways, intra- and extra-cellular functions, and/or protein/cell interactions, we were able to uncover novel relationships between groups of RNA and protein regulatory mechanisms and reward-seeking behaviors. These data, therefore, can facilitate hypothesis generation for new neurobiological research of reward-seeking behaviors, while also guiding a fundamentally data-driven prioritization approach to performing causal studies of identified genes in the future.

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Poster

089. Appetitive and Incentive Learning and Memory I

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Topic: F.02. Animal Cognition and Behavior

Title: Isoflurane/nitrous oxide anesthesia during the first week of life can cause long-lasting cognitive deficits in rhesus monkeys

Authors: *M. G. PAULE;
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Abstract: Isoflurane/nitrous oxide anesthesia during the first week of life can cause long-lasting cognitive deficits in rhesus monkeys M. G. Paule[1], M. Li[1], X. Zhang[1], S. Liu[1], J.P. Hanig[2], W. Slikker, Jr.[1] and C. Wang[1] [1]Division of Neurotoxicology, National Center for Toxicological Research, FDA, Jefferson, Arkansas, and [2]Center for Drug Evaluation and Research, FDA Previously our laboratory has shown that ketamine exposure (24 hours of clinically relevant anesthesia) causes significant increases in neuronal cell death and seemingly permanent cognitive deficits in rhesus monkeys. In the present study, eight monkeys were exposed on PND 5 or 6 to isoflurane (1%)/nitrous oxide (70%) anesthesia (ISO/N_2O; maintenance of a light surgical plane) for 8 hrs: eight control animals were unexposed. At 7 months of age all animals were weaned and began training to perform a series of cognitive function tasks as part of the

National Center for Toxicological Research (NCTR) Operant Test Battery (OTB). The OTB tasks used here included those for assessing aspects of learning, motivation, color discrimination, and short-term memory. Subjects responded for banana-flavored food pellets by pressing response levers and press-plates during daily (M-F) test sessions (50 min) and were assigned training scores based upon their individual task performance. Beginning as early as 8 months of age_and continuing for at least the following year--control animals earned more reinforcers in a task assessing appetitive motivation than animals exposed to ISO/N_2O (OTB assessments are continuing). At about 14 months of age, controls also began outperforming ISO/N_2O exposed animals in the OTB learning and visual discrimination tasks: exposed animals responded more slowly in the color discrimination task and completed less of the learning task--, responding more slowly and less accurately--and these effects have continued until the present (for at least six months). Performance in the short-term memory task is no different between the groups. These long-term cognitive impairments seen after an 8 hour exposure to ISO/N_2O, while slightly different from those noted in our previous ketamine studies, provide additional evidence that a single 8-hr episode of general anesthesia, occurring during a sensitive period of brain development, results in very long-lasting deficits in brain function in primates. Supported by CDER/FDA and NCTR/FDA.

Disclosures: M.G. Paule: None.

Poster

089. Appetitive and Incentive Learning and Memory I

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Topic: F.02. Animal Cognition and Behavior

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Rita Allen Foundation

Klingenstein Fund

Title: OpenMaze: A new resource for open source hardware and software for rodent behavioral neuroscience

Authors: C. O. LACEFIELD, *C. RODGERS, R. M. BRUNO;
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Abstract: Neuroscientists have always needed to be creative in the construction of novel experimental apparatus. In the present day, however, most systems for behavioral experiments are costly and require extensive engineering and programming skills. Recently, a rich landscape of open-source hardware and software tools, requiring only minimal background in electronics and computing, has emerged. Devices such as the Arduino and the Raspberry Pi, essentially miniature computers, have been adopted by immense communities of users who maintain online forums dedicated to their operation. In addition, many devices have been designed to interface with these platforms, including low-cost sensors and motors. We have launched the OpenMaze.org website as a flexible platform to support and guide the easy construction of a diverse array of experimental apparatus. On the hardware side, the OpenMaze.org website contains tips and techniques for the construction of interactive behavioral apparatus. For instance, we have designed a series of attached prototyping boards, or "shields", for the Arduino microcontroller system that contain simple circuitry necessary for presenting simple sensory stimuli, controlling motors to alter the animal's environment, and reading out behavioral responses. These animal interaction boards are complemented by serial communication with more powerful processors such as the Raspberry Pi, which allow computer vision applications such as animal tracking, as well as more complex visual and auditory stimuli. On the software side, OpenMaze.org contains a repository of fully functional code for the Arduino, as well as additional applications in the Processing and Python programming languages. This code accommodates a variety of experimental paradigms, such as two-alternative forced choice, but can also be adapted to other tasks. Our current focus is on head-fixed mouse behavior, but the principles apply to other animal and even human models. We hope that the OpenMaze website and platform will help neuroscientists, even those with minimal technical background, take full advantage of the current rich landscape of open source hardware and software for the study of animal behavior. User contributions are highly encouraged.

Disclosures: **C.O. Lacefield:** None. **C. Rodgers:** None. **R.M. Bruno:** None.

Poster

090. Memory Consolidation and Reconsolidation

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1R01DA034582

Title: Learning and memory impairment in HIV-1-transgenic (Tg)26 mice: Potential regulation by Toll-Like receptors

Authors: *S. MOIDUNNY[1], M. A. BENNEYWORTH[1], T. MACHEDA[2], S. METZGER[2], U. SHARMA[2], J. MEINTS[2], M. J. THOMAS[2], S. ROY[2];
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Abstract: Toll-like receptors (TLRs) are a family of innate immune system receptors that respond to pathogen-associated and damage-associated molecular patterns. Recent evidence suggests that TLRs play a significant role in normal development of the central nervous system (CNS), and regulate neuroplasticity and behavior. We hypothesized that impaired memory associated with HIV-1 infection of the CNS is regulated by altered TLR expression. 18 weeks old littermate wild-type (C57BL6/J) and transgenic (Tg)26 mice, which express all viral genes that are known to mediate neuropathogenesis associated with HIV-1 infection, were assessed for altered behavior using Barnes maze test. We provide the first evidence that both male and female Tg26 mice show severe impairment in learning and spatial reference memory, compared to wild-type mice. Both Tg26 and wild-type males showed similar locomotor activity, whereas Tg26 females showed increased locomotor activity than the wild-type. Furthermore, preliminary data suggests an altered TLR expression in Tg26 mice brain, compared to wild-type. A detailed immunohistochemical, biochemical and molecular analyses would help us to understand the potential role of TLRs in impaired memory and synaptic plasticity associated with HIV-1 infection of the CNS.

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Poster

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Topic: F.02. Animal Cognition and Behavior

Support: R00MH093459 to NCT

Title: Sex differences in molecular mechanisms underlying consolidation of memory

Authors: *A. A. SCHMELING, I. C. SPEIRS, E. J. DONZIS, L. M. TURNBULL, N. C. TRONSON;
Univ. of Michigan, Ann Arbor, MI

Abstract: Molecular mechanisms of context fear conditioning have been well studied in male animals, however females have been largely overlooked. There is some evidence that males and females differ in both learning about context and context fear conditioning, with males exhibiting stronger memory than females. In contrast, other studies have observed no sex differences in fear conditioning. The few studies examining mechanisms of fear conditioning in both males and females have also demonstrated striking sex differences. Disruption of GluR1 and calcium calmodulin (CaM) signaling impair context fear conditioning in males but not females. In addition, activation of extracellular signal regulated kinase (ERK)/CREB signaling after fear conditioning differs between males and females. Here, we extended these findings by determining sex differences in multiple signaling cascades known to be important for consolidation of context fear conditioning in males. Female and male mice were conditioned across a range of shock intensities and either tested for freezing the following day, or brains were dissected for molecular analyses. To identify patterns of kinase activity in consolidation of fear-associated memory we used western blot analysis and immunohistochemistry to determine activation of ERK, AKT, mTOR, and CREB in males and females. Pinpointing sex differences in patterns of intracellular signaling will allow us to better understand how fear-associated memories are consolidated and modulated in females. These findings suggest the need for sex-specific strategies for treatment and prevention of memory-related disorders such as post-traumatic stress disorder.

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Poster

090. Memory Consolidation and Reconsolidation

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Topic: F.02. Animal Cognition and Behavior

Title: Single and double alternation in the adult female C57BL/6 mouse: Non-knockout versus estrogen receptor knockout

Authors: *J. D. ROWAN[1], M. K. MCCARTY[2], A. BAJRACHARYA[3], H. BOETTGER-TONG[4];
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Abstract: Studies of sequence learning dating back to the 1930s have repeatedly found that a single alternation pattern (SA) is more difficult to learn than a double alternation pattern (DA). Because these SA/DA task uses the same basic procedure while manipulating the complexity of the patterns, it could be an ideal for comparing deficits in cognitive function. Recent research has found that male rats learn structured sequences faster than females, indicating that estrogen may play an important role in rule learning. The goal of this study is to compare female C57BC/6 mice, with and without the gene knock (KO)-out for sensitivity of estrogen receptors, on acquisition of SA and DA patterns in an attempt to determine whether estrogen plays a role in rule learning. The subjects were 9 naïve female C57BC/6 mice (5 non-KO and 4 KO). The test chamber used was rectangular in shape (32.5cm x 23cm x 25.5cm) and was composed of clear Plexiglass walls and a floor of wire mesh. One wall of the chamber was equipped with 2 infrared emitter-detectors with a water delivery system. Before training, all mice required shaping for 3 days during which they repeated 120 nosepokes per day in only one of the locations. An indicator light in each nosepoke recess indicated the start of each trial. At the beginning of the experiment, all lights were illuminated. A correct response resulted in reinforcement with approximately 0.2 mL of water. All of the lights were then extinguished for 1 s and then again illuminated. When an incorrect response was made, all the lights were turned off except the correct one. After a correct response was made, the mouse was then reinforced with water. This procedure assured that the mice received feedback regarding the correct response on each trial. Each day, for 14 days, mice performed 10 repetitions of the SA pattern for a total of 160 correct responses per day. Using the same procedure, the same 2 groups of mice were then transferred to the DA pattern for an additional 14 days of acquisition. Analysis of revealed no differences in acquisition rates for the SA pattern, {$F(1, 13) = 1.45$, $p = 0.268$} and both groups were performing at below chance levels by Day 3 of the experiment. Interestingly, following the transfer to the DA patterns, the C57BC/6 Normal mice performed significantly fewer errors than the C57BC/6 KO group {$F(1, 13) = 8.8262$, $p = 0.035$}. On Day 14, the non-KO C57BC/6 mice generated 19.5% errors compared to the 42.5% errors of the KO group. These finding support the idea that estrogen plays an important role in learning patterned sequences. They also demonstrate the ability of the SA/DA task to identify deficits in cognitive functions such as simple rule learning.

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Poster

090. Memory Consolidation and Reconsolidation

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Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant R00MH093459

Title: Dissociation of mechanisms underlying context fear conditioning and inhibitory avoidance

Authors: L. M. TURNBULL, I. C. SPEIRS, A. A. SCHMELING, N. NEVÁREZ, E. J. DONZIS, D. M. DUBOIS, *N. C. TRONSON;
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Abstract: Memory retrieval triggers a variety of active processes including reconsolidation to update or re-stabilize the memory, and extinction or learning about new stimulus contingencies. The precise mechanisms that mediate whether extinction or reconsolidation is engaged after retrieval remain unclear. Previous studies have demonstrated that contextual fear memory (CFC) but not inhibitory avoidance (IA) is sensitive to disruption of memory reconsolidation by propranolol. Given that both CFC and IA are examples of Pavlovian fear conditioning protocols with context acting as the conditioned stimulus, the difference in memory reconsolidation is somewhat surprising. In this study, we aimed to use this differential susceptibility to understand what mechanisms may trigger memory reconsolidation. To do this, we delineated molecular mechanisms after training and retrieval of several different fear conditioning protocols. C57Bl6 mice were trained on CFC or IA and brains were dissected post training or retrieval and immunoblotting and immunohistochemistry were used to examine the difference in circuits and molecular mechanisms activated after memory retrieval. We demonstrate that CFC and IA differentially activate ERK, EGR1, and pCREB activity, and that these differences occur across hippocampus, Anterior Cingulate Cortex and Nucleus Accumbens. These differences in circuit and mechanism active during retrieval may be key to understanding requirements for initiation of reconsolidation. Furthermore, these results suggest that what animals learn and what information is retrieved upon re-exposure to a conditioned context differs despite the similarity between fear conditioning protocols.

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Poster

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Title: HDAC3 in the dorsal hippocampus negatively regulates long-term memory consolidation for context fear

Authors: *J. L. KWAPIS, Y. ALAGHBAND, D. P. MATHEOS, R. M. BARRETT, A. SYLVAIN, A. E. CARL, M. A. WOOD;
Neurobio. and Behavior and Ctr. for the Neurobio. of Learning & Memory, Univ. of California, Irvine, Irvine, CA

Abstract: Understanding how aversive events are consolidated into lasting fear memory is a key step in treating anxiety-based disorders, including post-traumatic stress disorder. In rodents, aversive memory is often studied using Pavlovian fear conditioning, in which an initially neutral conditional stimulus (CS), like a tone or context, is paired with an aversive unconditional stimulus (UCS), like a footshock. Although it is clear that gene expression in both the amygdala and hippocampus is required for the formation of long-term context fear memory, it is less clear how gene expression is coordinated by the cell to produce the robust and persistent memory characteristic of fear learning. Epigenetic mechanisms (changes in gene expression that occur through alterations in chromatin structure) may produce lasting changes in cellular function that give rise to enduring memory for fear conditioning. One major epigenetic mechanism important for memory is histone acetylation, in which acetyl groups are added or removed from histone tails by histone acetyltransferases (HATs) or deacetylases (HDACs), respectively. Increasing histone acetylation by blocking HDAC activity generally enhances both gene expression and long-term memory. HDAC3, the most highly expressed Class I HDAC in the brain, appears to be

particularly important for long-term memory formation. Blocking HDAC3 produces persistent object location memory following subthreshold training. Here, we tested whether hippocampal HDAC3 also negatively regulates fear memory formation. First, we demonstrated that cFos expression rapidly and robustly increases in both the dorsal and ventral hippocampus after fear conditioning, confirming that context fear learning induces new gene expression throughout the hippocampus. Next, we identified subthreshold conditioning parameters that are normally insufficient for long-term context fear memory formation. Finally, we tested whether HDAC3 inhibition could transform this subthreshold context fear into lasting long-term memory by virally overexpressing a dominant negative form of HDAC3 in the dorsal hippocampus. Consistent with our hypothesis, blocking HDAC3 in the hippocampus transformed subthreshold conditioning into long-term memory for context fear. This is consistent with the idea that HDAC3 negatively regulates memory formation for aversive events. We are currently working to understand how HDAC3 regulates gene expression dynamics, which appears to be key to the formation of robust and persistent memories following HDAC3 inhibition.

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Poster

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MH101491

DA025922

DA036984

DA031989

Title: Subdomain 2 of the neuron-specific chromatin remodeling subunit BAF53b is required for synaptic plasticity and memory

Authors: *A. VOGEL-CIERNIA[1], D. P. MATHEOS[1], E. KRAMAR[2], B. TRIEU[2], C. COX[2], C. MAGNAN[3], M. ZELLER[3], A. TRAN[1], A. LOPEZ[1], K. SAKATA[1], S. AZZAWI[1], R. DANG[1], R. BARRETT[4], P. BALDI[3], G. LYNCH[2], M. WOOD[1];
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Abstract: Gene expression is considered a key step for long-term memory processes. Transcription does not occur on naked DNA, but rather in the context of chromatin, the protein complex that condenses and organizes genomic DNA. The repeating unit of chromatin is called a nucleosome, and nucleosome positioning along the DNA can be altered by nucleosome remodeling complexes to regulate gene expression. Recent human exome sequencing studies have implicated polymorphic Brg1-Associated Factor (BAF) complexes (mammalian SWI/SNF chromatin remodeling complexes) in several intellectual disabilities and cognitive disorders, including autism. However, it is unclear how mutations in BAF complexes result in impaired cognitive function. Post-mitotic neurons express a neuron-specific assembly, nBAF, characterized by the neuron-specific subunit BAF53b. Mice harboring mutations of BAF53b show severe defects in long-term memory and long-lasting forms of synaptic plasticity (Vogel-Ciernia, et al., 2013), but the neuron-specific function of BAF53b is unknown. BAF53b shares 93% similarity with its non-neuronal homologue BAF53a, which is expressed in all non-neuronal cells. The most divergent region between BAF53b and BAF53a is within subdomain 2. Subdomain 2 of BAF53b has previously been shown to be critical for dendritic arborization during neuronal development (Wu, et al., 2007), hinting at a unique role for subdomain 2 in neuronal function. To examine the role of subdomain 2 of BAF53b in long-term memory formation, we generated transgenic animals that over-express a dominant negative BAF53b with a deletion of subdomain 2 (BAF53bΔSB2). BAF53bΔSB2 animals show long-term memory impairments, but normal short-term memory. Targeting BAF53b subdomain 2 also impairs maintenance of long-term potentiation and activity-dependent gene expression. These findings indicate that BAF53b, and specifically subdomain 2, confers a unique function to the nBAF complex for regulating neuronal gene expression required for long-term memory formation.

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Poster

090. Memory Consolidation and Reconsolidation

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Title: Bi-directional pharmacogenetic manipulation of the CA1 using DREADDs leads to modulation in object location memory, but not object recognition memory

Authors: *A. J. LOPEZ, A. O. WHITE, A. VOGEL-CIERNIA, M. A. WOOD;
Dept. of Neurobio & Behavior; Ctr. for the Neurobio. of Learning & Memory, Univ. of California, Irvine, Irvine, CA

Abstract: Pharmacogenetics can lead to a dramatic change in how neural circuits are studied and understood. Particularly, Designer Receptors Exclusively Activated by Designer Drug (DREADDs) are a novel tool with the potential to bi-directionally drive cellular, circuit, and, ultimately, behavioral changes. These receptors have no endogenous ligand and can only be activated with exposure to clozapine-n-oxide (CNO), which is otherwise an inert ligand. To examine how this technique can be used to manipulate the activity of specific cell populations, we used this pharmacogenetic system to test memory formation in a hippocampus-dependent task. Here, we use DREADD technology to show the explicit role of the CA1 hippocampal subfield in object location memory (OLM), but not object recognition memory (ORM) formation. Excitatory DREADD (HM3D), inhibitory DREADD (HM4D), or GFP control were expressed virally in the CA1 subfield of the hippocampus. The lab has previously shown that for both OLM and ORM tasks, a 3 minute training session is a subthreshold event for long-term memory (LTM) formation, while a 10 minute training session is sufficient for LTM formation. For activation experiments, mice were given a 3 minute training session in the OLM and ORM tasks paired with systemic CNO administration (I.P., 3 mg/kg, 0.25% DMSO, 0.9% saline). For inactivation experiments, mice were given a 10 minute training session in the OLM and ORM tasks paired with systemic CNO administration. Mice were tested for LTM 24 hours following each respective training event. Compared to control animals, HM3D mice showed dramatic increase in LTM formation in the OLM task, but not ORM task. In contrast, HM4D mice showed LTM impairments in the OLM task, but showed no such impairments in LTM formation for the ORM task. Using c-Fos as a marker for neuronal activation, RT-qPCR revealed an increase in expression in CA1 of HM3D mice, while showing a decrease in activity of HM4D mice following CNO injections. Together, these experiments show a novel way of modulating LTM

formation in behaving animals while further demonstrating hippocampal dependence in OLM but not ORM task LTM formation. Moreover, this provides evidence for DREADDs being a robust and reliable means of modulating circuit function to manipulate behavior.

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Poster

090. Memory Consolidation and Reconsolidation

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Topic: F.02. Animal Cognition and Behavior

Support: Region rhone Alpes cluster Arc2

Title: Molecular mechanisms of spatial working memory and reference memory in the hippocampus: A western blot analysis

Authors: ***N. FRAIZE**[1,2], M. HAMIEH[2], M. JOSEF[2], M. TOURET[2], P. SALIN[2], G. MALLERET[2];
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Abstract: Working Memory (WM) and Reference Memory (RM) are two processes aimed to store information. WM is a specific form of short-term memory that refers to the ability to retain information within a single trial. This information can then be stored into long-term/reference memory. RM refers to the long-term storage of information that remains constant over time, that is gradually acquired over many training sessions and is widely believed to be dependent on long term potentiation (LTP) of the synaptic efficacy. However, not all information that comes from working memory needs to be transferred into long-term memory. Insignificant data is better to be erased in order not to overload the brain with irrelevant things. It has been demonstrated that WM is very sensitive to proactive interference (PI) which is a phenomenon whereby information learned in the past interferes with the learning of more recently presented material. Consequently, forgetting this old information would be necessary to perform everyday tasks requiring WM abilities. Work from our team suggest that long term depression (LTD) of synaptic efficacy may serve to weaken previous memory traces, thus preventing them to interfere with new ones. In order to assess post-synaptic changes in receptors trafficking and signaling involved in LTP or LTD, we performed a selective WB analysis in the different areas of the

hippocampus in control and test rats submitted to 10 days of training in a RM or WM task with or without interference. Our results show that, in the dentate gyrus (DG), the WM task with high level of interference induces a selective increase in the phosphorylation of the CamKinaseII (CamKII) as compared to controls and rats tested in RM or WM task with low interference. This increase was not observed in CA1 and CA3 area of the hippocampus. CamKII is a key protein involved in long-term synaptic plasticity and is activated by a synaptic increase in calcium concentration. Thus, our results suggest that long-term synaptic plasticity occurs selectively in the DG during the WM task with high interference, and that, in this task that requires a high level of cognitive flexibility, the memory trace must be quickly formed as well as quickly erased.

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Poster

090. Memory Consolidation and Reconsolidation

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 90.09/QQ24

Topic: F.02. Animal Cognition and Behavior

Support: NSERC

St. Boniface Hospital Research

Everett Endowment Fund

Title: Spatial learning capabilities and mitochondrial function in creatine-supplemented mice

Authors: W. M. SNOW[1,4], C. CADONIC[2,4], S. K. ROY CHOWDHURY[4], E. THOMSON[4], S. ALASHMALI[3], E. PLATT[4], M. SUH[3], P. FERNYHOUGH[1,4], *B. C. ALBENSI[1,2,4]; [1]Pharmacol. & Therapeut., [2]Biomed. Engin., [3]Human Nutritional Sci., Univ. of Manitoba, Winnipeg, MB, Canada; [4]Div. of Neurodegenerative Disorders, St. Boniface Hosp. Res., Winnipeg, MB, Canada

Abstract: Objective: Dietary supplementation with creatine, a neuroprotective organic acid, has been shown to improve memory in humans and animals. Whether creatine enhances the acquisition and retention of spatial memory in the Morris water maze (MWM), a well-characterized rodent memory assay, is unknown. Further, the physiological mechanisms for the memory enhancements reported thus far are unclear. The transcription factor nuclear factor

kappa B (NF-κB) has been implicated in creatine-induced enhancements in cultured neurons. CREB signaling is implicated in creatine-enhanced spatial memory assayed using the Barnes maze in mice. We have shown a role for NF-κB in maintaining synaptic plasticity, memory, and neuronal energy homeostasis. Thus, this study sought to investigate: 1) the effects of creatine supplementation on hippocampal-dependent spatial memory in the MWM; 2) the effects of creatine on neuronal mitochondrial function *in vitro;* 3) CREB and NF-κB levels in the hippocampi of creatine-fed mice; and 4) the NF-κB complex as a possible target of creatine-induced memory enhancements and mitochondrial function. **Methods:** C57BL/6 male mice (age 7 mos) were fed either a creatine-supplemented (3% w.w.) or control diet for 8-9 wks. During wk 8, mice underwent MWM training to assess spatial learning and memory. In a subset of animals, mitochondrial function was assessed (i.e., oxygen consumption rates (OCR); XF24 Analyzer, Seahorse Biosciences)) following MWM training. Cortical neurons from embryonic mice were cultured. Mitochondrial bioenergetics were measured in creatine-treated (1 mM) vs. untreated neurons alone and in the presence of sulfasalazine, an NF-κB blocker. Western blot experiments were used to determine hippocampal protein levels of CREB and NF-κB with and without creatine. **Results:** *In vivo*, creatine dietary supplementation significantly enhanced spatial learning in the MWM ($p<0.05$) relative to control-fed mice. *In vitro*, creatine increased OCR in cultured cortical neurons vs. untreated neurons ($p<0.05$). Blockade of NF-κB with sulfasalazine, however, resulted in diminished mitochondrial OCR ($p<0.05$). Western blot experiments to determine NF-κB and CREB levels are ongoing. **Conclusions:** These data establish creatine-induced enhancements in learning and neuronal cellular bioenergetics in mice and suggest the involvement of NF-κB as a key regulator of energy homeostasis. Such research has important implications for the treatment of disorders affecting memory, including Alzheimer's disease. Funding from Natural Sciences and Engineering Research Council (NSERC), the St. Boniface Hospital Foundation, and the Everett Endowment Fund

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Poster

090. Memory Consolidation and Reconsolidation

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Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 90.10/QQ25

Topic: F.02. Animal Cognition and Behavior

Title: Reelin supplementation rescues synaptic plasticity and cognitive deficits in a mouse model for Angelman syndrome

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Univ. of South Florida, Tampa, FL

Abstract: The reelin pathway has implications in synaptic plasticity, through the modulation of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor insertion and ion conductance through N-methyl-D-aspartate (NMDA) receptors, and in hippocampal learning and memory. Past research has demonstrated that through a single application of reelin, long-term potentiation (LTP) can be enhanced, dendritic spine density augmented, and associative and spatial learning and memory improved. Angelman Syndrome (AS) is a neurological disorder that presents with an overall change in synaptic function, including decreased LTP, reduced dendritic spine density, and deficits in learning and memory, making it an attractive model to examine reelin's ability to recover synaptic function and cognitive deficits. In this study, we investigated the effects of reelin on synaptic plasticity and cognitive function in a mouse model for AS, demonstrating that a single *in vivo* injection of reelin can enhance LTP, increase dendritic spine density, and rescue learning and memory associated with hippocampal dependent behavior. Furthermore, we detected alterations to the reelin profile in AS, both in mouse and human tissue, alluding to reelin as a possible therapeutic in the AS community.

Disclosures: **W. Hethorn:** None. **S.L. Blankenship:** None. **E.J. Weeber:** None.

Poster

090. Memory Consolidation and Reconsolidation

Location: Halls A-C

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Program#/Poster#: 90.11/QQ26

Topic: F.02. Animal Cognition and Behavior

Title: Role of neurobeachin in fear memory reconsolidation

Authors: *B. LEE, T. CHO, E. BANG, A. PAYDAR, S. LEE, W.-S. YANG, H.-S. SHIN;
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Abstract: Memory consolidation is a process that stabilizes a memory trace into long term memory after the initial learning. This consolidated memory becomes vulnerable after memory retrieval and either reinforced or weakened through reconsolidation. Many reports have

suggested that de novo protein synthesis is required for both consolidation and reconsolidation. However, the underlying mechanisms of the newly synthesized proteins in memory reconsolidation were not fully understood. Here, we show that neurobeachin (Nbea), a regulator of synaptic protein trafficking, plays a critical role in fear memory reconsolidation. First, in proteomic analysis Nbea was identified to be increased in mouse hippocampus after contextual fear memory retrieval. We studied the temporal expression of Nbea after fear conditioning and retrieval. Interestingly, Nbea was gradually increased after fear conditioning but was rapidly and transiently increased after fear memory retrieval suggesting different roles of Nbea during consolidation and reconsolidation. Next, we utilized shRNA knockdown (KD) system to understand the role of Nbea in the hippocampus during consolidation and reconsolidation. Nbea KD mice showed no significant differences for fear memory acquisition and consolidation from control group. However, Nbea KD mice clearly showed enhanced fear memory reconsolidation suggesting a specific role of Nbea during reconsolidation. Due to the enhanced fear memory with inhibition of Nbea expression in hippocampus, we suggest neurobeachin as a negative regulator for maintenance or stabilization of context-dependent fear memory during reconsolidation.

Disclosures: **B. Lee:** None. **T. Cho:** None. **E. Bang:** None. **A. Paydar:** None. **S. Lee:** None. **W. Yang:** None. **H. Shin:** None.

Poster

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NIH RO1 DA 016765 DA 023555 (PI AJE)

Title: Space radiation improves pattern separation in older mice without influencing gross hippocampal function

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BIRNBAUM[1], B. P. C. CHEN[2], A. J. EISCH[1];
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Abstract: High atomic weight and energy (HZE) particles are a major component of space radiation, and chronic low doses may be detrimental to astronauts during deep space missions by decreasing adult hippocampal neurogenesis (Rivera et al., 2013) and impairing cognitive ability (Raber et al., 2011). This raises concerns that HZE radiation may diminish hippocampal function (e.g. by decreasing memory or mood control) and thus compromise mission success. Furthermore, it is unknown how HZE radiation alters subtle aspects of hippocampal function such as pattern separation, the ability to distinguish unique patterns or contexts sharing overlapping features, in "astronaut-age" equivalent mice. To fill these knowledge gaps, we exposed C57BL/6J mice (9-week old [Young Adult] or ~6-month-old [Mature] mice) to HZE particles (^{28}Si, 275 MeV/n, 72.1 KeV/μm LET or ^{56}Fe, 600 MeV/n, 174.1 KeV/μm LET) and examined general behavior (e.g. locomotion) and 2 hippocampal-dependent functions, contextual fear conditioning (CFC) and pattern separation (contextual discrimination fear conditioning, CDFC) 2 to 6 months post-irradiation. For the ^{28}Si HZE particle radiation experiment, Young Adult and Mature mice received a single exposure of 20 cGy, a single exposure of 100 cGy, or 0 cGy (Sham). For the ^{56}Fe HZE particle experiment, mice received a single exposure of 20 cGy (Non-fractionated), a protracted dose of 20 cGy (Fractionated, 6.7 cGy x 3 days, 48h intervals), or 0 cGy (Sham). After ^{28}Si or ^{56}Fe irradiation, Young Adult mice showed normal general behavior, but diminished CFC and dose-dependent effects on CDFC. In contrast, Mature mice displayed normal locomotor activity and normal CFC. Interestingly, Mature irradiated mice that received ^{28}Si or ^{56}Fe showed improved CDFC compared to Sham. The improvement in CDFC in Mature mice was unexpectedly neurogenesis-independent, as doublecortin-immunoreactive immature neurons were lower than Sham in irradiated Young Adult and Mature mice. Thus, ^{28}Si and ^{56}Fe HZE particle radiation enhance pattern separation in mice of astronaut-equivalent age, but decrease hippocampal function in younger mice. We are currently exploring changes in local hippocampal neural circuitry as an explanation for the age-related improvement in pattern separation after HZE radiation exposure.

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Poster

090. Memory Consolidation and Reconsolidation

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Topic: F.02. Animal Cognition and Behavior

Support: NSF IOS 1121866

R21 MH083188

NSF 1258111

Title: Sparse encoding of a memory of a meal in the dorsal hippocampus as revealed with Arc expression

Authors: *M. B. PARENT[1], Y. O. HENDERSON[1], A. VAZDARJANOVA[2];
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Abstract: Our overarching hypothesis is that hippocampal neurons form a memory of a meal and inhibit meal onset during the period following a meal. In support of this, we discovered that inactivation of dorsal hippocampal neurons after a sucrose meal decreases the interval between two meals and increases total intake. Moreover, sucrose consumption is associated with small, but significant increases in the expression of the plasticity-related immediate early gene *Arc* (activity-regulated cytoskeleton-associated protein) in the dorsal hippocampus. It is possible that sucrose consumption produced low levels of *Arc* activation in dorsal hippocampal neurons because the rats had been given repeated exposure to the sucrose solution. That is, we hypothesize that extensive familiarity with the sucrose solution decreased the mnemonic demands associated with that meal, resulting in sparse encoding of the memory of the meal as revealed by Arc expression. To test this hypothesis, experimental rats were trained daily to consume a 32% sucrose solution at the same time and location and latency to contact the sipper tube during training was used to manipulate the degree of familiarity with the sucrose solution. The "more familiarized" group was trained until their latencies were less than 30-sec for 2 consecutive days and the "less familiarized" group was trained using a 60-sec criterion. The day after the criterion was reached, the experimental rats were given the sucrose solution for 7 min, euthanized immediately, and their brains were harvested. Caged control rats were handled and transported in a similar manner during training and on the experimental day, but were never given the sucrose solution. *Arc* mRNA expression in dorsal hippocampal neurons was measured using fluorescence *in situ* hybridization. Our preliminary findings based on a small sample size suggest that familiarity with the sucrose solution decreases sucrose-associated *Arc* activation in dorsal hippocampal neurons. Specifically, the less-familiarized group had more *Arc* activation than the more familiarized group, and the more familiarized group had more *Arc* activation than the caged controls. Additionally, when both groups were combined, the percentage of *Arc* expression in the dorsal hippocampus decreased as the number of training days increased. These findings suggest that an eating episode induces synaptic plasticity in dorsal hippocampal

neurons, which is consistent with our overarching hypothesis that dorsal hippocampal neurons form a memory of a meal. Moreover, our findings suggest that familiarity with the meal produces sparse encoding of the memory of that meal.

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Poster

090. Memory Consolidation and Reconsolidation

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Topic: F.02. Animal Cognition and Behavior

Support: CONACYT 155242

DGAPA-UNAM IN209413/25

UNAM 10113

Title: Post-retrieval hippocampal infusions of HDACi enhance spatial memory reconsolidation

Authors: *I. BALDERAS, G. CHAVEZ-MARCHETTA, A. AGOITIA-POLO, C. M. GOMEZ, A. DIAZ-GONZALEZ, F. BERMUDEZ-RATTONI;
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Abstract: It has been proposed that a short retrieval induces impairments in reconsolidation or enhancement of extinction. Reconsolidation refers to the destabilization/re-stabilization process upon memory reactivation. We tested the effect of a short retrieval on spatial memory reconsolidation in the Morris water maze. We found that infusions of an inhibitor of HDAC in the hippocampus immediately after a short retrieval enhanced memory, when tested 24 h and 7 days after. Moreover, CNQX infusion in the hippocampus blocked retrieval; however, CNQX did not impede memory enhancement by infusions of HDACi. We observed that infusions of HDACi in the hippocampus enhanced reconsolidation in the absence of retrieval. The results indicate that short retrieval not necessarily produce memory impairments in a reconsolidation protocol in a declarative memory model. Moreover, the HDACi induces a reliable spatial memory reconsolidation enhancement.

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Poster

090. Memory Consolidation and Reconsolidation

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Title: PDE11A4, a phosphodiesterase enriched in the ventral hippocampus, is required for consolidation of social memories and normal social approach behaviors

Authors: *M. P. KELLY, S. HEGDE;
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Abstract: Social deficits are key features of several neuropsychiatric disorders, such as autism, schizophrenia, and PTSD, yet no medicines are available to remedy these symptoms. Despite the fact that appropriate social behaviors are vital to thriving in one's environment, little is understood of the molecular mechanisms that control social behaviors or how social experiences modify these signaling pathways. Here, we show that Phosphodiesterase 11A (PDE11A), an enzyme that is restricted to the hippocampus and that breaks down cAMP and cGMP, may be a fundamental molecular mechanism of social behavior. First, we tested male and female PDE11A knockout (KO) and sex-matched wild-type (WT) littermates for social vs non-social odor recognition (SOR vs nSOR) using odor-saturated wooden beads as stimuli. Male and female PDE11A KO mice exhibited intact short-term memory (STM, 1 hour after training) for SOR but absolutely no long-term memory (LTM, 24 hours after training). This LTM deficit was reversed when PDE11A4 (the PDE11A isoform expressed in brain) was delivered back to the hippocampus of KO mice. Although PDE11A KO mice showed no LTM for SOR, they exhibited robust LTM for nSOR. Next, we determined if PDE11A KO mice would form memories for non-social odors if they learned about them via social interactions instead of odor-

saturated wooden beads. To test this, we used the social transmission of food preference (STFP) assay. Similar to results obtained in SOR, PDE11A KO mice demonstrated intact STM for STFP but no LTM. These results suggest that PDE11A4 is specifically required for the consolidation of social memories. Next, we tested PDE11A WT and KO mice in a social approach/social avoidance assay. When given the choice of exploring a cagemate versus a novel PDE11A WT mouse, neither male nor female PDE11A KO mice differed relative to WTs with regard to their approach behavior. When given the choice between a cagemate and a C57BL/6J, however, both male and female PDE11A KO mice approach the C57BL/6J mouse significantly less than do PDE11A WT mice. Since PDE11A appears to be required for intact social behaviors, we next asked if social experience might feed back to regulate PDE11A4 . We found that 1 month of social isolation, vs. group housing, changed the compartmentalization of PDE11A4 protein within both the dorsal and ventral hippocampus. This isolation-induced shift in the compartmentalization of PDE11A4 was mimicked by phosphorylation of serine 162 (see Pathak et al., SFN 2014). Together, our findings suggest that PDE11A4, a highly druggable target, regulates social behavior and is a key mechanism by which social experience modifies the brain.

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Poster

090. Memory Consolidation and Reconsolidation

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R01-MH100822

Title: Memory traces and their underlying mechanisms during the infantile amnesia period

Authors: *A. TRAVAGLIA, R. BISAZ, C. M. ALBERINI;
New York Univ., New York, NY

Abstract: Clinical and basic research over the past century has demonstrated that the developing brain is extraordinarily sensitive to environmental influences and that early life aversive

experiences profoundly affect the neural systems critically involved in cognition and emotions, resulting in lifelong consequences on brain and behavior. On the other hand, there is very little recollection in adulthood of memories occurred during the early period of life, a phenomenon known as infantile amnesia. We have used a negatively reinforced one-trial operant conditioning paradigm (inhibitory avoidance, IA) in rats to investigate the expression, temporal dynamics and mechanisms of memory formation and retention in infant rats. In agreement with previous literature, we found that rats trained at postnatal day (PN) 17, which corresponds to early childhood in humans, acquired and expressed the memory immediately after training. This memory was then very rapidly forgotten, and no long-term memory was detected, in line with the theory of infantile amnesia. We will refer to this as infantile memory trace. However, a reminder experience (combination of context and footshock), which per se does not evoke any IA response, given at later times (up to at least one month after training) produced a significant IA memory, which was very long lasting and specific to the training context. Infantile amnesia was not found in rats trained at PN24, which showed a very strong and long lasting IA memory similar to that of adult rats. We asked whether the hippocampus is involved in establishing the infantile memory trace. Inactivation of the hippocampus with muscimol at the time of training prevented memory reinstatement, suggesting the functional involvement of the hippocampus in the acquisition of the memory. As expected, muscimol at the time of training of PN24 rats blocked long-term memory formation suggesting that at this age, like in adulthood, the hippocampus is required to form IA memory. Quantitative western blot analyses of activity, plasticity and memory markers in the dorsal hippocampus indicated that training at PN17 leads to the activation of the immediate early genes Egr-1 and Arc, as well the BDNF/TrkB pathway. We conclude that the hippocampus is critically engaged in the formation of memories during the period of infantile amnesia, and that, despite the memories are not expressed, traces of the experience must be stored long term and later can be reactivated by reminders.

Disclosures: **A. Travaglia:** None. **R. Bisaz:** None. **C.M. Alberini:** None.

Poster

090. Memory Consolidation and Reconsolidation

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Support: NIH Grant MH 074736

Title: Insulin-like growth factor 2 prevents memory decay associated with normal aging

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Ctr. for Neural Sci., New York Univ., New York, NY

Abstract: Memory loss is a common problem in aging that manifests as rapid forgetting of newly learned information. This suggests that memory consolidation, the process that occurs after learning and leads to stable memory retention, is likely compromised by aging. Targeting consolidation mechanisms may therefore prove to be an effective strategy for preventing or reversing age-related memory deficits. Work from our laboratory has shown that memory consolidation in young adult rats requires the induction of insulin-like growth factor 2 (IGF-II) expression in the dorsal hippocampus. Furthermore, administration of recombinant IGF-II during the early stages of consolidation enhances memory and prevents forgetting (Chen et al 2011; Stern et al 2014). Here we sought to determine whether dysregulation of hippocampal IGF-II expression contributes to age-related changes in memory formation and persistence, and whether targeting consolidation with exogenous IGF-II can prevent age-related memory loss. Young adult (4 months) and aged (26 months) male Fischer 344 x Brown Norway (FBN) F1 hybrid rats were injected with IGF-II or vehicle in the dorsal hippocampus immediately after inhibitory avoidance (IA) training. When tested 14 or 28 days after training, aged rats injected with vehicle showed memory impairment relative to young adult rats. IGF-II injections significantly prevented this memory loss. Quantitative western blot analyses revealed that expression of IGF-II in hippocampal synaptic fractions is reduced in aged relative to young adult rats, supporting the hypothesis that a decrease in hippocampal IGF-II function contributes to age-related cognitive impairment. Further, in aged rats, IA training did not lead to induction of plasticity mechanisms such as Arc in the dorsal hippocampus; however, a significant rescue of training-induced Arc expression was observed in aged rats injected with IGF-II. Our results indicate that administering IGF-II within the initial consolidation period can effectively prevent memory deficits in aged rats, potentially by targeting Arc-dependent mechanisms.

Disclosures: **S.A. Johnson:** None. **C.M. Alberini:** None.

Poster

090. Memory Consolidation and Reconsolidation

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Support: NIMH R01 MH100822 to CMA

McKnight Award to CMA

NIMH F30 MH098570 to VG

Title: β-adrenergic receptors and memory consolidation: the role of astrocytic mechanisms

Authors: *V. GAO[1,2], A. SUZUKI[3], S. LENGACHER[4], P. J. MAGISTRETTI[4], C. M. ALBERINI[1];
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Abstract: Emotionally charged events generate strong memories. Norepinephrine (NE) modulates the strength of emotional memories by binding to β-adrenergic receptors on target cells. Antagonists of β-adrenergic receptors such as propranolol are known to block memory consolidation. β-adrenergic receptors are expressed on astrocytes in addition to neurons. In fact, stimulation of astrocytic β-adrenergic receptors *in vitro* promotes glycogenolysis, a process that occurs in astrocytes but not in neurons in the adult brain. Using inhibitory avoidance (IA) training in rats, we previously reported that learning requires glycogenolysis, which results in release of lactate from astrocytes and its transport into neurons. This lactate-mediated astrocytic-neuronal coupling is required for long-term memory formation, long-term potentiation, and underlying molecular mechanisms required to mediate memory consolidation. Here we tested the hypothesis that β-adrenergic receptors in the hippocampus mediate memory formation via astrocytic-neuronal lactate coupling. We found that the hippocampal increase in lactate after IA training is completely blocked by intra-hippocampal administration of propranolol. Infusion of propranolol into the hippocampus before training blocks long-term, but not short-term memory, and this memory impairment is rescued by co-administration of L-lactate. Induction of long-term plasticity markers including Arc, phosphorylated CREB, CCAAT-enhancer-binding protein β (C/EBPβ), phosphorylated Ca2+/calmodulin-dependent protein kinase II (CaMKII), and phosphorylated cofilin after training are also blocked by propranolol and rescued by co-administration of L-lactate, as measured by quantitative Western blot analysis. The effect of propranolol on memory does not appear to be mediated by β1-adrenergic receptors, as intrahippocampal injections of β1 blocker betaxolol had no effect on memory. We conclude that β-adrenergic receptors mediate memory consolidation by critically recruiting astrocytic mechanisms.

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Poster

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Title: A critical role for the hippocampus-prelimbic/infralimbic cortex-amygdala circuit in retrieval-mediated memory strengthening

Authors: *X. YE[1], C. INDA[2], N. HUMALA[1], C. M. ALBERINI[1];
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Abstract: Memory impairment is often observed in aging as well as many cognitive disorders. It is an important yet challenging goal for mental health research to develop effective treatments for memory disorders. Several current strategies for memory improvement target the initial learning and consolidation period, which is temporally limited and requires de novo gene expression. It was once thought that following its consolidation memory is stable. However, many studies showed that a consolidated memory can become labile again if reactivated, such as by retrieval, and requires again gene expression to be re-stabilized. This process, referred to as reconsolidation, offers another opportunity for changing memory strength. Using the inhibitory avoidance task in rats, we have reported that three brief and spaced reactivations of a recent memory (3R) significantly enhance memory strength via reconsolidation processes (Inda et al., 2011). In the current study, we sought to determine the neural circuitry and molecular mechanisms underlying the retrieval-dependent memory strengthening. Using endogenous immediate early gene expression as tracers of brain activation, we found that memory reactivation induces Arc expression in dorsal hippocampus (dHC), basolateral amygdale (BLA), prelimbic/infralimbic cortex (PrL/IL) and anterior cingulate cortex (ACC). Blocking reactivation-induced Arc induction in different brain regions produces distinct effects on memory strength: (1) in the dHC, PrL or IL, it completely prevents memory enhancement evoked by 3R without affecting the memory strength induced by training; (2) in the BLA it causes memory loss indicating a role in reconsolidation; (3) in the ACC, it has no effect. We examined the molecular mechanisms in the dHC and PrL/IL accompanying retrieval-mediated

memory enhancement. We found that 3R significantly and selectively increases the phosphorylation levels of the transcription factor cAMP response element binding protein (CREB) and of the actin-binding protein cofilin in the dHC and PrL/IL. 3R also lead to significant expression changes in the PrL/IL region of several synaptic proteins, including AMPA receptors and the cell adhesion molecule neuroligins, Finally, blocking reactivation-induced Arc expression in dHC not only prevents molecular changes induced by 3R in dHC, but also in the PrL/IL, suggesting that dHC crosstalk to PrL/IL to promote memory enhancement. Taken together, these findings suggest a critical role for dHC-PrL/IL-BLA circuit in retrieval-dependent memory strengthening, and pave the way for developing and understanding retrieval-based strategy for memory improvement.

Disclosures: **X. Ye:** None. **N. Humala:** None. **C.M. Alberini:** None. **C. Inda:** None.

Poster

090. Memory Consolidation and Reconsolidation

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 90.20/QQ35

Topic: F.02. Animal Cognition and Behavior

Support: NIA Grant AG035379

Title: Cell specific knockout of Disabled-1 reveals novel approach to examine the role of the Reelin signaling pathway on synaptic plasticity and learning and memory

Authors: *A. L. LUSSIER[1], J. H. TROTTER[1], H. L. MAHONEY[1], G. D'ARCANGELO[2], E. J. WEEBER[1];
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Abstract: The intracellular protein Disabled-1 (Dab1) acts downstream of the Reelin signaling pathway to promote dendritic outgrowth and neuronal migration in the developing brain. In the adult brain the Reelin signaling pathway is important in synaptic plasticity, dendritic arborization, and learning and memory. Using novel Dab1 knockout mice in excitatory (eKO; Camk2 promoter) and inhibitory (iKO; GAD2 promotor) cells, we examined the role of Dab1 in adult synaptic plasticity and learning and memory. We found that Dab1 eKO mice had deficits in hippocampal synaptic function, dendritic spines size, and associative and spatial learning. Dab1 iKO mice had significant increases in the levels of several excitatory synaptic proteins, while the

levels of other excitatory and inhibitory synaptic proteins were normal. Glutamatergic synapse alterations were supported by CA1 field recordings which revealed increases in both the presynaptic activation as well as postsynaptic excitatory neurotransmission in the Dab1 iKO mice. However, the iKO mice had impairments in theta-burst stimulated long term potentiation. Collectively, these data demonstrate an important new method role for examining Reelin-Dab1 signaling in the adult brain, and underscore the importance of this pathway in learning and memory.

Disclosures: **A.L. Lussier:** None. **J.H. Trotter:** None. **H.L. Mahoney:** None. **G. D'Arcangelo:** None. **E.J. Weeber:** None.

Poster

090. Memory Consolidation and Reconsolidation

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 90.21/QQ36

Topic: F.02. Animal Cognition and Behavior

Support: NIMH RO1 MH069558

Title: The role of protein synthesis and protein degradation in the amygdala during fear extinction

Authors: ***E. K. ROTONDO**, N. C. FERRARA, F. J. HELMSTETTER;
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Abstract: The amygdala is important for fear memory consolidation, reconsolidation, and extinction. Evidence indicates that both protein degradation and synthesis in the amygdala are critical processes during the consolidation and reconsolidation of cued and contextual fear memories. It has been suggested that protein degradation by the ubiquitin-proteasome system (UPS) may mediate reorganization of the post-synaptic density as well as remove transcriptional repressors to promote *de novo* protein synthesis (Jarome et al., 2011). While the administration of protein synthesis inhibitors in the amygdala prior to extinction training impairs later retention of extinction learning, post-training protein synthesis inhibition does not appear to impair extinction memory consolidation. (Duvarci, ben Mamou, & Nader, 2006; Lin et al., 2003). Similarly, infusion of protein degradation inhibitors in the amygdala prior to extinction training impairs the consolidation of extinction learning but it remains unknown if blocking protein degradation in the post-extinction period impairs consolidation (Mao et al., 2006). To test

whether protein synthesis and degradation in the amygdala are critical for time-dependent consolidation after extinction training, animals were trained using auditory fear conditioning. The next day, fear was extinguished with 40 CS presentations. Bilateral amygdala infusions of the protein synthesis inhibitor anisomycin (ANI), the proteasome inhibitor clasto-Lactacystin β-lactone (βlac), or vehicle were given immediately after the extinction session. At a retention test 24 hours later, rats infused with ANI, βlac, and vehicle all showed similarly low freezing, indicating that the extinction memory had successfully consolidated regardless of drug infusion. These results suggest that, following extinction training, neither protein synthesis nor degradation in the amygdala is necessary for the consolidation of extinction. However, given evidence that pre-training protein synthesis or degradation inhibition in the amygdala impairs extinction retention, the critical factor in determining the effect of protein synthesis inhibition on extinction learning may be when this inhibition occurs relative to CS exposure. Current work is focused on understanding the sequence of cellular events in the amygdala that influence the induction of extinction-related plasticity and control post-training consolidation of memory for extinction.

Disclosures: **E.K. Rotondo:** None. **N.C. Ferrara:** None. **F.J. Helmstetter:** None.

Poster

090. Memory Consolidation and Reconsolidation

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Program#/Poster#: 90.22/RR1

Topic: F.02. Animal Cognition and Behavior

Support: NIH MH069558

Title: Activity dependent proteolysis in the amygdala modulates protein synthesis in the amygdala and dorsal hippocampus during consolidation of fear conditioning

Authors: *D. S. REIS, M. SEHGAL, F. J. HELMSTETTER;
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Abstract: The requirement for protein synthesis in the consolidation of fear memories in the amygdala is well documented. Post-training infusions of the translation inhibitor anisomycin into the amygdala impair the formation of both auditory and contextual fear memory. Recent work from our lab has shown that protein degradation mediated by the ubiquitin-proteasome system (UPS) is also critically involved in the consolidation of auditory fear memories in the amygdala.

Some evidence suggests that UPS-mediated protein degradation may drive the requirement for de novo protein synthesis during the consolidation period. However, the specific relationship between synthesis and degradation remains unclear. Here, animals were trained with auditory fear conditioning and given immediate post-training intra-amygdala infusions of vehicle, the protein synthesis inhibitor anisomycin, or the proteasome inhibitor clasto-lactacystin-β-lactone. Using a modified version of the surface sensing of translation (SUnSET) assay, we measured the level of protein synthesis in the amygdala and dorsal hippocampus of each rat at 60 min after fear conditioning. Our results indicate that inhibition of UPS-mediated protein degradation in the amygdala significantly reduces the amount of protein synthesis. In addition, we found that inhibition of protein synthesis or UPS-mediated protein degradation in the amygdala immediately after fear conditioning dramatically reduces the amount of global protein synthesis in the dorsal hippocampus as compared to the vehicle infused group. In fact, these manipulations seem to reduce protein synthesis in the dorsal hippocampus back to basal levels, as seen in the untrained vehicle group. Together these data suggest that the activity of the ubiquitin-proteasome system in the amygdala may have regulatory influence on the rate of mRNA translation in both the amygdala and dorsal hippocampus following auditory fear conditioning. These results provide *in vivo* evidence of an interaction between UPS-mediated protein degradation and de novo protein synthesis in memory and support the idea that UPS-mediated protein degradation may be a primary regulatory mechanisms critical to the initial formation and consolidation of auditory fear memory. Finally, the finding that inhibition of protein synthesis or degradation in the amygdala impairs global protein synthesis in the dorsal hippocampus lends further support to the idea that the amygdala is a primary site of synaptic plasticity during fear conditioning and may regulate specific mechanisms of memory consolidation, like protein synthesis, in other supporting neural structures.

Disclosures: **D.S. Reis:** None. **M. Sehgal:** None. **F.J. Helmstetter:** None.

Poster

090. Memory Consolidation and Reconsolidation

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Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 90.23/RR2

Topic: F.02. Animal Cognition and Behavior

Support: NIMH R01 MH069558

Title: ERK-mTOR interactions in the lateral, basolateral, and central amygdala during fear memory consolidation

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Abstract: The amygdala receives projections from the thalamus and hippocampus and is generally considered a critical locus of neural plasticity following fear conditioning. The different subnuclei within the amygdala interact through intrinsic connections and have different roles during the consolidation and expression of aversive learning. The details about how the lateral, basolateral, and central nuclei interact during long term memory (LTM) formation are not well understood. ERK and mTOR are two major signaling pathways that regulate synaptic plasticity supporting memory consolidation in the amygdala. mTOR complex 1 regulates downstream translational machinery, and the phosphorylation of ERK regulates transcriptional and translational processes. There is some evidence supporting an interaction between ERK- and mTOR-mediated translation during activity dependent synaptic plasticity (e.g.,Tsokas et al., 2007). The current study focused on amygdala-subnuclei specific interactions between ERK and mTOR during LTM consolidation after fear conditioning. Consistent with our prior work, results suggest that bilateral microinfusion of pharmacological inhibitors of ERK (U0126) or mTOR (rapmycin) phosphorylation into the lateral amygdala, leaving the central nucleus of the amygdala unaffected, is sufficient to prevent memory formation when assessed 24-hours after training. Conversely, blocking ERK phosphorylation within the central nucleus of the amygdala did not impact fear memory. Immunohistochemistry results revealed that mTOR inhibition reduced phosphorylated ERK in the lateral amygdala, phosphorylated p70s6k in the lateral and basolateral amygdala, and increased phospho-ERK immunopositive cells in the central amygdala. Additionally, inhibition of ERK resulted in a significant reduction of both phosphorylated p70s6k in the basolateral and ERK in the lateral amygdala. This effect suggests a bi-directional interaction between the ERK and mTOR pathways that is dependent on the specific population of cells within the amygdala.

Disclosures: **N. Ferrara:** None. **M.R. Gilmartin:** None. **D.S. Reis:** None. **J.L. Lee:** None. **F.J. Helmstetter:** None.

Poster

090. Memory Consolidation and Reconsolidation

Location: Halls A-C

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Topic: F.02. Animal Cognition and Behavior

Support: NIMHRO1MH069558

Title: Hippocampal protein degradation is required for context memory formation

Authors: *P. K. CULLEN, N. C. FERRARA, B. CALLIF, F. J. HELMSTETTER;
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Abstract: Simple exposure to a novel context results in the formation of a memory for that environment. Animals that have formed this memory do not show the deficits in context fear conditioning typically seen with immediate shock presentations, a phenomenon known as the context-preexposure facilitation effect (CPFE). Forming a representation of a context following chamber exposure requires de novo protein synthesis in the CA1 region of the dorsal hippocampus (dHPC) (Barrientos, et al., 2002; Huff, et al., 2006). However, it remains unclear whether hippocampal activity-dependent protein degradation, which has been shown to underlie the consolidation and retrieval of context-shock associations (Jarome, et.al, 2014; Lee, et al., 2008), is required for the formation of context memory in the absence of shock. We predicted that the synaptic plasticity required here would also require ubiquitin-proteasome system (UPS) mediated protein degradation. In Experiment 1, rats were placed into an observation chamber and allowed to explore for 5 min and then returned to their home cage. Animals were sacrificed 30 min, 60 min, 90 min or 2 hrs following this exposure. We quantified activity of the 20S proteasome by measuring three types of proteolytic activity (chymotrypsin-, trypsin-, and peptidylglutamyl-like) in synaptosomal fractions from the dHPC and ventral hippocampus (vHPC). We found that exposure to the context resulted in different changes in proteasome activity between the dHPC and vHPC. In Experiment 2, we verified the functional role of hippocampal protein degradation by blocking UPS activity in the period after context exposure. Animals received a 5-minute exposure to the context followed by an immediate intra-dHPC infusion of the UPS inhibitor clasto-lactacystin β-lactone (βLac) (32ng/μl), anisomycin (ANI) (125 μg/ μl), or vehicle. Twenty-four hours later, rats were returned to the context and received 5 immediate shocks (1-sec, 1mA; 1-sec ITI). Context conditioning was assessed 24-hours after shock delivery in the training context. Animals that received either ANI or βLac after initial exposure exhibited impaired context conditioning compared to vehicle controls. These data suggest that both protein synthesis and protein degradation are required for the formation of a context representation in the absence of the UCS. Taken together, these data suggest that the formation of a context memory requires UPS-mediated protein degradation within the hippocampus and that the dHPC and vHPC undergo biochemically distinct degradation processes.

Disclosures: **P.K. Cullen:** None. **N.C. Ferrara:** None. **B. Callif:** None. **F.J. Helmstetter:** None.

Poster

090. Memory Consolidation and Reconsolidation

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Topic: F.02. Animal Cognition and Behavior

Support: Japan Society for the Promotion of Science

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Title: Stathmin-dependent changes in microtubule stability are critical for memory formation

Authors: *G. P. SHUMYATSKY[1], S. UCHIDA[1], G. MARTEL[1], A. PAVLOWSKY[2], S. TAKIZAWA[1], C. HEVI[1], Y. WATANABE[3], E. KANDEL[4], J. ALARCON[2];
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Abstract: Changes in microtubule stability, ubiquitous cytoskeletal structures, regulate many biological processes, but their role in memory remains unclear. Here we show that learning causes biphasic changes in the microtubule-associated network in the hippocampus. In the early phase, dephosphorylation regulating microtubule-destabilizing activity of stathmin, stathmin-tubulin binding and microtubule instability are increased, whereas in the late phase these processes are reversed leading to an increase in microtubule/KIF5-mediated localization of the GluA2 subunit of AMPA receptors at the synaptic sites. A microtubule stabilizer paclitaxel decreases or increases memory when applied at the early or late phases, respectively. Stathmin mutations disrupt changes in microtubule stability, GluA2 localization, synaptic plasticity and memory. Aged wild-type mice show impairments in stathmin levels, changes in microtubule stability, and GluA2 localization. Blocking GluA2 endocytosis rescues memory deficits in

stathmin mutant and aged wild-type mice. These findings demonstrate the role for microtubules in memory in young adult and aged individuals.

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Poster

090. Memory Consolidation and Reconsolidation

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Title: A new mechanism of translational control of hippocampal mGluR-dependent long-term depression and object-place learning

Authors: *G. VIANA DI PRISCO[1], W. HUANG[1], S. BUFFINGTON[1], C.-C. HSU[1], P. BONNEN[1], A. PLACZEK[1], C. SIDRAUSKI[2], K. KRNJEVIC[3], R. J. KAUFMAN[4], P. WALTER[2], M. COSTA-MATTIOLI[1];
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Abstract: At hippocampal synapses, activation of group-I metabotropic glutamate receptors (mGluRs) induces long-term depression (LTD), which requires new protein synthesis. However,

the underlying mechanism remains elusive. Here we describe the translational program that underlies mGluR-LTD and identify the translation factor eIF2α as its master effector. Genetically reducing eIF2α phosphorylation, or specifically blocking the translation controlled by eIF2α phosphorylation, prevents mGluR-LTD and the reduction of surface AMPA receptors (sAMPARs). Conversely, direct phosphorylation of eIF2α, bypassing mGluR activation, triggers a sustained LTD and removal of sAMPARs. Combining polysome-profiling and RNA-sequencing, we identify the mRNAs translationally up-regulated during mGluR-LTD. Translation of one of these mRNAs mediates the LTD induced by eIF2α phosphorylation. Remarkably, mice with deficient p-eIF2α-mediated translation are impaired in object-place learning, a behavioral task that induces hippocampal mGluR-LTD *in vivo*. Our findings identify a novel model of mGluR-LTD, which promises to be of value in the treatment of mGluR-LTD-linked cognitive disorders.

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Poster

090. Memory Consolidation and Reconsolidation

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Support: NIH Grant MH096120 (D.L.G.)

NIH Grant NS029563 (D.L.G.)

Title: Inhibiting histone deacetylation overrides the erasure of long-term memory caused by inhibition of PKM in *Aplysia*

Authors: *D. CAI[1], K. PEARCE[1], S. CHEN[1], J. PARK[1], D. L. GLANZMAN[1,2,3,4]; [1]Integrative Biol. and Physiol., Univ. California LA, LOS ANGELES, CA; [2]Neurobio., David Geffen Sch. of Med. at UCLA, Los Angeles, CA; [3]Brain Res. Inst., [4]Integrative Ctr. for Learning and Memory, David Geffen Sch. of Med. at UCLA, Los angeles, CA

Abstract: A major outstanding question in neuroscience is how memories persist in the brain over years to decades. Studies in mammals have shown that inhibiting PKMζ, the constitutively

active catalytic fragment of the atypical PKC isoform PKCζ, appears to erase consolidated memories. Previously, we reported that inhibition of PKM Apl III, the *Aplysia* homolog of PKMζ, appears to erase the memory for long-term sensitization (LTS) in *Aplysia*, as well as long-term facilitation (LTF), the specific form of synaptic plasticity that underlies LTS (Cai et al., 2011). Moreover, we found that inhibition of PKM Apl III reverses the synaptic growth associated with long-term memory (LTM) (Chen et al., unpublished). Recently, however, we discovered that LTM is not actually eliminated following inhibition of PKM Apl III by the PKC inhibitor chelerythrine, as indicated by our ability to fully restore LTM by retraining with a sensitization regimen that is insufficient to induce LTS in naïve animals (Pearce et al., 2014). This result is surprising, because, according to standard electrophysiological and morphological criteria, the memory should be fully erased after the chelerythrine treatment. How can LTM persist despite chelerythrine's reversal of synaptic facilitation and sensitization-related synaptic growth? Possibly, LTM is maintained in *Aplysia* by epigenetic changes. To test this idea, we examined whether inhibition of histone deacetylases (HDACs) with trichostatin A (TSA) can override chelerythyrine's disruption of LTM. There were five experimental groups: a Control-Veh group (untrained plus injection of vehicle), a Trained-Veh group (sensitization trained plus injection of vehicle), a Trained-TSA group (sensitization trained plus injection of TSA), a Trained-Chel group (sensitization trained plus injection of chelerythrine), and a Trained-Chel-TSA group (sensitization trained plus injection of TSA prior to injection of chelerythrine). The sensitization training consisted of five bouts of electrical shocks, spaced 20 min apart, delivered to the tail via implanted electrodes. The drug/vehicle solution was injected into an animal's hemocoel after the 24 hr posttest. Animals in all four trained groups exhibited significant sensitization at 24 hr after training. As previously reported, the Trained-Chel animals did not exhibit LTS at 48 hr, while the Trained-Veh animals did. By contrast, the Trained-Chel-TSA group did exhibit significant sensitization at 48 hr. These results indicate that the TSA treatment overrode chelerythrine's reversal of LTM, and suggest that histone acetylation plays a critical role in the maintenance of long-term memory in *Aplysia*.

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Poster

090. Memory Consolidation and Reconsolidation

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NIH Grant NS029563 (D.L.G.)

Title: Reinstatement of long-term memory after its apparent erasure by inhibition of PKM or blockade of reconsolidation in *Aplysia*

Authors: K. PEARCE[1], D. CAI[1], S. CHEN[1], R. CHOE[1], R. SINGH[2], M. KIMBROUGH[1], K. SARMIENTO[1], X. ZHAO[1], T. DEHGHANI[1], *D. L. GLANZMAN[3,4,5];
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Abstract: Reactivation of consolidated long-term memory (LTM) in *Aplysia*, as in mammals, is thought to trigger its reconsolidation, during which it is, apparently, vulnerable to disruption by protein synthesis inhibition (Cai et al., 2012; Lee et al., 2012). Moreover, inhibition of PKM Apl III, a homolog of PKMζ, appears to erase LTM in *Aplysia* (Cai et al., 2011). But do these two disruptive manipulations actually eliminate LTM or do they merely suppress its expression? To address this question, we asked whether the memory for long-term sensitization (LTS) can be reinstated following its disruption by PKM inhibition. There were two groups. The first received 5 bouts of tail shocks (full sensitization training), followed by an injection of chelerythrine at 24 hr, and 3 bouts of shocks (truncated sensitization training), which does not induce LTS in naïve animals, at 48 hr (5XTrained-Chel-3XTrained group). Animals in the second (control) group were given a vehicle injection at 24 hr and the truncated training at 48 hr. Animals in the 5XTrained-Chel-3XTrained group exhibited significant sensitization at 24 hr, but not at 48 hr, compared to the control group. However, at 72 hr LTM was reinstated in the 5XTrained-Chel-3XTrained group. Next we tested whether LTM can be reinstated after reconsolidation blockade. There were four groups: (i) a control group that received an injection of the vehicle solution at 48 hr and truncated sensitization training (3 bouts of tail shocks) at 72 hr (Control-Veh-3XTrained group); (ii) a group that initially received full sensitization training (5 bouts of tail shocks) followed by an injection of anisomycin at 48 hr (Trained-Aniso group); (iii) a group that was initially given full sensitization training, and the reminder stimulus followed by anisomycin injection at 48 hr (5XTrained-Reminder-Aniso group); and (iv) a group that was initially given full sensitization training, followed by the reminder stimulus and anisomycin injection at 48 hr, and, finally, truncated training at 72 hr (5XTrained-Reminder-Aniso-3XTrained group). There was significant sensitization at 48 hr in all three groups that received the initial training compared with the Control-Veh-3XTrained group. The Trained-Aniso group also exhibited sensitization at 72 hr and 96 hr. Sensitization was absent in the Trained-Reminder-Aniso group during the 72 and 96 hr posttests. But, although absent at 72 hr, sensitization reappeared at 96 hr

in the 5XTrained-Reminder-Aniso-3XTrained group. Taken together with our previous work, these data indicate that LTM can be fully restored despite elimination of the synaptic expression of LTM, and argue against the idea that memories can be erased.

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Poster

091. Animal Models: Anatomical Connections in Learning and Memory Circuits

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Topic: F.02. Animal Cognition and Behavior

Support: ANR Neurovasc

ANR VSDIR

Title: A subpopulation of cortical fast spiking parvalbumin interneurons surrounded by perineuronal net (PNN) expresses various metallopeptidases including Adamts8, Adamts15 and Neprilysin. Their possible importance in plasticity and long-term memory

Authors: *J. P. ROSSIER[1], A. URBAN[2], A. SAVOYE[2], A. BERNARD[3], M. HAWRYLYCZ[3], E. LEIN[3];
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Abstract: The *in situ* hybridization (ISH) Allen Mouse Brain Atlas was mined for proteases genes expressed in the somatosensory cerebral cortex. Among the 480 genes coding for protease/peptidases in the mouse genome, 4 were found enriched in cortical interneurons: *reln* coding for reelin; *Adamts8* and *Adamts15* belonging to the class of metzincin proteases involved in reshaping the perineuronal net (PNN) and *Mme* encoding for Neprilysin, the enzyme degrading amyloid beta peptides accumulating in Alzheimer's plaques. The pattern of expression of these proteases in interneurons defines a new class of interneurons. In this report, single cell RT multiplex PCR (scRT-mPCR) after patch-clamp was performed for the simultaneous expression of 27 genes including these 4 peptidases,10 commonly accepted interneurons markers, 12 additional genes from the Allen database that are expressed in cortical interneurons

and 1 marker for excitatory neurons. Clustering of these genes by K-means algorithm displays five distinct clusters. The SOM cluster expresses *Sst* coding for somatostatin. Two clusters are co-expressing *Kit,* a tyrosine kinase and *Sema3c,* a semaphorin guiding growth cone molecule with VIP (KS-VIP) or without VIP (KS-neurogliaform). Two clusters of fast spiking (FS) interneurons expressing the calcium-binding protein *Pvalb* were identified, one co-expressing *Pvalb* with *Sst* (PV-SOM) and another co-expressing *Pvalb* with three metallopeptidases *Adamts8, Adamts15* and *Mme* (PV-MP). PV-MP interneurons are surrounded by a PNN whose role in plasticity is established. The three MPs identified in PV-MP cluster are secreted and may reshape the PNN highlighting the importance of FS interneurons in plasticity and long-term memory.

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Poster

091. Animal Models: Anatomical Connections in Learning and Memory Circuits

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Title: Direct examination of how supramammillary activity alters neurotransmission in hippocampal area CA2/CA3a

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Abstract: The experiments presented in this poster rely on a combination of recently developed methods to examine and better understand how the supramammillary nucleus (SuM), a hypothalamic region that is known to be active during reward and emotional-laden behaviors, alters hippocampal activity. The SuM sends axonal projections to area CA2/CA3a and the dentate gyrus of the hippocampus. It is well established that the SuM plays a significant role in

controlling the hippocampal theta rhythm, a phenomenon critical for memory formation. While the SuM-hippocampal projection has been investigated *in vivo* and at an anatomical level, several large and important questions remain. Using stereotaxic injection of viral vectors in combination with transgenic mouse lines in order to express light-activated channels in a precise location- and cell-specific way, we have been able to selectively stimulate SuM fibers in the hippocampus, allowing for the direct examination of synaptic transmission. Whole-cell recordings of principal cells and interneurons have been performed in acute hippocampal slices in order to elucidate the precise cellular targets of SuM fibers, and to determine which neurotransmitters or neuropeptides are released. We have found strong evidence of direct glutamatergic transmission onto principal cells in area CA2/CA3a, as well as release of the neuropeptide substance P (SP). Our experiments reveal that SP appears to be acting on gabaergic transmission in this area. Thus, we are currently investigating which class of interneuron in modulated by SP and the resulting effect on CA2 transmission.

Disclosures: **V. Robert:** None. **R.A. Piskorowski:** None. **V. Chevaleyre:** None.

Poster

091. Animal Models: Anatomical Connections in Learning and Memory Circuits

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 91.03/RR10

Topic: F.02. Animal Cognition and Behavior

Support: BFU 2009-14705

BFU 2006-12964

Title: Comparison of VTA and SN ascending projections to the Hippocampal Formation in the monkey (Macaca fascicularis). An anterograde tracer study

Authors: *R. INSAUSTI[1], D. HERNÁNDEZ[2], M. UBERO[2], M. LEGIDOS[3], M. ARROYO[3], M. MARCOS[3], E. ARTACHO[3], M. IÑIGUEZ DE ONZOÑO[3], M. MUÑOZ[3], H. EVRARD[4], N. LOGOTHETIS[4];
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Abstract: The Hippocampal Formation (HF) is the brain system that supports declarative memory in human and nonhuman primates. The HF is made up of dentate gyrus (DG), Cornu

Ammonis fields CA3, CA2 and CA1, subiculum (S), presubiculum (PrS), parasubiculum (PaS) and entorhinal cortex (EC). The unidirectional circuit that links all these structures through several synaptic steps ultimately, end up in stable memories, very likely, in the cerebral cortex. This consolidation process takes place once the brainstem activity is cancelled (Logothetis et al., 2012). The HF receives cortical input from polymodal association areas, as well as subcortical brain centers, which are monoaminergic brainstem nuclei. The main centers with direct access to the HF, are dopamine containing cell groups such as the ventral tegmental area (VTA) and the substantia nigra (SN) and adjacent mesencephalic reticular formation, serotonergic (centralis superior and dorsal raphe nuclei), and noradrenergic (Locus coeruleus) as retrograde tracing studies demonstrated. The paths and termination of those brainstem projections to the different HF fields are unknown. In the course of an ongoing study aiming at the study of brainstem projections to the HF, we describe the resulting labeling after deposits into the SN and the in different parts of the VTA. 1) Direct nigro-hipocampal fibers are scarce, but present in all components of the HF in particular rostrally, while VTA are somewhat denser. 2) Both VTA and SN fibers were observed in non-cellular strata of the HF. 3) Both SN and VTA present fibers that change course and adopt a direction in a transversal plane to the main axis of the hippocampus, in stratum radiatum and lacunosum-moleculare, usually orthogonal to the direction of the dendrites. 4) The SN, was observed giving off fibers to the polymorphic cell layer of the DG, which crossed the granule cell layer, into innermost portion of the molecular layer of the DG. Our results are in agreement with retrograde studies in which scarce retrograde labeled neurons were found, and suggest that direct and presumably through modulation of the excitability of the dendritic field of neurons in the HF, they possibly produce an effect on the HF function in memory, as it has been shown already in the monkey (Logothetis et al., 2012). (Supported by Grant BFU 2009-14705, MINECO, Spain)

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Poster

091. Animal Models: Anatomical Connections in Learning and Memory Circuits

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Program#/Poster#: 91.04/RR11

Topic: F.02. Animal Cognition and Behavior

Support: NINDS-R21NS085502

Title: Re-emergence of cholinergic/nestin cells in the medial septum/diagonal band after exercise

Authors: *J. M. HALL, L. M. SAVAGE, C. D. ALVARADO;
Binghamton Univ., Binghamton, NY

Abstract: It is well known that exercise leads to increases in a variety of neurotrophin and growth factors within the hippocampus and other brain regions that subsequently lead to cognitive improvements. Our laboratory has shown that exercise recovers spatial memory performance in an animal model (pyrithiamine-induced thiamine deficiency [PTD]) of the amnestic disorder Korsakoff syndrome. Our recent data indicate that exercise enhances behaviorally-evoked acetylcholine (ACh) efflux in the hippocampus of both healthy and PTD-treated rats. We hypothesize that exercise mediates the hippocampal cholinergic response by eliciting remodeling of cholinergic neurons within the medial septum/ diagonal band (MS/DB). Interestingly, a subpopulation of nestin+ cholinergic neurons have been discovered within in the basal forebrain and these neurons retain greater plasticity compared to nestin- cholinergic neurons. We hypothesized that exercise mediates the hippocampal cholinergic response by eliciting differential effects on nestin- and nestin+ cholinergic neurons. It was found that exercise increased the soma size of cholinergic neurons, but increases the number immunopositive nestin+ neurons in the MS/DB. Understanding the molecular mechanisms of activating these nestin+ cholinergic neurons could lead to pharmacological therapies in neurological disorders afflicted by cholinergic dysfunction.

Disclosures: **J.M. Hall:** None. **L.M. Savage:** None. **C.D. Alvarado:** None.

Poster

091. Animal Models: Anatomical Connections in Learning and Memory Circuits

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Topic: F.02. Animal Cognition and Behavior

Support: Mercator Stiftung

SFB 874

International Graduate School for Neuroscience, Ruhr-University Bochum

Title: A new fMRI compatible set-up to study perception and memory of odors in anesthetized and awake rats

Authors: *C. CHWIESKO[1], B. BOULAT[2], D. WIEDERMANN[3], M. HOEHN[3], M. SAUVAGE[4];
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Abstract: Many studies investigating memory function in humans use functional magnetic resonance imaging (fMRI). Recent translational studies have shown that the adaptation of human memory tasks to rodents is especially helpful to study memory processes by extrapolating results obtained in animals to humans, while potentially applying invasive methods. However, set-ups that allow for fMRI studies in awake animals are very rare to date. Here, we developed a new fMRI compatible setup designed to study perception and memory performances based on the recognition of odors. This setup includes an olfactometer that can release an unusually high number of odors (40 versus 3-4 published), an new animal holder that includes a head fixation system, a steady, but comfortable attachment solution of the coil to the rats head, and a respiration mask that allows for the rat to sample different odors while being head fixed. Furthermore, a safe, but effective, body restraining technique was developed to reduce stress on the head fixation, hence motion artefacts. The setup was first tested by imaging the olfactory bulb during the delivery of odors in sedated rats and is currently used to test odor memory performance within the frame of human to rat translational projects.

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Poster

091. Animal Models: Anatomical Connections in Learning and Memory Circuits

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Topic: F.02. Animal Cognition and Behavior

Support: DAAD

NRF

Title: Organization and neuroanatomy of the Cetartiodactyl hippocampus: an examination of 18 species

Authors: *N. PATZKE[1,2], K. Æ. KARLSSON[3], A. N. ALAGAILI[4], O. B. MOHAMMED[4], N. C. BENNETT[5], P. R. MANGER[2];
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Abstract: Cetaceans (whales, dolphins and porpoises) and Artiodactyls (even-toed ungulates) belong in the same mammalian order the Cetartiodactyla. While cetacean brains are generally larger than other mammals, cetaceans have absolutely and relatively small hippocampi in comparison to other mammals. The cetacean hippocampus exhibits a disorganized architecture and displays no evidence of adult hippocampal neurogenesis, which is present in all mammals analysed to date. A chemoarchitectonic description of the cetartiodactyl hippocampus is absent from the literature. Here we provide the first detailed chemoarchitectonic description of the hippocampus in 18 Cetartiodactyla species, 2 cetaceans: the northern minke whale (Balaenoptera acutorostrata) and the harbour porpoise (Phocoena phocoena) and 16 artiodactyls: Scimitar-horned oryx, (Oryx dammah), Arabian oryx (Oryx leucoryx), Black wildebeest (Connochaetes gnou), Blue wildebeest (Connochaetes taurinus), Blesbok (Damaliscus pygargus phillipsi), Arabian Ibex (Capra ibex), Springbok (Antidorcas marsupialis), Sand gazelle (Gazella subgutturosa), African buffalo (Syncerus caffer), Eland (Taurotragus derbianus), Kudu (Tragelaphus strepsiceros), Nyala (Tragelaphus angasii), Hippopotamus (Hippopotamus amphibious), Pig (Sus scrofa), Camel (Camelus dromedaries), using immunohistochemical staining for the calcium binding proteins parvalbumin, calbindin and calretinin and histological staining for Nissl. We reiterate that the cetacean hippocampus is small and architecturally disorganized, the most prominent difference being the loosely packed granular layer of the dentate gyrus. In all artiodactyls analysed the molecular layer could be clearly separated into an outer and inner layer, a feature not obvious in the cetaceans. In the cetaceans the stratum radiatum and stratum lacunosum moleculare of the hippocampus could not be clearly separated chemoarchitectonically as it was the case in artiodactyls. In most artiodactyls the parahippocampus was clearly delineated from the neighbouring areas by a strong immunoreactivity to calbindin and/or calretinin, but this was not present in cetaceans. Taken together the small size, undefined architecture and the lack of adult neurogenesis in the hippocampus of cetaceans, question the current assumptions regarding cognitive abilities associated with hippocampal function in the cetaceans.

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Poster

091. Animal Models: Anatomical Connections in Learning and Memory Circuits

Location: Halls A-C

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Program#/Poster#: 91.07/RR14

Topic: F.02. Animal Cognition and Behavior

Title: Anatomical Biomarkers of Learning in a murine model

Authors: S. KULASON, D. TWARD, K. NG, Y. ZHANG, J. ZHANG, J. W. KRAKAUER, *J. T. RATNANATHER, R. O'BRIEN, M. MILLER;
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Abstract: Motor training is thought to lead to structural changes in regions of the brain involved in motor control. While murine models are more convenient for studying brain development and its cellular and molecular underpinnings compared to human models, detecting small structural changes of the mouse brain is challenging. We hypothesized that combining high resolution MRI data with a sophisticated surface-based analysis tool would detect statistically significant structural changes. The mice were subjected to a 28-day motor training regimen of a skilled prehension task involving the right paw. Images were taken before and after training with *in vivo* longitudinal T2-weighted images acquired on a 9.4T MRI scanner with spatial resolution of 0.125 mm x 0.125 mm x 0.2mm. In order to segment the brain, the images were skull-stripped, rigidly aligned, and then mapped to an atlas using single-channel Large Deformation Diffeomorphic Metric Mapping (LDDMM). The 46 previously hand-segmented structures of the murine atlas were mapped onto each subject. Each region's bounding surface was calculated through a process of tessellation, smoothing, and downsampling. For each structure, surface driven transformations (LDDMM surface mapping) were calculated between the atlas and each subject to analyze changes in shape. We tested for differences between control and experimental populations using a global (volume of each structure), and local (log-Jacobian, or local volume change, at each surface vertex) measure of structural changes. Statistical significance was analyzed without making assumptions about the data's distribution by using permutation testing, which corrects for multiple comparisons by controlling the familywise error rate at $p<0.05$. The results show significant volume change in the left and right neocortex, as well as significant volume change and local expansion in left inferior and superior colliculus, left thalamus, and right piriform cortex. These regions may underlie brain changes important in motor learning. Our results suggest that subtle morphometric changes can be detected quantitatively in a murine

model to identify individual regions that are influenced by motor training. This indicates a role for MRI and quantitative surface-based analysis in studying brain plasticity.

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Poster

091. Animal Models: Anatomical Connections in Learning and Memory Circuits

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Topic: F.02. Animal Cognition and Behavior

Support: Medical Research Council UK, G0800329

Wellcome Trust WT090051MA

Title: Tracking value in an uncertain environment: Contrasting functional roles of primate mediodorsal thalamus and perirhinal cortex during learning

Authors: ***A. S. MITCHELL**[1], S. CHAKRABORTY[2], M. J. BUCKLEY[1], M. E. WALTON[1]; [1]Oxford Univ., Oxford, United Kingdom; [2]Bioengineering, Imperial Col., London, United Kingdom

Abstract: Recent evidence from monkey models of amnesia supports a more critical role for the magnocellular mediodorsal thalamus (MDmc) in new learning than in retention of previously acquired information (Mitchell & Gaffan, 2008). However, there are several ways in which animals can learn about object-reward relationships in complex environments and exactly how MDmc lesions affect these processes remains unclear. Primate MDmc has strong reciprocal connections with the orbitofrontal cortex (OFC), which has been shown to be necessary to enable appropriate, flexible value assignment and value-based choices. Primate MDmc and OFC also receive inputs from anterior temporal lobe structures such as perirhinal cortex (PRh). The current study compared functional roles of MDmc and PRh when flexibly assigning values to stimuli. Macaque monkeys were preoperatively trained on a decision making task using three objects per session that required the animals to learn and track probabilistic object-reward associations. In some task conditions, the value probabilities linked to each object would change dynamically during the session whereas in others the probabilities were fixed throughout. After receiving bilateral neurotoxic injections into MDmc, animals were able to learn to choose the highest value

option and to track fluctuations in its value, just like the unoperated control monkeys. However, MDmc lesion animals were impaired at updating their behaviour following reversals in the identity of the best option. By contrast, PRh lesioned animals exhibited a small but significant decrease in learning object-reward values, both before and after reversals. Regression analyses suggest that MDmc animals exhibited a selective reduction in the influence of recent choices on current learning and decision-making, whereas PRh lesions reduced the influence of the most recently selected object-reward combination. These results provide new evidence about specific functional roles of MDmc and PRh in one aspect of associative learning, where the object-reward associations are changing within session.

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Poster

091. Animal Models: Anatomical Connections in Learning and Memory Circuits

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Title: Differences in network activation patterns may underlie learning enhancement with spaced training

Authors: *C. A. KARSTEN[1], C. D. COX[1], K. WANG[1], G. LYNCH[1,2], C. M. GALL[1,3]; [1]Anat. and Neurobio., [2]Psychiatry and Human Behavior, [3]Neurobio. and Behavior, Univ. of California, Irvine, Irvine, CA

Abstract: First described by Ebbinghaus in the 1800s, and routinely demonstrated in different experimental animals and humans, training that is spaced across multiple trials is more effective than a single massed training session for memory encoding. Nevertheless, the mechanisms underlying the potency of spaced training remain unknown. Recent studies in our lab showed that spaced training in mice improves encoding of object location memory. The present studies, using immunolabeling of the immediate early gene product Fos to identify recently activated neurons, tested the hypothesis that spaced training engages different network patterns than a single (massed) learning episode. Cohorts of mice were trained in the object location memory task using either a single session or three spaced sessions with total training times being equal between paradigms. Animals were sacrificed 60-90 minutes after the final, or single, training episode, and spaced series of tissue sections through the forebrain were processed for immunofluorescence localization of Fos. In-house software was designed to align images - montages of full coronal cross sections - to the Allen Mouse Brain Reference Atlas and then automatically count highly active cells within specific fields. Correlation matrices were used to approximate network coherence. Mice that received effective spaced training exhibited robust patterns of correlated forebrain activity that were significantly different from patterns in the massed training group. As notable examples, the massed training group had greater coherence between hippocampal subfields whereas the spaced animals had a statistically greater correlation between orbital frontal cortex and hippocampus. Forebrain-wide descriptions of neuronal activation, as presented here, suggest a new interpretation of why distributed practice is so effective in encoding new information. The relationship of this hypothesis to psychological theories for the spaced trials effect will be discussed.

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Poster

091. Animal Models: Anatomical Connections in Learning and Memory Circuits

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Topic: F.02. Animal Cognition and Behavior

Support: Mercator Stiftung

SFB 874

Title: Differential contribution of the medial and lateral entorhinal cortices, the perirhinal and postrhinal cortices and the hippocampal subfields to familiarity

Authors: *A. MALET-KARAS[1], N. NAKAMURA[1], T. KITSUKAWA[2], M. SAUVAGE[1]; [1]Ruhr Univ. Bochum, Bochum, Germany; [2]Osaka university, osaka, Japan

Abstract: Recognition memory is known to rely on two distinct processes recollection and familiarity. It is well-accepted that the hippocampus supports recollection. However, the specific neural basis for familiarity remains elusive. Indeed, a major controversy in recognition memory is whether only the parahippocampal region supports familiarity or whether the hippocampus also does in addition to recollection. This discrepancy emerged principally because of the lack of tools with spatial resolution high enough to dissociate activity occurring in adjacent regions in humans (for example the hippocampus and the parahippocampal region). Also, a growing number studies have reported a functional segregation within the parahippocampal region itself, e.g. between the lateral and medial entorhinal cortices and the perirhinal cortex and postrhinal cortices. Yet, the specific contribution of those areas to familiarity remains unclear. Moreover, the medial entorhinal cortex was recently found to selectively contribute to recollection and not familiarity, suggesting that not all parahippocampal areas would support familiarity. Here, we tested this hypothesis by combining a rat behavioral memory paradigm shown to yield familiarity-only judgments and high resolution molecular imaging to map the activity of the different MTL areas (the lateral and medial entorhinal cortices, the perirhinal cortex and postrhinal cortices, CA1 and CA3) during the retrieval of odor memory. The neuronal activation is assessed by the detection by fluorescent in-situ hybridization of the immediate-early gene Arc which is tightly linked to plasticity processes. Preliminary results indicate a functional segregation of the MTL areas in terms of their contribution to the familiarity process.

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Poster

091. Animal Models: Anatomical Connections in Learning and Memory Circuits

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Topic: F.02. Animal Cognition and Behavior

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Title: Topographical and functional innervation of non-canonical back-projection from the subiculum to hippocampal CA1

Authors: *Y. SUN, T. IKRAR, A. J. LÓPEZ, X. XU;
Anat. & Neurobio., Univ. of California, Irvine, Irvine, CA

Abstract: While the hippocampal formation largely has a feedforward, unidirectional circuit organization, we recently established the existence of an under-described pathway, the direct backward projection from the subiculum (Sub) to CA1, using monosynaptic rabies tracing (Sun et al., 2014 Cell Reports). In the present study, topographical and functional relationships of the projection from Sub to CA1 are addressed through multiple new and effective approaches. We first examined if Sub-> CA1 connections follow any topographical rules and whether its connectivity strength varies with proximal and distal CA1. Subicular cells retrogradely labeled from rabies tracing targeting excitatory pyramidal neurons in proximal versus distal CA1 *in vivo* were examined, and we found that distal CA1 receives stronger subicular connections than proximal CA1 as determined by the measurement of the relative abundance of connected populations. The ratios of labeled pre-synaptic subicular neurons relative to the number of starter neurons in CA1 increased from 0.12 to 1.15 with targeting locations shifting from proximal to distal CA1. In addition, we used fast voltage sensitive dye imaging to detect CA1 excitatory ensemble responses to subicular photostimulation via glutamate uncaging in slice preparations. The imaging experiments revealed an exponential decay relationship between CA1 activation strength and the subicular photostimulation distance from Sub/CA1 border with the stronger Sub-evoked responses in the more distal region of CA1. To characterize subicular innervation of single CA1 neurons, we performed microiontophoretic injection of anterograde AAV expressing channelrhodopsin-2 (ChR2) fused with YFP in Sub and conducted subcelluar ChR2-assisted circuit mapping (sCRACM). The YFP-expressing subicular axons were distributed across CA1 laminae with prominent labeling in stratum oriens (SO), lacunosum moleculare (SLM) and radiatum (SR). Whole-cell recordings from CA1 excitatory neurons in slice preparations were combined with focal photoactivation of subicular axons in CA1. The sCRACM approach mapped the spatial distribution of subicular inputs to the recorded CA1 neurons; both excitatory and inhibitory inputs were robustly detected around their perisomatic regions as well as in SO and SR. Together these data provide new and important information on the non-canonical Sub-> CA1 circuit connections, and this work has laid a foundation for further functional studies of this pathway.

Disclosures: **Y. Sun:** None. **T. Ikrar:** None. **A.J. López:** None. **X. Xu:** None.

Poster

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Topic: F.02. Animal Cognition and Behavior

Support: KAKENHI #24223004

Title: Topographical organization of multisynaptic inputs to the hippocampus: Dual transsynaptic tracing with rabies virus vector in the rat

Authors: *S. OHARA[1], S. SATO[1], K.-I. TSUTSUI[1], M. P. WITTER[2], T. IIJIMA[1];
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Abstract: Behavioral, anatomical, and gene expression studies have shown functional dissociations between the dorsal and ventral hippocampus with regard to their involvement in spatial cognition, emotion, and stress. In this study we examined the difference of multisynaptic inputs to the dorsal and ventral hippocampus in the rat by using dual transsynaptic tracing which employs two recombinant rabies viruses expressing different fluorescent proteins. This method allows us to distinctly label two neural circuits and visualize the divergent projection of these two circuits from a single neuron by the co-expression of the two fluorescent proteins (double labeling). In a previous study, we injected two rabies virus vectors into the dorsal and ventral dentate gyrus (DG) respectively (Ohara et al., PLoS One 8(11), 2013). We reported topographically arranged monosynaptic inputs from entorhinal cortex layer II, medial septum, diagonal band, and supramammillary nucleus. Disynaptic inputs from the piriform and medial prefrontal cortices, endopiriform nucleus, claustrum, cortical amygdala, medial raphe nucleus, medial habenular nucleus, interpeduncular nucleus, and lateral septum were also topographically organized. Here we report the results of injecting the two virus vectors into the dorsal and ventral pole of the DG and CA1 regions. In addition to the regions labeled following DG-injection, a two day survival resulted in labeled neurons in entorhinal cortex layer III, cortical amygdala, and the thalamic nucleus reuniens. After five days survival, labeled neurons were additionally present in the anterior cingulate cortex and presubiculum. In all areas we observed two differently situated populations of labeled neurons, showing almost no double labeled cells. These results indicate that the cortical and subcortical inputs to the dorsal and ventral CA1, like those to DG, are conveyed through parallel disynaptic pathways. This second-order input difference in the dorsal and ventral hippocampus is likely to contribute to the functional differentiation of the hippocampus along the dorsoventral axis.

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Poster

091. Animal Models: Anatomical Connections in Learning and Memory Circuits

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Topic: F.02. Animal Cognition and Behavior

Support: Supported by NIMH/IRP

Title: Anatomical pathways for auditory memory II: Information from rostral superior temporal gyrus to dorsolateral temporal pole and medial temporal cortex

Authors: *M. MUNOZ[1], R. INSAUSTI[1], A. MOHEDANO-MORIANO[1], M. MISHKIN[2], R. C. SAUNDERS[2];
[1]Univ. of Castilla-La Mancha Sch. of Med., Albacete, Spain; [2]Lab. of Neuropsychology, Natl. Inst. of Mental Hlth., Bethesda, MD

Abstract: Monkeys' auditory recognition memory differs from recognition memory in other sensory systems, in that they unable to store auditory stimuli in long-term memory. Monkeys learn the rule for tactile and visual delayed matching-to-sample within a few sessions, and then show one-trial recognition memory lasting 10-20 minutes. By contrast, monkeys require hundreds of sessions to master the rule for auditory recognition and then show retention lasting no longer than 30-40 seconds. Moreover, unlike the severe effects of rhinal lesions on visual memory, such lesions have no effect on the monkeys' auditory memory performance. In vision, long-term recognition memory relies on anatomical connections from the visual association area TE with areas 35 and 36 of the perirhinal cortex (PRC). We wondered whether a similar anatomical route for auditory processing existed or was the lack of auditory recognition memory a result of the lack of such a pathway. We hypothesized that an auditory pathway for recognition memory would originate in the higher order processing areas of the rostral superior temporal gyrus (rSTG) and then connect via the lateral temporal pole to access the rhinal cortex of the medial temporal lobe. To test this, we placed retrograde (3% FB and 2% DY) and anterograde (10% BDA 10,000 MW) tracer injections in rSTG and dorsolateral area of the temporal pole. Results showed, that area 38DL of the lateral temporal pole receives dense projections from auditory association areas Ts1, TAa, TPO, of the rSTG and from the rostral parabelt and to a lesser extent from areas Ts2-4 and PGa. In addition, area 38DL projects densely to area 35 of PRC, area 28, and to areas TH/TF of the parahippocampal cortex, while the projection avoids most of area 36r/c of the PRC. The results suggest that auditory information can access the

perirhinal cortex of the medial temporal lobe but taken together with the previous evidence this is not sufficient to support recognition memory.

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Poster

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Topic: F.02. Animal Cognition and Behavior

Support: NSF IOS-1150292

Title: The nucleus reuniens and perirhinal cortex are critical to memory for sequences of events

Authors: *C. R. QUIRK[1,2], T. A. ALLEN[1,2], N. J. FORTIN[1,2];
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Abstract: Memory for sequences of events is fundamental to episodic memory. However, the neurobiology of this form of sequence memory is poorly understood. Using a cross-species sequence memory task we developed (Allen et al., 2014), our previous work in rodents has shown that neurons in the hippocampus (HC) and prefrontal cortex (PFC) code for sequences of events. Additionally, we have shown that infusions of fluorescent muscimol (FCM), a $GABA_A$ agonist, into the HC or PFC impaired sequence memory. Further, HC-PFC disconnection inactivations also impaired performance on the task, suggesting that interactions between the HC and PFC are critical to sequence memory. Similarly, our ongoing work using BOLD fMRI imaging in humans indicates that the HC and PFC regions are strongly engaged during task performance. Collectively, these findings suggest that the HC and PFC are part of a system that underlies the memory for sequences of events. However, little is known about the functional roles of distinct pathways that connect the HC and PFC. To begin to address this issue, we temporarily inactivated the nucleus reuniens or perirhinal cortex using FCM while rats performed the sequence task. The nucleus reuniens is reciprocally connected with the HC and PFC forming a *thalamic route* of communication. The perirhinal cortex is also reciprocally connected with the HC and the PFC forming part of the *cortical route* of communication. In the sequence task, rats are presented with a sequence of four odors through an odor port. On most

trials, odors are presented in the correct sequence (InSeq; ABCD). On probe trials, an odor is presented out of sequence (OutSeq; e.g., ABDD). Rats are required to hold in the odor port for ≥1sec for InSeq odors and withdraw from the port in <1sec for OutSeq odors. Sequence memory is demonstrated when rats correctly discriminate between InSeq and OutSeq odors. We report that inactivations of the nucleus reuniens or perirhinal cortex impaired sequence memory, indicating that both structures are critical to memory for sequences of events. Overall, these findings support the hypothesis that the HC and PFC communicate through both the thalamic and cortical routes in support of sequence memory. Further, these data motivate further examination of the roles of the thalamic and cortical pathways in memory for sequences of events.

Disclosures: **C.R. Quirk:** None. **T.A. Allen:** None. **N.J. Fortin:** None.

Poster

091. Animal Models: Anatomical Connections in Learning and Memory Circuits

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 91.15/RR22

Topic: F.02. Animal Cognition and Behavior

Support: RIKEN Brain Science Institute

Title: Island cells control temporal association memory

Authors: ***T. KITAMURA**[1], M. PIGNATELLI[1], J. SUH[1], K. KOHARA[1], A. YOSHIKI[2], K. ABE[2], S. TONEGAWA[1];
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Abstract: Episodic memory requires associations of temporally discontiguous events. In the entorhinal-hippocampal network, temporal associations are driven by a direct pathway from layer III of the medial entorhinal cortex (MECIII) to the hippocampal CA1 region. However, the identification of neural circuits that regulate this association has remained unknown. In layer II of entorhinal cortex (ECII) we report clusters of excitatory neurons called Island Cells, which appear in a curvilinear matrix of bulb-like structures, directly project to CA1 and activate interneurons that target the distal dendrites of CA1 pyramidal neurons. Island Cells suppress the excitatory MECIII input through the feedforward inhibition to control the strength and duration of temporal association in trace fear memory. Together, the two EC inputs comprise a control circuit for temporal association memory.

Disclosures: **T. Kitamura:** None. **M. Pignatelli:** None. **J. Suh:** None. **K. Kohara:** None. **A. Yoshiki:** None. **K. Abe:** None. **S. Tonegawa:** None.

Poster

091. Animal Models: Anatomical Connections in Learning and Memory Circuits

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 91.16/RR23

Topic: F.02. Animal Cognition and Behavior

Support: BMRC 10/1/21/19/645

NMRC IRG10nov104

Title: Nucleus incertus and hippocampal prefrontal cortical pathway - a stress responsive circuit in rats

Authors: *G. S. DAWE, Y. WU, W. TAN, J. KUMAR, U. FAROOQ, N. RAHADI, R. RAJKUMAR;
Natl. Univ. Singapore, Singapore, Singapore

Abstract: The nucleus incertus (NI) is the major brainstem source of the relaxinergic projection system in the mammalian brain. Recent reports suggest that the NI projects to the hippocampus and prefrontal cortex, structures that play a key role in cognition. The NI expresses corticotropin-releasing factor type 1 (CRF1) receptors and responds to water restraint stress with increased c-Fos expression. Previous studies from our lab showed that the NI projects to medial prefrontal cortex and that infusion of CRF into nucleus incertus suppresses medial prefrontal cortical activity and hippocampo-medial prefrontal cortical (HP-mPFC) long-term potentiation (LTP). The current study investigated the role of the NI in stress and synaptic plasticity in the HP-mPFC pathway in male Sprague-Dawley rats. Effects of various types of stress - repetitive (10-10 min in 24 ºC) and prolonged swim (30 min in 24 ºC) stress, and exposure to an elevated platform (30 min) - on c-Fos and relaxin-3 expression in the NI were examined using immunofluorescence and western blotting techniques, respectively. Serum corticosterone levels were measured to confirm stress induction. Further, we explored the effects of these stressors on LTP in the HP-mPFC pathway. The results showed that the NI responds to the stressors as indicated by increased c-Fos and relaxin-3 expression. LTP in the HP-mPFC pathway was significantly suppressed by 30-min swim and elevation stress. Finally, infusion of antalarmin, a CRF1 receptor antagonist, into the NI before or after elevation stress, partially but significantly

reversed the suppression of LTP in the HP-mPFC pathway. These results indicate that the NI responds to stress and could contribute to stress-induced impairment of cognitive processes involving the hippocampus and prefrontal cortex.

Disclosures: G.S. Dawe: None. **Y. Wu:** None. **W. Tan:** None. **J. Kumar:** None. **U. Farooq:** None. **N. Rahadi:** None. **R. Rajkumar:** None.

Poster

091. Animal Models: Anatomical Connections in Learning and Memory Circuits

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 91.17/RR24

Topic: F.02. Animal Cognition and Behavior

Support: Burroughs Wellcome Fund Postdoctoral Enrichment Grant

NIH T32 Training Grant

Title: Catecholamine release from the locus coeruleus to the dorsal hippocampus mediates the selective attention underlying spatial learning and memory

Authors: *K. A. KEMPADOO, E. R. KANDEL;
Columbia Univ., New York, NY

Abstract: Catecholamine signaling in the hippocampus mediates aspects of attention and arousal, however the precise roles of dopamine and norepinephrine in driving the selective attention that underlies spatial learning and memory are largely underexplored. We attempt to probe these questions by utilizing optogenetics to selectively stimulate dopamine release from the ventral tegmental area (VTA) to the hippocampus (HPC). Surprisingly, our immunohistochemistry results suggest that the locus coeruleus (LC), not the VTA, may provide the main source of dopamine to the HPC. We have therefore functionally assayed the role of LC catecholamine release in the HPC during learning and memory tasks. Photostimulation of the LC-to-hippocampal catecholamine pathway enhances performance in a spatial recognition task and attenuates performance in a novel object recognition task. These findings suggest dichotomous hippocampal function as a direct result of catecholamine release and therefore provide a framework for further exploring catecholamine anatomy and function in the HPC.

Disclosures: K.A. Kempadoo: None. **E.R. Kandel:** None.

Poster

092. Animal Models: Impairments in Learning and Memory

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 92.01/RR25

Topic: F.02. Animal Cognition and Behavior

Support: Research & Scholarship Grant, Bloomsburg University

Title: A high-fat diet impairs hippocampal-dependent learning but spares hippocampal-independent learning

Authors: *E. M. STOUFFER, E. E. WARNINGER, P. N. MICHENER;
Bloomsburg Univ. of PA, Bloomsburg, PA

Abstract: Two experiments were conducted to examine the effects of consumption of a high-fat diet on a hippocampal-dependent and hippocampal-independent latent learning task (Experiment 1) and classical conditioning task (Experiment 2; in progress). In both experiments, 5-month old male Sprague-Dawley rats were first given free access to a high-fat diet (45% of calories from fat; Research Diets D12492) or a low-fat diet (10% of calories from fat; Research Diets D12450J) for 8 weeks prior to training. Experiment 1 involved the use of two versions of the Latent Cue Preference (LCP) task, in which water-replete rats sampled water (a neutral stimulus) in 1 compartment of a 3-compartment LCP box on 1 day, and then had no water in a second compartment of the LCP box the following day (1 training trial), for a total of 4 training trials over 8 days. In the hippocampal-dependent version of this task, there were several multi-modal (visual, tactile, olfactory) cues in each of the 3 compartments, while the hippocampal-independent version used a single (visual) cue in each of the 3 compartments. Rats were then water-deprived prior to a compartment preference test, during which they were allowed to move freely among the compartments with the water removed. Latent learning was demonstrated during the preference test if rats spent more time in the compartment that previously contained the water. Experiment 2 was similar to Experiment 1, but involved the use of two versions of a Conditioned Cue Preference task. Training and testing was identical to the LCP task, except that rats were water-deprived during both training and testing, making the water an unconditioned stimulus. Just as in Experiment 1, the 3-compartment box either had several multi-modal cues (hippocampal-dependent version) or a single cue (hippocampal-independent version) in each of the compartments. The results of Experiment 1 showed that the rats that consumed the high-fat diet prior to training showed impaired latent learning in the multi-cue LCP task compared to rats

that consumed the low-fat diet. However, rats that consumed either diet showed intact latent learning in the single-cue LCP task. These results indicate that consumption of the high-fat diet impaired hippocampal-dependent latent learning but did not impair hippocampal-independent latent learning. We are predicting the same pattern of results for Experiment 2. The hippocampal-dependent learning impairment induced by consumption of a high-fat diet was most likely due to increased oxidative stress, decreased antioxidant activity, decreased levels of brain-derived neurotrophic factor, and/or disrupted glutamate transmission in the hippocampus.

Disclosures: **E.M. Stouffer:** None. **E.E. Warninger:** None. **P.N. Michener:** None.

Poster

092. Animal Models: Impairments in Learning and Memory

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 92.02/RR26

Topic: F.02. Animal Cognition and Behavior

Title: Impaired hippocampal place cell stability and local field potential characteristics in a mouse model of Fragile X mental retardation

Authors: *T. ARBAB[1], C. A. BOSMAN[1], R. WILLEMSEN[2], F. P. BATTAGLIA[3], C. M. A. PENNARTZ[1];
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Abstract: Hippocampal pyramidal cells fire strongly when the animal is at a specific location of the environment: the cell's place field. Proper spatial learning and memory consolidation have been associated with temporal stability of place fields, as well as certain characteristics of local field potentials (LFPs) and sharp-wave ripple events (SWRs) during subsequent sleep. Fragile X syndrome (FXS) is an inherited mental retardation, caused by a mutation that silences the X-chromosomal Fragile X Mental Retardation 1 gene (Fmr1). This leads to disturbed synaptic structure and function, which affects hippocampal synaptic plasticity. We have investigated in Fmr1-KO mice (Mientjes et al., 2006, Neurobiol Dis 21, 549-555) how the compromised synaptic function affects hippocampal network and single cell activity *in vivo* in a spatial exploration paradigm. These animals show impaired spatial and reversal learning deficits; however, little is known about how the mutation affects the functioning of hippocampal networks during cognitive processing *in vivo*. Multitetrode recordings were done in animals

freely exploring an open field arena surrounded by visual cues to evaluate the organization of hippocampal neural coding. We observed no differences between Fmr1-KO (n=6) and control (n=6) animals with regards to behavior and basic neurophysiological properties. We find that temporal stability of place fields, LFP power amplitude dynamics at different frequency bands, and properties of SWRs associated with proper spatial memory consolidation, are altered in Fmr1-KO animals. Together, these results suggest impaired mechanisms of spatial information processing and memory formation, linking the FXS genotype and its cognitive phenotype *in vivo*.

Disclosures: **T. Arbab:** None. **C.A. Bosman:** None. **C.M.A. Pennartz:** None. **R. Willemsen:** None. **F.P. Battaglia:** None.

Poster

092. Animal Models: Impairments in Learning and Memory

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 92.03/RR27

Topic: F.02. Animal Cognition and Behavior

Title: NMDA antagonist MK-801 leaves latent inhibition intact in rats

Authors: A. S. ADAMS[1], *J. A. WILLNER[2], A. G. ADAMS[1];
[1]Psychology, [2]Radford Univ., RADFORD, VA

Abstract: Prior exposure to a stimulus retards later conditioning of that stimulus, an outcome known as “latent inhibition”. Although the hippocampus has long been thought to be critical for latent inhibition, studies employing neurotoxin lesions suggest that an intact hippocampus is not needed for the effect. The present study investigated this question by examining whether MK-801, a non-competitive NMDA antagonist that impairs hippocampal synaptic plasticity, would affect latent inhibition for a pre-exposed stimulus. Young adult male Long-Evans rats (N=24) were reduced to 90% of their free-feeding weights and assigned to groups that differed in pre-session drug treatment (subcutaneous injection of 0.05 or 0.1 mg/kg MK-801, or isotonic saline 20 min before all daily sessions). Rats were initially trained to retrieve food pellets from a feeder in a cylindrical chamber. They then received 4 sessions of non-reinforced exposure to either a 2 Hz clicker or a white noise in the chamber, each session consisting of 20 presentations of the 10-sec stimulus. Finally, the rats received 4 conditioning sessions during which the exposed and non-exposed auditory cues were individually presented and paired with delivery of two food pellets (15 trials with each stimulus per session). Videos recorded just before (PreCS) and during

each conditioning trial (CS) were scored every 2 sec by one of the authors for occurrence of five different behaviors (Magazine, Headjerk, Rear, Perambulate, Other), with excellent inter-rater reliability on 10 randomly selected sessions scored by another author (r = .96). The relative frequency of Magazine and Headjerk behaviors significantly increased from PreCS to CS periods over days, whereas the relative frequency of other behaviors decreased. Analysis of daily averages for Magazine and Headjerk behaviors during CS periods demonstrated a significant effect of stimulus exposure, with rats conditioning more rapidly to the novel auditory stimulus than they did to the pre-exposed auditory stimulus, thus demonstrating latent inhibition. There was no overall effect of drug treatment, however, nor were there any interactions between drug treatment and stimulus exposure (no impairment or enhancement of latent inhibition). These results suggest that NMDA receptors are not critical for the basic latent inhibition effect. Whether NMDA receptors play a role in other aspects of latent inhibition tied to hippocampal function (e.g., context dependence) remains to be determined.

Disclosures: **A.S. Adams:** None. **J.A. Willner:** None. **A.G. Adams:** None.

Poster

092. Animal Models: Impairments in Learning and Memory

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 92.04/RR28

Topic: F.02. Animal Cognition and Behavior

Support: NIH R15 Grant HD060122

Title: LLTP stimulation causes long term depression in conditional neuronal Deaf1 knockout mouse hippocampus

Authors: ***A. GHOSH**[1], S. RAJAMANICKAM[1], P. JENSIK[1], M. COLLARD[1], G. ROSE[2]; [1]Physiol., [2]Physiology, Anatomy, CIR-CNS, Southern Illinois Univ. Carbondale, Carbondale, IL

Abstract: Mutations within the DNA binding domain of the DEAF1 gene in humans result in severe intellectual disabilities. Conditional neuronal knockout of Deaf1 in mice result in anxiety like behavior and impaired contextual memory (Am. J. Human Genetics., 2014). This led us to analyze hippocampal plasticity in the knockouts. Briefly, hippocampal slices from male C57Bl/6J mice with floxed Deaf1 allele and positive for Nes-cre (Deaf1fl/fl;Nes-cre) or NKOs and controls (lacking Nes-cre), median age 14 months were used. The animals were anesthetized using isoflurane, after which the brain was quickly extracted and placed in artificial

cerebrospinal fluid (aCSF, composition in mM: 124 NaCl, 3 KCl, 2.5 CaCl2, 1.3 MgSO4, 1.4 NaH2PO4, 26 NaHCO3, 10 dextrose) bubbled with 95% O2 / 5% CO2, pH ~7.3, 0°C. 400 µm-thick coronal slices containing the middle one-third of the hippocampus were cut into 0°C aCSF using a Vibratome. The slices were trimmed to isolate the hippocampus and overlying neocortex and then transferred to an interface-style recording chamber and maintained at 32.5 °C. The slices were perfused with 95% O2/5% CO2-bubbled aCSF (2.5 mL/min flow rate) and humidified 95% O2/5% CO2 gas flowed continuously over their exposed surfaces. Slices were equilibrated for at least 2 hours before recordings began. Schaffer/commissural afferents to CA1 were stimulated using a concentric bipolar stimulating electrode (25µm center diameter, 0.033 Hz base rate). Negative field EPSP slopes were recorded from stratum radiatum using 3-5 MΩ glass recording electrodes filled with aCSF. Baseline responses were set at approximately half-maximal amplitude. Protocols for plasticity studies were: ELTP - a single burst of 15 pulses @ 100 Hz; LLTP - 4 trains of 100 pulses @ 100 Hz with a 5 minute inter-train interval. Baseline responses and ELTP (~120% of baseline 30 minutes after stimulation) were not different between Control and NKO mice. However, LLTP stimulation produced divergent effects in the two groups. In Control mice, fEPSP slopes were enhanced in 70% of recordings (average increase to 190% of baseline, measured 30 and 180 minutes after stimulation). In NKO mice the predominant response was a lasting depression of fEPSP slope, observed in 60% and 90% of cases at 30 and 180 minutes, respectively. Average slopes were reduced to 50% and 30% of baseline at the two time points. Further work is being done to try to explain this paradoxical finding of induction of LTD following LLTP stimulation.

Disclosures: **A. Ghosh:** None. **S. Rajamanickam:** None. **P. Jensik:** None. **M. Collard:** None. **G. Rose:** None.

Poster

092. Animal Models: Impairments in Learning and Memory

Location: Halls A-C

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Program#/Poster#: 92.05/RR29

Topic: F.02. Animal Cognition and Behavior

Support: NIH COBRE 1P20GM103653 - 01A1

Title: Delay dependent effect of Nucleus Reuiens/Rhomboid inactivation on working memory performance

Authors: *D. M. LAYFIELD, M. PATEL, H. L. HALLOCK, A. GRIFFIN;
Univ. of Delaware, Newark, DE

Abstract: Electrophysiological evidence has shown that the hippocampus (HPC) and prefrontal cortex (PFC) functionally synchronize during working memory tasks in rodents, indicating that the two brain structures form a neural circuit that is important for working memory performance (Jones and Wilson 2005, Gordon 2011). Recent evidence suggests a time-dependent functional relationship between HPC and PFC, with functional inactivation studies suggesting that HPC and PFC act together during tasks that require working memory over long delays, and operate in parallel over short delays (Churchwell & Kesner 2011). The nucleus reuniens and rhomboid nucleus (RE/Rh) of the thalamus are anatomically connected to both the HPC and PFC and are thus well positioned to gate the flow of information between them (Vertes, 2006). RE/Rh have been shown to be necessary for the performance of spatial working memory tasks (Hembrook & Mair 2011); however, the extent to which RE/Rh are necessary for the transfer of information between HPC and PFC over both long and short delays remains unclear. If inactivation of RE/Rh produces delay length-specific impairments that parallel those following hippocampal-prefrontal disconnection in working memory tasks, then RE/Rh inactivation should cause a delay-dependent impairment on working memory task performance. To test this prediction, we inactivated RE/Rh using the GABAA agonist muscimol while rats performed one of 3 different tasks: Delayed alternation with a 30-second inter-trial delay period (DA30), delayed alternation with a 5-second inter-trial delay period (DA5), or continuous alternation with no inter-trial delay period (CA). All 3 tasks require rats to alternate between a left and a right goal arm to obtain a reward, relying on their previous choice of direction to determine the location of the next reward, with the difference being the duration over which the rat must remember trial-specific information. We found that RE/Rh inactivation produced a deficit in both DA30 and DA5 groups, but no deficits in the CA group. These results are consistent with previous hippocampal-prefrontal disconnection studies and suggest that the dependence on RE/Rh for working memory tasks increases as the delay period for the tasks increases, providing evidence for a time-dependent component of RE/Rh activity in working memory.

Disclosures: **D.M. Layfield:** None. **M. Patel:** None. **A. Griffin:** None. **H.L. Hallock:** None.

Poster

092. Animal Models: Impairments in Learning and Memory

Location: Halls A-C

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Program#/Poster#: 92.06/RR30

Topic: F.02. Animal Cognition and Behavior

Support: NSF IOS1120395

Neuroplasticity of Aging Training Grant

Medical Research Service of the Department of Veterans Affairs

Title: Rodents with lesions of the medial prefrontal cortex path integrate normally despite working memory deficits

Authors: *M. SAPIURKA[1,4], A. OCAMPO[4,1], L. R. SQUIRE[4,1,2,3], R. E. CLARK[4,1];
[1]Psychiatry, [2]Neurosciences, [3]Psychology, UCSD, San Diego, CA; [4]VAMCSD, San Diego, CA

Abstract: Path integration refers to the ability to use self-motion cues to keep track of a reference location. Earlier studies showed that the hippocampus is critical for path integration in the rodent, even for very short, simple paths that are traversed in 3 seconds. In contrast, humans with damage that includes the hippocampus performed normally on path integration tasks when the paths were simple, presumably by relying on their intact working memory (Kim, Sapiurka et. al. 2013). We explored the possibility that rats with hippocampal damage fail at path integration because the information needed for path integration exceeds the capacity of working memory in the rat and that intact rats must therefore rely on long-term memory to path integrate. We lesioned the medial prefrontal cortex (mPFC) in rats, a structure thought to be important for working memory. Following recovery, we first tested the rats on the forced-choice alternation task. In this task, animals must remember whether they turned left or right in a T-maze on the previous trial in order to enter the alternate arm and receive a food reward. An intertrial interval of 5 seconds was used. Performance was measured as the percentage of correctly alternated trials. We then tested rats on path integration. Rats were placed on a circular table (2m diameter) with eight holes around the perimeter that had removable screens to provide access to a refuge box below the table. On each trial, one box was opened and the rat placed inside. Different holes were used for each trial. In complete darkness the rat left the box and explored the environment until it located a piece of food that had been placed in the interior of the table. The rat then spontaneously returned to the start box in order to consume the food. The time, distance, and number of turns needed to find the food was recorded as well as the accuracy of the return path. Rats with mPFC lesions were impaired on the delayed alternation task but not on path integration. We suggest that in rats path integration requires long-term memory because in the rat the complexity of the spatial information exceeds the capacity of working memory.

Disclosures: **M. Sapiurka:** None. **A. Ocampo:** None. **L.R. Squire:** None. **R.E. Clark:** None.

Poster

092. Animal Models: Impairments in Learning and Memory

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 92.07/RR31

Topic: F.02. Animal Cognition and Behavior

Title: Frustration-based anxiety and magnitude of reward-based pattern separation

Authors: *R. P. KESNER;
Univ. Utah, SALT LAKE CTY, UT

Abstract: In a previous study Salinas et al., 1996) ran rats in a runway task for food reward and focused on recovery time associated with reduced reward in order to measure frustration-based anxiety. Based on previous findings that ventral dentate gyrus (vDG) lesions disrupt anxiety, seven rats with colchicine lesions of the vDG and ten vehicle control rats were trained to run down a runway for 20 food pellets. After reaching running speed asymptote, the two groups of rats received on five consecutive trials 1, 9, or 17 food pellets and at the same time running speed was measured. The order of pellet presentations was counterbalanced. Usually 2-3 days for the vehicle controls were required to re-reach asymptote. The results for the vehicle control rats indicated a linear decrease in running speed as a function of an increase in reward magnitude change. In contrast, vDG lesioned rats maintained the same running speed independent of a change in reward magnitude. The results suggest that the vDG support a frustration-based anxiety effect and a possible frustration-based anxiety pattern separation effect.

Disclosures: R.P. Kesner: None.

Poster

092. Animal Models: Impairments in Learning and Memory

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 92.08/RR32

Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant R01 HD29792

Title: Externalilty in obesity: A hippocampal-dependent phenomenon

Authors: C. H. SAMPLE[1], J. MAK[1], P. MACIVER[1], *L. E. JARRARD[2], T. L. DAVIDSON[1]; [1]Psychology, American Univ., Washington, DC; [2]Washington & Lee Univ., Lexington, VA

Abstract: Hypersensitivity to external food-related cues and hyposensitivity to interoceptive satiety signals has been suggested to play an important role in the development and maintenance of obesity. Previous research showed that consuming a diet high in saturated fat and carbohydrate (i.e., Western diet (WD)) promotes excessive body weight gain and impairs performance on hippocampal-dependent learning and memory problems. Other research showed that hippocampal lesions impaired rats' ability to use interoceptive cues arising from different levels of food deprivation as discriminative signals relative to exteroceptive (e.g., auditory and visual) cues. The current research assessed whether stimulus control of appetitive behavior by food deprivation cues would be reduced relative to control by external stimuli following maintenance of rats on the WD. In Experiment 1, rats were first trained to solve a food deprivation intensity discrimination problem using only interoceptive cues arising from 0- and 24- hr food deprivation as discriminative stimuli for the delivery of sucrose pellets. The rats were then assigned to two groups matched on terminal discrimination performance; one group was maintained for 6 weeks on ad libitum WD and the other on standard lab chow ad libitum. All rats were then tested with their original discriminative deprivation cue contingencies. Following testing, rats were then trained with both external cues and deprivation stimuli as compound discriminative cues. The results showed that discrimination performance based on interoceptive food deprivation cues was impaired only for WD-fed rats. WD-fed rats' performance remained intact when external cues could be used to solve the discrimination. Experiment 2 investigated the effects of exposure to WD prior to the beginning of discrimination training with deprivation and external cues as compound discriminative stimuli. Furthermore, following 20 days on WD or chow, rats were categorized as diet-induced obese (DIO) or diet-resistant (DR). All rats were then tested with and without external cues. WD-fed rats (DIO and DR) were impaired in discriminating based on their food deprivation cues, but maintained significant discrimination when external cues were also present. CHOW rats maintained discrimination throughout the experiment. These results suggest that WD consumption diminished rats' ability to use energy state cues relative to food cues in discriminative control. A WD-induced impairment in the ability to use deprivation cues to control appetitive behavior could underlie excess intake and obesity. This effect may represent a hippocampal-dependent mechanism for externality.

Disclosures: C.H. Sample: None. **L.E. Jarrard:** None. **T.L. Davidson:** None. **J. Mak:** None. **P. MacIver:** None.

Poster

092. Animal Models: Impairments in Learning and Memory

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 92.09/RR33

Topic: B.08. Synaptic Plasticity

Support: Mount Sinai seed fund

Title: Role of FosB/JunD transcription factors in male pathological aggressive behavior in mice

Authors: *H. ALEYASIN[1,2], S. A. GOLDEN[2], M. E. FLANIGAN[2], M. L. PFAU[2], G. E. HODES[2], M. HESHMATI[2], S. J. RUSSO[2];
[1]Dept. of Neurol. and Neurosci., Mount Sinai Sch. of Med., New York, NY; [2]Neurosci., Icahn Sch. of Med. at Mount Sinai, New York, NY

Abstract: Male aggression is an innate social behavior that helps individuals to defend their territory against competitors, as well as increase the probability of successful mating and reproduction with females. Indeed, adaptive aggressive behavior is conserved across most mammalian species. However, the use of extreme violence during aggression is considered pathological and can have devastating consequences on society. In the past couple of years a number of studies implicate reward circuitry as an important modulator of aggressive behavior. However, little is known about the molecular mechanisms modulating the initiation and motivation for aggressive behavior. Our study provides experimental evidence that supports the role of Fos/Jun transcription factors in nucleus accumbens, a key brain reward region, in regulating pathological aggression. Considering the role of these transcription factors in other reward-related behaviors, such as drug addiction, sexual pleasure and alcohol drinking, our data help to understand molecular basis for maladaptive motivational aspects of aggressive behavior in mice.

Disclosures: **H. Aleyasin:** None. **S.A. Golden:** None. **M.E. Flanigan:** None. **M.L. Pfau:** None. **G.E. Hodes:** None. **M. Heshmati:** None. **S.J. Russo:** None.

Poster

092. Animal Models: Impairments in Learning and Memory

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 92.10/RR34

Topic: F.02. Animal Cognition and Behavior

Support: CIHR Grant 37754

Title: *In vivo* MRI studies of structural changes in mouse brain following forced treadmill training

Authors: *B. D. ADDISON[1], A. C. EVANS[2], B. J. BEDELL[2];
[1]McGill Univ., Verdun, QC, Canada; [2]McGill Univ., Montreal, QC, Canada

Abstract: Introduction Environmental enrichment and exercise are known to promote brain plasticity. The cellular underpinnings associated with plasticity-related changes of brain structure, however, remain poorly understood. Previous studies have demonstrated volumetric changes in particular regions of the rodent brain by histological methods. However, a comprehensive assessment of structural alterations following exercise has not been performed. As such, the objective of this study was to perform an exploratory analysis of changes in regional cortical thickness and neuroanatomical volumes from longitudinal, *in vivo*, mouse brain magnetic resonance imaging (MRI) data following a short-term exercise regimen. Methods Three month-old, male, CD1 mice were randomly assigned to two groups. The exercise group (n =15 mice) was exposed to six weeks of forced treadmill (FT) exercise. The baseline treadmill speed was 24 cm/s, which was increased by 1cm/s per week. The control group (n = 12 mice) did not undergo treadmill exposure (NX) during this six week interval. Anatomical MRI scans were acquired at baseline and following completion of the six week exercise regimen. Scanning was performed on a 7T Bruker Pharmascan MR system. The MRI data was processed using a fully-automated image processing pipeline to generate regional cortical thickness (5 regions) and neuroanatomical volumetric (7 regions) measures. Group differences were analyzed by two-tailed t-tests with Bonferroni correction for multiple comparisons. Results Analysis of volume measurements taken from baseline and post-exercise scans revealed that six weeks of FT exercise resulted in a significantly greater increase in hippocampal volume (13.74%) compared to NX controls (4.13%) ($p < 0.001$). The amygdala showed an increase in volume in the FT group (6.21%) compared to a slight decrease in the NX controls (-1.39%) ($p < 0.01$). No significant differences in cortical thickness changes were identified. Conclusions We observed a statistically significant increase in the volumes of the mouse hippocampus and amygdala following six weeks of FT exercise. Interestingly, none of the cortical regions, including motor cortex, demonstrated significant differences between FT and NX groups. Correlative quantitative immunohistochemistry (qIHC) studies are currently underway to elucidate the cellular changes (*e.g.* increased synaptic density, astroglial activation) underlying the MRI-defined increases in regional brain volumes.

Disclosures: **B.D. Addison:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Biospective Inc. **B.J. Bedell:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Biospective Inc. **A.C. Evans:** E.

Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Biospective Inc.

Poster

092. Animal Models: Impairments in Learning and Memory

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 92.11/RR35

Topic: F.02. Animal Cognition and Behavior

Support: NSERC

Title: Retrograde ventral hippocampal lesions after visual discrimination training impair context-specific conditioned inhibition

Authors: *R. J. BALOG[1], D. BENIS[2], N. S. HONG[2], J. TROW[2], S. H. DEIBEL[2], R. J. MCDONALD[2];
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Abstract: It is well documented that during simple discrimination learning an excitatory association is accrued to the reinforced cue and an inhibitory association is acquired to the non-reinforced cue. We have utilized a visual discrimination task developed for the 8-arm radial maze and shown that excitatory instrumental conditioning was dependent on the dorso-lateral striatum and was not context specific. The inhibitory association acquired to the non-reinforced cue was mediated by the ventral hippocampus and was context-specific. The latter finding suggested that the ventral hippocampus had to be intact for rats to acquire this kind of context-specific conditioning. However, it was unclear what would happen if the lesion was induced in the retrograde direction. Our hypothesis was that the ventral hippocampus is both the storage site and key retrieval portal for these types of inhibitory associations and so we predicted that ventral hippocampal lesions induced after visual discrimination training should impair the expression of context-specific inhibitory associations as well. In the present study we trained a group of normal rats to asymptotic levels of performance on the visual discrimination task and then half of the animals were given neurotoxic lesions of the ventral hippocampus. After recovery, all groups were given reversal learning in the same context as original training. Rats with ventral hippocampal lesions reach asymptote on the reversal phase of training faster than normal rats. We have previously shown that normal rats needed extensive amounts of training in the original training context compared to rats reversed in a different context. This effect, combined with

other evidence, suggested that the non-reinforced cue during original training became a conditioned inhibitory and that it was context-specific. Combined with other work, these results suggest that the ventral hippocampus is required for the acquisition and expression of context-specific conditioned inhibition.

Disclosures: **R.J. Balog:** None. **D. Benis:** None. **N.S. Hong:** None. **J. Trow:** None. **S.H. Deibel:** None. **R.J. McDonald:** None.

Poster

092. Animal Models: Impairments in Learning and Memory

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Topic: F.02. Animal Cognition and Behavior

Title: Behavioral phenotypes differ depending on background mouse strain

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Abstract: This research follows in the footsteps of Wimer, Wimer, Crawley, Belknap, Wahlsten and others that have published data indicating differences between inbred strains of mice on both behavioral and histological variables. As researchers continue to create genetically manipulated rodent models of disease and disorders, and to assess the impact of endocrine disrupting chemicals (EDC) and toxins in our environment, it is increasingly important to know the abilities of your underlying background strain. EDC are known to reverse sex differences, but that means there has to be a sex difference in the strain you are treating to begin with, for example. We have tested hundreds of animals from a variety of strains on a basic battery of open field activity, simple water maze escape, Morris water maze and complex pattern discrimination. Data indicate that the strains do not have similar performance across the board. The background strain for a transgenic model of Alzheimer's Disease outperform even the standard C57BL/6 workhorse - this has serious implications for the impact of the AD genes. If you have a "bright" strain, there is perhaps less of a chance that the genetic manipulation will impair performance. In addition, the sex differences across the strains are not similar, despite blanket statements in the literature that males and females differ on some tasks. Data on several strains will be presented along with discussion of having a strong foundation of knowledge on background strain and behavioral testing ability.

Disclosures: **A. Stavnezer:** None.

Poster

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Title: Adult behavioral consequences of prenatal nanomaterial exposure

Authors: *E. B. ENGLER-CHIURAZZI[1,2], J. J. STALNAKER[1,2], X. REN[1,2], H. HU[1,2], S. N. SARKAR[1,2], S. JUN[1,2], D. D. QUINTANA[1,2], P. A. STAPLETON[1], T. R. NURKIEWICZ[1], C. M[1], J. YI[1], J. W. SIMPKINS[1,2];
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Abstract: Engineered nanomaterials (ENM), such as titanium dioxide (TiO_2), are commonly used and have tremendous clinical and commercial potential. However, systemic and central toxicities during the critical period for early nervous system development are unclear. Prenatal ENM exposure (maternal TiO_2 inhalation) alters the uterine microvascular environment and influences birth outcomes (Stapleton et al., 2013). Further, prenatal TiO_2 via maternal injection accumulates in cranial nerves (Takeda et al., 2009), is associated with alterations in genes related to cell death, mitochondrial function, oxidative stress, and apoptosis, and increases dopamine in the prefrontal cortex and striatum of 3-6 week old offspring (Shimizu et al., 2009; Takahashi et al., 2010). Only one study has evaluated the effects of prenatal TiO_2 inhalation exposure on adult cognitive outcomes, finding that exposure was associated with decreased center time in the open field (a measure of increased anxiety) and altered pre-pulse inhibition (Hougaard et al., 2010). The objective of this study was to determine if prenatal ENM exposure (via maternal TiO_2

inhalation during gestation) affects adult brain and behavior. Pregnant rats were exposed to aerosolized TiO_2: 11.3 +/-0.039 mg/m^3 for 5 hours/day for eight days. The final maternal deposition was 85 +/-3 µg. At five months, cognitive behavior was assessed in male pups with a battery of cognitive tests selected to tap into several unique mnemonic functions, including the anxiety-like behavior (open field, elevated plus maze), spatial navigation memory (Morris water maze, radial arm water maze), locomotor coordination (rotarod), and depressive-like behavior (forced swim test). While we observed no impact of exposure to prenatal TiO_2 via maternal inhalation on measures of anxiety-like behavior, locomotor coordination, nor depressive-like behavior, we did observe a spatial navigation memory impairment on the water radial arm maze. Specifically, animals that experienced prenatal TiO_2 exposure had higher working memory correct errors relative to unexposed control rats. This behavioral change could be accounted for by the dysregulation of synaptic plasticity-related proteins and proteins mediating inflammation. We therefore assessed the potential dysregulation of these proteins by measuring the expression of microRNA in serum. Preliminary data indicate that microRNA associated with the inflammatory response are altered in TiO_2-exposed animals. These findings are consistent with the notion that prenatal ENM exposure imparts alterations in cognitive behaviors that ultimately manifest in adulthood.

Disclosures: **E.B. Engler-Chiurazzi:** None. **J.J. Stalnaker:** None. **X. Ren:** None. **H. Hu:** None. **S.N. Sarkar:** None. **S. Jun:** None. **D.D. Quintana:** None. **P.A. Stapleton:** None. **T.R. Nurkiewicz:** None. **C. M:** None. **J. Yi:** None. **J.W. Simpkins:** None.

Poster

092. Animal Models: Impairments in Learning and Memory

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Topic: F.02. Animal Cognition and Behavior

Support: CIHR 133693

NSERC 386686-10

CFI-LOF 25026

Title: The lateral entorhinal cortex exhibits a highly selective code that integrates physical and relational aspects of sensory stimuli

Authors: *M. PILKIW[1], N. INSEL[2], Y. CUI[3], S. SARKAR[2], M. MORRISSEY[2], K. TAKEHARA-NISHIUCHI[2];
[1]The Psychology Dept., [2]Univ. of Toronto, Toronto, ON, Canada; [3]Med. Col. of Soochow Univ., Jiangsu, China

Abstract: The relevance of environmental correlations is often not immediately obvious, and it is therefore advantageous for the brain to build associative maps that are rich in incidental details. The lateral entorhinal cortex (LEC) may be a key hub for these maps. Lesions of the LEC disrupt behavioral expression of associations that predict upcoming reinforcers (Morrissey et al., 2012; Tanninen et al., 2013), as well as those associations that do not (Wilson et al., 2013). Here we examine how single neurons and neuron populations in the LEC encode both conditioned and non-conditioned associations and compare these patterns with those observed in the primary auditory cortex (AUC). Each recording session contained three conditioning epochs of 70 trials. In the first 20 trials, stimuli were presented alone. In the following 50 trials, a trace-eyeblink paradigm was used, in which a conditioned stimulus (CS) was paired with electrical stimulation of the eyelid (the unconditioned stimulus, or US) separated by a 500 ms trace interval. Between epochs, rats moved between two visually and spatially distinct conditioning chambers, and in the final epoch the CS was switched from an auditory to a visual stimulus. We found that about one half of the recorded LEC neurons changed firing rates upon CS presentation, and a large majority of these signaled a conjunction of trial type, conditioning chamber, and modality of the CS. In contrast, no neurons recorded from the AUC were selective for the conjunction of all three dimensions: most fired at the onset of the auditory CS and a small, partially overlapping set discriminated between environmental contexts. These patterns were also apparent at the neuron population level: in the LEC, the population state vectors during CS presentation were relatively uncorrelated between epochs and trial types, while in the AUC, state vectors were highly correlated for trials in which the auditory CS was used. These results suggest that the LEC forms a highly conjunctive code that integrates the relational and physical features of environmental stimuli, including information that is not necessarily predictive of upcoming reinforcers.

Disclosures: **M. Pilkiw:** None. **N. Insel:** None. **Y. Cui:** None. **S. Sarkar:** None. **M. Morrissey:** None. **K. Takehara-Nishiuchi:** None.

Poster

092. Animal Models: Impairments in Learning and Memory

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Program#/Poster#: 92.15/RR39

Topic: F.02. Animal Cognition and Behavior

Support: NSERC 386686

CFI-LOF 25026

Title: Cholinergic, but not NMDA, receptors in the lateral entorhinal cortex mediate acquisition in trace eyeblink conditioning

Authors: *K. TAKEHARA-NISHIUCHI, X. YU, T. GIRITHARAN, L. TRAN, R. BAKIR, M. D. MORRISSEY, S. N. TANNINEN;
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Abstract: Anatomical and electrophysiological studies collectively suggest that the entorhinal cortex consists of several sub-regions, each of which is involved in the processing of different types of information. Consistent with this idea, we previously reported that the dorsolateral portion of entorhinal cortex (DLE), but not the caudomedial portion, is necessary for the expression of memory association between two temporally discontiguous stimuli in trace eyeblink conditioning (Morrissey et al., 2012; Tanninen et al., 2013). The present study examined whether memory acquisition depends on the DLE and what types of local neurotransmitter mechanisms are involved in acquisition and expression. Male Long-Evans rats received trace eyeblink conditioning, in which an auditory conditioned stimulus (CS) was paired with a mildly aversive electric shock to the eyelid (US), following a microinfusion of neuro-reactive substances into the DLE. Reversible inactivation of the DLE with GABAA receptor agonist, muscimol, significantly impaired memory acquisition when the interval between the CS and US was 500 msec, but not when the interval was shortened to 250 msec. Furthermore, blockade of local muscarinic acetylcholine receptors (mACh) with scopolamine retarded the acquisition of CS-US association with a 500-msec interval whereas blockade of local NMDA receptors with APV had no effect. In contrast, memory expression was not impaired by either type of receptor blockers. These results suggest that the DLE is necessary for memory acquisition only when the length of temporal gap between the paired stimuli is sufficiently long, and that acquisition depends on the integrity of local cholinergic, but not NMDA, receptors.

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Poster

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Topic: F.02. Animal Cognition and Behavior

Support: Alzheimer Association Grant NIRG-12-237032

CFI Grant CFI-LOF 25026

CIHR Grant 133693

Title: The overexpression of mutated tau in the entorhinal cortex: Its effects on local neurons, cortical theta oscillations, and memory

Authors: *S. E. TANNINEN[1], M. D. MORRISSEY[1], R. L. KLEIN[2], K. TAKEHARA-NISHIUCHI[1];
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Abstract: The accumulation of hyper-phosphorylated tau protein in the entorhinal cortex is one of the first abnormalities observed in Alzheimer's disease (AD). The entorhinal cortex is reciprocally connected with many cortical regions critical for memory formation and expression. Entorhinal tau pathology would therefore disrupt the transfer of information within the cortical memory network, resulting in abnormal neural activity which may lead to memory impairments in AD. To test this hypothesis, we expressed, through transduction with a viral vector, an excess of human tau with the P301L mutation (Tau rats) or green fluorescent protein as a control (GFP rats), specifically in the entorhinal cortex of adult rats. We then tested the rats' ability to acquire an associative memory in trace eyeblink conditioning while monitoring local field potentials in the hippocampus, a major efferent target of the entorhinal cortex. One month after transduction, approximately 20% of entorhinal neurons were filled with hyper-phosphorylated tau and the area adjacent to the injection sites showed signs of astrogliosis and neurofibrillary tangles. When tested in trace eyeblink conditioning, the Tau rats formed the association between a tone and eyelid stimulation in a comparable manner to the GFP rats; however, the hippocampus of the Tau rats had relatively smaller amplitude of theta oscillations during conditioning in comparison to GFP rats. Thus, minor entorhinal tau pathology alters hippocampal neuronal activity even before memory impairments become apparent.

Disclosures: **S.E. Tanninen:** None. **M.D. Morrissey:** None. **R.L. Klein:** None. **K. Takehara-Nishiuchi:** None.

Poster

092. Animal Models: Impairments in Learning and Memory

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Support: NIMH Z01-MH-002498

NIEHS Z01-ES-100222

Title: Role of the vasopressin 1b receptor in rodent aggressive behavior and synaptic plasticity in hippocampal area CA2

Authors: *S. YOUNG[1], J. H. PAGANI[1], M. ZHAO[2], Z. CUI[1], S. K. WILLIAMS AVRAM[1], D. A. CARUANA[2,3], S. M. DUDEK[2];
[1]NIMH, NIH, DHHS, Bethesda, MD; [2]NIEHS, NIH, DHHS, Triangle Park, NC; [3]Keele Univ., Keele, Staffordshire, United Kingdom

Abstract: The vasopressin 1b receptor (Avpr1b) is critical for social memory and social aggression in rodents, yet little is known about its specific roles in these behaviors. Some clues to Avpr1b function can be gained from its profile of expression in the brain, which is limited to the pyramidal neurons of the CA2 region (and immediately adjacent CA3 region) of the hippocampus, and from experiments showing that inactivation of the gene or antagonism of the receptor leads to a reduction in social aggression. Here we show that partial replacement of the Avpr1b through lentiviral delivery into the dorsal CA2 region restored the probability of socially motivated attack behavior in total Avpr1b knockout mice, without altering anxiety-like behaviors. To further explore the role of the Avpr1b in this hippocampal region, we examined the effects of Avpr1b agonists on pyramidal neurons in mouse and rat hippocampal slices. We found that selective Avpr1b agonists induced significant potentiation of excitatory synaptic responses in CA2, but not in CA1 or in slices from Avpr1b knockout mice. In a way that is mechanistically very similar to synaptic potentiation induced by oxytocin, Avpr1b agonist-induced potentiation of CA2 synapses relies on NMDA receptor activation, calcium and calcium/calmodulin-dependent protein kinase II activity, but not on cAMP-dependent protein kinase activity or presynaptic mechanisms. Our data indicate that the hippocampal CA2 is important for attacking in response to a male intruder and that the Avpr1b, through its role in regulating CA2 synaptic plasticity, is a necessary mediator. This research was supported by the NIMH (Z01-MH-002498-24) and the NIEHS (Z01-ES-100222) Intramural Research Programs.

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Poster

092. Animal Models: Impairments in Learning and Memory

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Topic: F.02. Animal Cognition and Behavior

Support: East of Scotland Bioscience Doctoral Training Partnership (EASTBIO) BBSRC

Scottish Government's Rural and Environment Science and Analytical Services Division (RESAS)

Title: Episodic-like memory is rapidly compromised by a high-fat diet in C57Bl/6 mice and is associated with markers of hippocampal neuronal damage identified by proteomics

Authors: *F. H. MCLEAN[1], R. F. LANGSTON[2], F. M. CAMPBELL[1], A. LORENZO-ARRIBAS[3], L. M. WILLIAMS[1];
[1]RINH, Univ. of Aberdeen, Aberdeen, United Kingdom; [2]Div. of Neurosci., Univ. of Dundee, Dundee, United Kingdom; [3]Biomath. and Statistics Scotland, Univ. of Aberdeen, Aberdeen, United Kingdom

Abstract: Obesity and type 2 diabetes are associated with increased risk of dementia and Alzheimer's disease (Luchsinger et al 2009 J Alzheimer's Dis 16:693-704). A high-fat diet (HFD) is known to cause memory loss in rodents; however, complex, episodic-like memory has not been tested in response to HFD. Episodic memory is the recollection of events using a "what-where-which" experience and is lost early in Alzheimer's disease (Eacott et al 2004 J Neurosci 24:1948-1953, Easton et al 2012 J Learn and Mem 19:146-150, Swainson 2001 Dem and Ger Cog Dis 12:265-280). To identify a link between a HFD and episodic memory loss, 12 week old, male, C57Bl/6 mice, were fed either a HFD (60% energy from fat) or a LFD diet (10% energy from fat) *ad libitum* and tested daily for 2 weeks with an object-place-context (OPC) recognition memory task. This task challenges episodic-like hippocampal dependent memory, with rats bearing hippocampal lesions showing OPC deficits (Langston et al 2010 Hippo 20:1139-1153), as well as the 3xTgAD mouse model of Alzheimer's disease being impaired in this task (Davis et al 2013 J Alzheimer's Dis 33:681-698). We also used additional behavioural tests alongside the OPC task; a novel object recognition task (NOR), an object-place task (OP) and an object-context task (OC). The NOR task uses the perirhinal cortex, and the OP and OC tasks have recently been hypothesised to activate the lateral entorhinal cortex and postrhinal cortex (Eacott et al 2004 J Neurosci 24:1948-1953, Wilson et al 2013 Hippo 23:352-366). We used the OP and OC tasks as they test components of the OPC task which do not depend on the hippocampus.

Animals were killed by exsanguination, under terminal anaesthesia, after 3 days, 1 week or 2 weeks on diet. Brains were rapidly removed and frozen over dry ice until the hippocampus was dissected for proteomic analysis. A separate group of mice underwent intraperitoneal glucose tolerance tests (IPGTTs) as a non-recovery procedure. We found that episodic-like memory is compromised after only 1 week of a HFD together with OP and OC, however, the ability to carry out the NOR was preserved. IPGTT showed that glucose tolerance was compromised after 3 days on HFD. These results indicate that functions of the lateral entorhinal and postrhinal cortex and hippocampus are compromised rapidly by HFD. Proteomic analysis of the hippocampus revealed changes in a number of proteins associated with neuronal damage after only 3 days on HFD. These data link HFD to the rapid induction of glucose intolerance, indices of hippocampal damage and memory deficit and have implications for the link between diet, obesity and cognitive decline.

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Poster

092. Animal Models: Impairments in Learning and Memory

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Topic: F.02. Animal Cognition and Behavior

Support: NSERC

Title: Antagonizing dorsal hippocampal dopamine D1-type receptors with SCH23390 affects social learning and social interactions but not food intake in male and female mice

Authors: *R. MATTA, A. N. TIESSEN, M. M. KIVLENIEKS, A. M. MEERSSEMAN, Y. O. ADJEI-AFRIYIE, E. CHOLERIS;
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Abstract: The neurotransmitter dopamine (DA) is involved in many motivationally relevant behaviors, such as drug/alcohol addiction, as well as social learning and feeding behavior. With systemic studies, our lab has previously implicated DA D1-type receptors in the social transmission of food preferences (STFP) (Choleris et al., 2011). However, where DA D1-type receptors are acting in the brain to influence social learning remains unknown. The ventral tegmental area has direct dopaminergic neuronal projections to many limbic structures, such as

the amygdala, nucleus accumbens, and the hippocampus. The latter is important for learning and memory, as well as social learning in the STFP. In the present study, we microinfused the DA D1-type antagonist SCH23390 (at 1, 2, 4 and 6 μg/μL) directly into the Cornu Ammonis 1 (CA1) region of the dorsal hippocampus of adult male and female CD-1 mice. Infusions were 15min before a 30 minute social interaction where mice had the opportunity to acquire a food preference from a recently fed same-sex conspecific. We found that females infused with 4 and 6 μg/μL and males infused with 1, 4 and 6 μg/μL of SCH23390 failed to acquire the socially learned food preference. In addition, the total amount of food consumed was not influenced by D1-type inhibition. Video analysis of the social interactions also revealed that the social learning impairment could not be explained by a reduced exposure to the food odor found on the breath of the demonstrator conspecific, since oronasal investigation was not influenced by SCH23390 treatment. Further analysis revealed that together with blocking social learning, SCH23390 also reduced agonistic related behaviors in males, and social investigatory related behaviors in females. A follow-up olfactory discrimination task also showed that mice infused with the highest dose of SCH23390, at 6 μg/μL, that blocked the STFP, could distinguish between the two food types used in the social learning test. Thus, DA D1-type receptors in the CA1 may be mediating social learning, specifically and not via other behavioral or sensory effects. This study highlights the potential importance that hippocampal dopamine plays in the 'social brain'.

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Poster

092. Animal Models: Impairments in Learning and Memory

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Topic: F.02. Animal Cognition and Behavior

Title: Neurostructural analysis of the hippocampal dentate gyrus neurons in the intraventricularly streptozotocin-injected rats

Authors: *A. S. SHINGO[1], S. KITO[2], T. MURASE[1];
[1]Okinaka Mem. Inst. For Med. Res., Minato-Ku, Tokyo, Japan; [2]Chigasaki Tokushu-kai Clin., Chigasaki, Japan

Abstract: Resent epidemiological studies have associated type 2 diabetes mellitus with an increased risk of developing Alzheimer's disease (AD). Previously, we examined the effects of

intracerebral administration of streptozotocin (STZ) on cognitive performance in rats. The STZ-treated rats, one of the AD models showed significant declines in all the parameters of the MWM task compared to control rats. Immunohistochemical analysis using hippocampal formations revealed decreases in pCREB, Akt, somatostatin, insulin receptor, and insulin-degrading enzyme immunoreactivities and a meaningful increase in amyloid beta immunoreactivity that was especially marked in the crest of the dentate gyrus. An intraventricular injection of a long acting insulin analogue improved these STZ-induced behavioural and immunohistochemical changes In this study, morphological analysis of the granule cell layer neurons of the dentate gyrus using COX-Golgi stain and spine density counting was done since Alzheimer disease is a disease of synaptic failure in the hippocampus. After the intracerebral STZ injection, there was the decrease of the ratio to which the granule cell layer fills the space that was recovered by intracerebral insulin administration, In addition, the STZ-3V-treated rats showed diminished spine densities in all the 3 types of spines. It is concluded that in the STZ-3V-rats, there are injuries in the primary recipients of excitatory input into the granule cell layer through the perforant pathway.

Disclosures: **A.S. Shingo:** None. **S. Kito:** None. **T. Murase:** None.

Poster

092. Animal Models: Impairments in Learning and Memory

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Topic: F.02. Animal Cognition and Behavior

Title: Damage to the retrosplenial cortex impairs context discrimination

Authors: J. S. ADELMAN, *S. ROBINSON;
Oberlin Col., Oberlin, OH

Abstract: The retrosplenial cortex (RSP) provides visuospatial sensory information to the hippocampal memory system in rodents and primates. Consistent with a role for RSP in hippocampal-dependent learning and memory, RSP lesions produce deficits in contextual fear learning and sensory preconditioning in rats. Together, these findings support the view that RSP may be involved in the formation and recollection of associations between diverse sensory stimuli which contribute to contextual representations. The present study combined electrolytic lesions of RSP and a context discrimination paradigm to further assess the contribution of RSP to context-based learning. During the first training phase of the procedure one auditory stimulus was presented in Context A whereas a second auditory stimulus was presented in Context B. In

the second training phase, one auditory stimulus was paired with footshock in Context C whereas the second auditory stimulus was presented in Context C, but was not paired with footshock. During the context test phase, rats were exposed to Context A and B and freezing levels were evaluated. During a fourth and final phase, freezing behavior in response to the auditory stimuli was evaluated while rats were in Context C. Control rats exhibited more freezing behavior in the context where they heard the auditory cue that was paired with footshock compared to the context where they heard the unpaired cue, indicating that stimulus-stimulus associations had been formed during the first phase of training. In contrast, RSP-lesioned rats failed to show context discrimination, but maintained the ability to discriminate between the individual auditory stimuli. These data support the notion that RSP is involved in forming associations between neutral stimuli even in the absence of reinforcement.

Disclosures: **J.S. Adelman:** None. **S. Robinson:** None.

Poster

093. Learning and Memory: Physiology I

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NSF IIS-0643995

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Title: Real-time decoding of hippocampal replay events

Authors: ***D. F. LIU**[1], X. DENG[3], M. KARLSSON[2], U. EDEN[4], L. FRANK[2];
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Abstract: The hippocampus plays an essential role in learning and memory-guided decision making. Previous results have established the importance of hippocampal activity during sharp-wave ripple (SWR) events. Disruption of SWRs during sleep can impair performance the next day, suggesting a role in consolidation. Similarly, disruptions of SWRs during awake behavior causes specific learning and memory deficits associated with trials when animals need to link multiple experiences across time. During SWRs, sequences of place cells are "replayed", recapitulating sequences seen during past experience. While the presence of replay during SWRs is well established, the role of the specific information content of these sequences for learning and decision making processes remains unknown. We have therefore developed a real-time decoding system that makes it possible to identify the content of SWRs with low latency and then interrupt them before they would naturally terminate, degrading the related information available in downstream regions. For this system we optimized the speed of algorithms for cluster-less spike encoding and decoding (Kloosterman et al., 2013). The software architecture utilizes high performance computing techniques, such as parallel computing across networked clusters implemented through the Message Passing Interface (MPI) Protocol. MPI allows the scalable distribution of calculations to encode and decode across many recording channels. A simulation framework was developed to evaluate the system on raw data collected from animals learning a hippocampal-dependent W-track alternation task. Using data collected from 6 tetrodes implanted in an animal, we are able to estimate the content being replayed with an average lag of ~15ms (~10ms mode). These results show we are capable of detecting and disrupting SWRs that encode information related to a specific experience, with short enough latency to disrupt the processing of that information in downstream areas. This technology will allow for investigations of the causal role SWR memory replay in learning, memory, and decision making processes. Kloosterman, F., Layton, S.P., Chen, Z., and Wilson, M.A. (2013). Bayesian Decoding using Unsorted Spikes in the Rat Hippocampus. J Neurophysiol.

Disclosures: **D.F. Liu:** None. **X. Deng:** None. **M. Karlsson:** None. **U. Eden:** None. **L. Frank:** None.

Poster

093. Learning and Memory: Physiology I

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Support: NIH-NIDA 5R01DA026297-04

NIH-NEI 5R01EY017658-05

Supplement from DA026297 to DS-M

Young Investigator Grant from the Brain & Behavior Research Foundation (NARSAD) to DS-M

Title: Neurons in the primate nucleus basalis signal error during associative learning

Authors: *C. MARTINEZ-RUBIO[1], O. J. AHMED[1], D. SIERRA-MERCADO[1,2], E. N. ESKANDAR[1];
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Abstract: Reward and motivation have an important role in learning. The Nucleus Basalis of Meynert (NBM) is a group of neurons found in the substantia innominata of the basal forebrain that provides cholinergic innervation to the neurons of the cerebral cortex. These cortical cholinergic innervations have been implicated in behavioral and cognitive functions, such as learning and motivation. However, the neurophysiological mechanisms by which the NBM is involved in learning and motivational control is unclear. To address this issue, we recorded neural activity in the NMB using single-unit recordings in behaving non-human primates (NHPs) during an associative learning task. During this task, the animal was required to learn, by trial and error, to associate a visual image with a specific direction of eye movement. Our results show that the firing rate of 34% of neurons in NBM differentiate between incorrect and correct choices. Together, these results provide physiological support for the idea that the NBM is necessary for associative learning.

Disclosures: C. martinez-Rubio: None. **O.J. Ahmed:** None. **D. Sierra-Mercado:** None. **E.N. Eskandar:** None.

Poster

093. Learning and Memory: Physiology I

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Program#/Poster#: 93.03/RR48

Topic: F.02. Animal Cognition and Behavior

Support: CONACyT Grant 155242

DGAPA Grant IN-209413

Title: Amygdalar NMDAr and AMPAr activity on aversive memory retrieval: neurochemical modulation in the insular cortex

Authors: *D. OSORIO-GÓMEZ[1], K. R. GUZMAN-RAMOS[2], A. LUYEN DIAZ[1], F. BERMUDEZ-RATTONI[1];
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Abstract: Memory retrieval involves the quick reactivation of neural circuits that were modified during learning. It is widely known that AMPA and beta-adrenergic receptors activity modulates aversive memories retrieval, whereas the activation of NMDA receptors takes part in the memory consolidation process. There is plenty of evidence that the insular cortex (IC) and the amygdala (Amy) have a key role in the acquisition, consolidation and retrieval of conditioned taste aversion (CTA), a learning paradigm where animals associate a novel taste (CS) with gastric malaise, decreasing CS intake in further presentations. Previously, we have demonstrated an interaction between Amy and IC which contributes to memory consolidation and retention of CTA, but it is still unknown whether this interaction participates in CTA retrieval. In this study, we measured through *in vivo* microdialysis, extracellular levels of Glu and norepinephrine (Ne) in the IC during CTA retrieval. Male Wistar rats were implanted with a guide cannula aimed to the IC, changes in extracellular level of Glu and Ne were monitored at CTA retrieval using a capillary electrophoresis method based on micellar electrokinetic chromatography. During CTA retrieval, significant increments in Glu and Ne within the IC were related to the exposure to CS. To evaluate the role of Ne and Glu in CTA retrieval, bilaterally infusions of saline solution, AP5 (10ug/uL), CNQX (1ug/uL) or Propranolol (5ug/uL) in the IC were given. Inactivation of AMPA receptors or beta-adrenergic receptors in the IC impaired memory retrieval, neither saline solution nor AP5 affected memory recall. Many studies have demonstrated that retrieval process in the amygdala is mediated by the activation of AMPA receptors, but not NMDA. Our data have shown that the IC also uses the same mechanisms for retrieval. However, the Amy-IC interaction seems to be very important for taste aversive information recovery, so we inactivated AMPA or NMDA receptors in the Amy before retrieval and measured extracellular levels of Glu and Ne within IC during presentation of the CS; our data showed that inactivation of amygdalar NMDA receptors impairs only Glu release but does not disrupt memory recall. Interestingly, infusion of AMPA receptors antagonist in the Amy inhibits memory retrieval, as well as Glu and Ne release disruption within IC. These results suggest that impairment in aversive memory retrieval with AMPA receptors inactivation within the amygdala is through inhibition of norepinephrine release in the insular cortex.

Disclosures: **D. Osorio-Gómez:** None. **K.R. Guzman-Ramos:** None. **A. Luyen Diaz:** None. **F. Bermudez-Rattoni:** None.

Poster

093. Learning and Memory: Physiology I

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 93.04/RR49

Topic: F.02. Animal Cognition and Behavior

Title: Neuronal oscillations in the rat hippocampus during object-in-location recognition memory

Authors: *J. B. TRIMPER[1], C. R. GALLOWAY[1], M. G. FARINA[2], N. A. HERNANDEZ[3], J. R. MANNS[1];
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Abstract: Neuronal oscillations in the hippocampus have been found previously to correlate with recognition memory performance in rats and monkeys. For example, in a study of rat novel object recognition memory (Trimper et al., 2014; Hippocampus, 24, 341-353), increased coherence between oscillations in hippocampal subregions CA3 and CA1 in the low gamma range (30-55 Hz) during novel object exploration correlated with subsequent memory for those objects. It was unclear whether the increase in CA3-CA1 low gamma coherence for a well-remembered object in that study reflected memory for the object's identity, for the object's location, or for an association between the object and its location. In the present study, we adapted the novel object recognition memory task used in that previous study to distinguish between objects that were subsequently remembered poorly (poor memory), objects for which the object identity but not location was remembered (object-only), and objects for which both the object identity and location were remembered (object-in-location). Local field potentials were recorded in the pyramidal layer of regions CA3 and CA1 in rats. Levels of CA3-CA1 low gamma coherence during novel object exploration were similar for object-only and object-in-location memory conditions. These results suggest that this particular correlate of recognition memory might not distinguish between different types of recognition memory judgments (e.g., object-only vs. object-in-location) and may instead reflect memory strength more generally.

Disclosures: J.B. Trimper: None. **C.R. Galloway:** None. **M.G. Farina:** None. **N.A. Hernandez:** None. **J.R. Manns:** None.

Poster

093. Learning and Memory: Physiology I

Location: Halls A-C

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Program#/Poster#: 93.05/RR50

Topic: F.02. Animal Cognition and Behavior

Support: FIRST program

Title: Brain structural changes through long-term learning of tool use supported by sustained motivation for tool use in adult non-human primates

Authors: *A. IRIKI[1], Y. YAMAZAKI[3,1], K. HIKISHIMA[4,5], M. SAIKI[1], M. INADA[1], E. SASAKI[4,5], R. LEMON[6], C. PRICE[7], H. OKANO[4,2];
[1]RIKEN Brain Sci. Inst., Wako-shi, Saitama, Japan; [2]RIKEN Keio Univ. Joint Res. Lab., RIKEN Brain Sci. Inst., Saitama, Japan; [3]Advanced Res. Centers, [4]Dept. of Physiol., Keio Univ., Tokyo, Japan; [5]Central Inst. for Exptl. Animals, Kanagawa, Japan; [6]Sobell Dept. of Motor Neurosci. and Movement Disorders, UCL Inst. of Neurol., London, United Kingdom; [7]Wellcome Trust Ctr. for Neuroimaging, Univ. Col. London, London, United Kingdom

Abstract: Acquisition of new behavior requires formation and activation of new brain networks, which are supported and maintained by active interaction between organisms and environments for long periods of time. We developed a step-by-step protocol to train common marmosets to use a rake-shaped tool to retrieve food: almost one year of training was needed to acquire this new skill. Then we explored volumetric changes in the brain structure of the trained marmosets as they learnt this new technology. Structural MRI scans were performed before, during, and after tool-use training. Long term training of tool-use behavior induced volumetric changes in several brain structures which clearly differed from those of short term training. The volume of nucleus accumbens (Acb) increased in the later phases of the training, and was significantly positively correlated with performance indices, but not with the number of reinforcers earned. So this change could reflect increased motivation for tool use as marmosets learned to use the rake effectively. We found other brain regions with increased volume including visual areas (V2-V3), superior temporal areas, and the anterior trunk of the corpus callosum. The latter has never previously been reported to increase through motor skill training. White matter changes also occurred in the middle and inferior cerebellar peduncles which could reflect the sustained capacity for tool use long after training was completed. These unique areas reflected the behavioral and motivational changes during long-term training, both for acquisition and maintenance of a newly developed skill.

Disclosures: **A. Iriki:** None. **K. Hikishima:** None. **M. Saiki:** None. **M. Inada:** None. **E. Sasaki:** None. **R. Lemon:** None. **C. Price:** None. **H. Okano:** None. **Y. Yamazaki:** None.

Poster

093. Learning and Memory: Physiology I

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Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant K99 MH100284

NIH R01 MH090188

EMBO Postdoctoral Fellowship

ELSC Postdoctoral Fellowship

Title: Coordinated awake reactivation of behaviorally related hippocampal-prefrontal ensembles

Authors: *S. P. JADHAV, G. ROTHSCHILD, D. K. ROUMIS, I. GROSSRUBATSCHER, L. M. FRANK;
Dept. of Physiology, Sandler Neurosci. Ctr., Univ. of California San Francisco (UCSF), San Francisco, CA

Abstract: Interactions between the hippocampus and medial prefrontal cortex (PFC) play a critical role in learning and memory-guided decision making, but the nature of these interactions remains unclear. We have previously shown that awake sharp wave ripple (SWR) events, during which hippocampal memory replay occurs, are critical for spatial memory. Here we show that there is coordinated and content-specific reactivation of hippocampal-prefrontal ensembles during awake SWRs. We simultaneously recorded activity of hippocampal (CA1) and prefrontal (prelimbic and infralimbic) cells in adult male Long Evans rats over multiple days of spatial learning. Distinct hippocampal patterns of activity, SWRs (150-250 Hz) and theta periods (6-12 Hz), were detected using local field potential and speed criteria during behavior. We found that a substantial fraction of PFC cells (36%) showed significant firing rate modulation during awake SWRs. Changes in rates included both excitation and inhibition. This SWR modulation was related to previously reported theta phase locking in PFC: the majority of SWR modulated cells were significantly phase-locked to hippocampal theta (74%), and SWR modulated cells showed significantly stronger theta modulation than non-SWR modulated cells. We also found coordinated reactivation of CA1-PFC neurons during awake SWRs, recapitulating activity seen during behavior. The SWR-triggered activity of 14% of CA1-PFC cell pairs was significantly

correlated, such that activity of one cell predicted the activity of the other cell. Across the population, the covariance of CA1-PFC cell pairs during theta periods was significantly correlated with their SWR correlations. Importantly, spatial coding properties were also preserved during awake SWR reactivation. CA1-PFC cell pairs that encoded similar locations were reactivated together, and their SWR correlations were significantly higher than pairs that encoded distinct spatial locations. Highly specific functional networks comprised of CA1-PFC ensembles underlie this structured reactivation, as revealed by generalized linear models (GLM) that significantly predicted PFC activity during SWRs from ensemble CA1 activity during both SWRs and theta periods. Our results establish awake SWRs as a prominent network activity pattern mediating hippocampal-PFC interactions during behavior. Further, our findings of coherent reactivation of behaviorally relevant information across CA1-PFC networks during awake SWRs suggests that this coordinated reactivation is well suited to support learning and memory-guided behavior.

Disclosures: **S.P. Jadhav:** None. **G. Rothschild:** None. **D.K. Roumis:** None. **I. Grossrubatscher:** None. **L.M. Frank:** None.

Poster

093. Learning and Memory: Physiology I

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 93.07/SS2

Topic: F.02. Animal Cognition and Behavior

Support: JSPS KAKENHI 26460320

Title: Inhibition of de novo synthesis of plasmalogens in the mice hippocampus results in the memory loss associated with the reduction of BDNF

Authors: *T. KATAFUCHI[1], S. M. HOSSAIN[2], S. Y. M. AHMED[2], K. MIAKE[3];
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Abstract: The special ether phospholipids Plasmalogens (Pls) are characterized by the presence of a vinyl ether linkage at the *sn-1* position of the glycerol moiety. The ethanolamine plasmalogens (EthPls) was found to be highly comprised in the hippocampus but its role was mostly elusive. A reduction of brain Pls in advanced Alzheimer's disease (AD) patients

suggested a possibility that Pls might be associated with the memory dysfunction. Our present study was aimed to see the memory performance of the adult mice after down-regulating the Pls synthesis enzymes, GNPAT (glyceronephosphate O-acyltransferase) and AGPS (alkylglyceronephosphate synthase) in the hippocampus. To knock down the Pls synthesis enzyme genes in the hippocampus, we have designed short hairpin RNAs (shRNAs) against mouse genes *GNPAT* and *AGPS*. Two individual lentiviral shRNAs for each gene was confirmed for their knockdown efficiencies both *in vitro* (in cells) and *in vivo* (in the hippocampus tissue). For the behavioral study, we injected the lentiviruses at the dose of ($5X10^5$ transduction unit) in the hippocampus directly by stereotaxic instrument. For the memory performance, Morris water navigation task was carried out using the control and Pls knock-down mice and showed a significant reduction in the memory from first week to the 5^{th} weeks of the injection. This prolong reduction of the memory in those mice was associated with the reduction in the expression of brain derived neurotrophic factor (BDNF) and its target genes Synapsin-1 and Synaptotagmin-1. Furthermore, knock down of Pls in the neuronal cells Neuro-2A also confirmed a reduction of BDNF, Synapsin-1 and Synaptotagmin-1 expression suggesting that Pls can maintain the expression of these memory-related genes in the neurons. Our present findings suggest for the first time that the endogenous Pls in the hippocampus are very important for memory and do so via regulating the expression of memory-related genes.

Disclosures: **T. Katafuchi:** None. **S.M. Hossain:** None. **S.Y.M. Ahmed:** None. **K. Miake:** None.

Poster

093. Learning and Memory: Physiology I

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Topic: F.02. Animal Cognition and Behavior

Support: National Research Council of Thailand (NRCT)

Suranaree University of Technology

Title: Mangosteen fruit hull extract improves memory and affects brain acetylcholinesterase activities in normal aged rats

Authors: *R. SRISAWAT[1], W. TONGJAROENBUANGAM[2], N. NONTAMART[1];
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[2]Preclinical Div., Fac. of Medicine, Mahasarakham Univ., Mahasarakham Province, Thailand

Abstract: Natural polyphenols have been reported to possess a wide variety of biological activities, including enhancement of neuroprotective, learning and memory, and acetylcholinesterase (AChE) inhibitory activities. They can be found in large quantities in the fruit hull of mangosteen. Thus, the effects of mangosteen fruit hull extract (GME) on learning and memory, and brain AChE activity were investigated in normal aging. Ten months old male Wistar rats received vehicle (10% tween80, 1 ml/kg), vitamin E (40 mg/ml/kg), GME (500 or 1000 mg/ml/kg) orally once daily for 30 days. Spatial memory and learning of rats were tested by Morris water maze test. The protocol consisted of 21 training trials (3 times per day for 7 days on day 24-30) and probe trial on day 30. Immediately after last trial, rats were decapitated and brains were removed. Cerebral cortex, hippocampus, and basal forebrain were dissected out and homogenated. AChE activity of homogenized brains was determined by colorimetric method. The Morris water maze test study showed that time to find platform on day 7 of training trial was significantly decreased from day 1 in all groups. Time spent in target quandrant was significantly increased in vitamin E-treated group and 500 mg/ml/kg GME-treated group, but not 1000 mg/ml/kg GME-treated group, when compared to vehicle treated group ($P<0.05$). All doses of GME and vitamin E tended to increase, but not significant difference, in number of entries into the target quadrant when compared to vehicle treated group. The AChE activity study showed that GME at 1000 mg/ml/kg, but not 500 mg/ml/kg and vitamin E, significantly increased AChE activity in hippocampus when compared to vehicle treated group ($P<0.05$). All doses of GME and vitamin E did not showed significant difference in AChE activity in both basal forebrain and cerebral cortex when compared to vehicle treated group ($P<0.05$). This study provides the first evidence of memory and learning enhancing effects and anti-acetylcholinesterase action of GME on selected brain areas in aging. GME may be useful for the prevention of the development or progression of cognitive impairment caused by natural aging, however, the underlying mechanisms are still not fully understood.

Disclosures: **R. Srisawat:** None. **W. Tongjaroenbuangam:** None. **N. Nontamart:** None.

Poster

093. Learning and Memory: Physiology I

Location: Halls A-C

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Program#/Poster#: 93.09/SS4

Topic: F.02. Animal Cognition and Behavior

Support: German Research Foundation, SFB874/B1

Title: Novel exploration of positional or directional spatial cues induces Arc mRNA expression in distinct hippocampal subfields

Authors: *D. MANAHAN-VAUGHAN[1], V. ALIANE[2];
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Abstract: The hippocampus is crucially involved in declarative or explicit forms of learning and memory. Spatial memory is presumably encoded by means of changes in hippocampal synaptic efficiency and neuronal networks. However, the specific roles of the CA1, CA3 and dentate gyrus subregions are still unclear. In the hippocampus, learning about navigationally relevant information facilitates log-term depression (LTD) in the dentate gyrus, whereas learning about discrete environmental content facilitates LTD in the CA1 region (Kemp & Manahan/Vaughan, 2007, Trends Neurosci. 30:111-118). We examined the impact of these kinds of spatial learning on hippocampal neuronal activity. We observed increased Arc mRNA in the CA1 region after exploration of discrete spatial features and in the dentate gyrus after exploration of large spatially-distinct landmarks. These observations are in line with the parallel map theory of spatial representations (Jacobs & Schenk, 2003, Psychol Rev. 110:285-315) and support that these hippocampal subfields may contribute different components of a spatial representation.

Disclosures: **D. Manahan-Vaughan:** None. **V. Aliane:** None.

Poster

093. Learning and Memory: Physiology I

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Topic: F.02. Animal Cognition and Behavior

Support: CAPES

CNPq

Faperj

SR-2 UERJ

Title: Protein malnutrition and caloric restriction during the lactation period affects NPY distribution in the rat hippocampus

Authors: *P. L. GUEDES DA SILVA, A. C. B. BARBOSA, M. L. M. ROCHA, P. C. BARRADAS, F. TENORIO;
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Abstract: Perinatal malnutrition can lead to permanent impairments in brain morphology, physiology and neurochemistry. The hippocampus is a very vulnerable structure that is selectively affected by alterations on food intake during the developmental period. Several molecules are involved in hippocampal development, maintenance and cognitive phenomena, such as neuropeptide Y (NPY). NPY is highly expressed in this structure and studies have shown its participation in neurogenesis, learning and memory. In this work we evaluated the effects of protein malnutrition and caloric restriction during the first 10 days of lactation on NPY expression in offspring hippocampus. This study was approved by our University Ethics Committee (CEA/055/2009). We used rats from 5 to 20 postnatal (P) days of age whose dams were either fed a 0% protein diet (MG) or a normoprotein diet (CG) from P1 to P10 (n=3 per group/age). To reproduce the same amount of calories ingested by the MG we used a pairfed group (PFG). Animals were anesthetized and perfused with 0.9% saline solution, 4% paraformaldehyde (PF) and PF plus 10% sucrose. Sections were immunostained using anti-NPY antibody, revealed with a secondary antibody conjugated with Alexa 488 and observed at a fluorescence microscope. At P5, NPY positive (NPY+) cell bodies were small and the processes were well stained in the three groups. The number of NPY+ cells reduces during development in CG whereas this reduction was not so conspicuous in MG and PFG. At P5, MG and PFG presented less stained cell bodies in the dentate gyrus (DG) than CG ($p<0.01$ and $p<0.05$). At P10, NPY+ cells were bigger and fusiform in DG and their number was decreased in PFG when compared to CG ($p<0.05$). In CA1 of MG and PFG it is observed that most of cell bodies are concentrated in the outer layer, in a different pattern than CG. In CA3, there was a decrease in the number of NPY+ cells that are placed in all layers of that region in the MG ($p<0.01$) and PFG ($p<0.001$). P20 animals showed rounded cell bodies, differing from the younger ages. At this age there was no difference concerning NPY+ cell number between groups in any region. Our results showed that both proteic and caloric restriction during lactation affects NPY distribution in the hippocampus. Furthermore the differences observed in the pattern of NPY+ cells distribution in CA1 may suggests a delay in cell migration during development.

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

Poster

093. Learning and Memory: Physiology I

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Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 93.11/SS6

Topic: F.02. Animal Cognition and Behavior

Support: Clark Foundation

Undergraduate Research Fellowship

American Tinnitus Association

Title: Memory-enhancing intra-amygdalar clenbuterol enhances intrinsic excitability of CA1 pyramidal neurons during consolidation

Authors: E. S. LOVITZ, M. CHAVEZ, D. PROCH, *L. T. THOMPSON;
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Abstract: Emotional arousal enhances memory formation, creating strong lasting memories. Stress from fear activates the amygdala, and in turn modulates memory consolidation via the hippocampus. Packard et al. (1994) reported bilateral amphetamine infusion into the amygdala after a hippocampal-dependent task enhanced memory compared to controls. Beta-adrenergic modulation of basolateral amygdala (BLA) directly alters consolidation: norepinephrine (NE) released in the BLA during training on an inhibitory avoidance (IA) task correlates with memory retention. Post-trial beta-adrenergic agonist clenbuterol infusion into BLA dose-dependently enhances, while propranolol, a beta-antagonist, impairs IA memory. During consolidation, hippocampal pyramidal cells exhibit reduced post-burst after-hyperpolarizations (AHPs). AHP plasticity enhances memory, with drugs reducing AHPs improving acquisition and consolidation in several different tasks. After acquisition of new spatial or trace eyeblink learning, CA1 pyramidal neurons exhibit reduced AHP amplitude and duration *in vitro* up to 72 hr later. AHP plasticity is also seen during consolidation of IA, up to 24 hr post-trial in CA1 and CA3, and 1 hr post-trial in BLA pyramidal cells. AHPs are generated by medium (mAHP) and slow (sAHP) currents, and both are reduced during consolidation. AHP plasticity also affects accommodation, another measure of intrinsic excitability. In the current study, rats received a single paired IA training trial then were immediately infused with clenbuterol into the BLA. Effects on both a behavioral measure of memory and on intrinsic excitability in the CA1 region of hippocampus were assessed 24 hr later. A dose-response curve for clenbuterol (bilaterally infused) was first determined, enhancing memory retention 24 hr later. Using the most effective dose (15 ng), we then compared AHP and accommodation measures from CA1 neurons in clenbuterol-infused hemispheres to those in contralateral vehicle-infused hemispheres in slices prepared 24 hr post-

trial, as well as to neurons from clenbuterol- and vehicle-infused untrained controls 24 hr post-infusion. Reductions in AHP amplitudes occurred in CA1 neurons from trained clenbuterol-infused hemispheres compared to untrained vehicle-infused hemisphere neurons, consistent with other findings linking amygdala activation to memory consolidation mediated by the hippocampus.

Disclosures: **E.S. Lovitz:** None. **L.T. Thompson:** None. **M. Chavez:** None. **D. Proch:** None.

Poster

093. Learning and Memory: Physiology I

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Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 93.12/SS7

Topic: F.02. Animal Cognition and Behavior

Support: Mercator stiftung

Title: Modulation of cellular properties by environmental enrichment that may underlie learning enhancement and seizure prevention

Authors: *M. J. VALERO-ARACAMA[1], M. M. SAUVAGE[2], M. YOSHIDA[1];
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Abstract: Animals housed in enriched environments (EE) show an improved learning and memory (L&M) performance and a reduced propensity to suffer from epileptic seizures. While an increased cellular excitability in the hippocampus is suggested to be a possible cellular underpinning of enhanced L&M, the literature in this area is controversial. In addition, it remains unclear how an EE can diminish the probabilities for the animals to develop epileptic seizures, which would require a decreased excitability. In order to better understand these issues, we conducted *in vitro* patch clamp recordings in hippocampal CA1 pyramidal cells from mice housed either in an enriched or control environment. We observed that an EE increased the cellular excitability of these cells after a short (< 40 days) but not after a long (> 40 days) period of enrichment. In addition, this increased excitability was mainly limited to the dorsal CA1 region. We also report that the increased excitability was supported by the modulation of the input resistance and spike threshold. Furthermore, the slow after hyperpolarization potential (sAHP), a property that is the focus of some anticonvulsant therapies, was larger in the cells from animals housed in the EE. In conclusion, controversial results among studies regarding cellular excitability may stem from the housing duration- and anatomical region-dependent effects of an

EE. Moreover, enhanced L&M performance and seizure prevention, which may require increased and decreased excitability, may be enabled by the combination of increased cellular excitability and increased sAHP.

Disclosures: **M.J. Valero-Aracama:** None. **M.M. Sauvage:** None. **M. Yoshida:** None.

Poster

093. Learning and Memory: Physiology I

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Program#/Poster#: 93.13/SS8

Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant NS079774

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NIH Grant MH060236

Title: Cardiac change-based fear index and its application for measuring fear memory consolidation

Authors: ***F. ZHAO**[1,2], J. LIU[1], W. WEI[1,2], J. Z. TSIEN[1];
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Abstract: Changes of heart rate (HR) and heart rate variability (HRV) have been identified as highly useful indicators for assessing emotion status. Yet cardiac changes upon the presentation of conditioned cues or unconditioned stimuli have not been systematically studied in mice. Here, we examined changes in heartbeat interval dynamics as physiological readout for assessing fearful reactions as mice were subjected to sudden air puff, free-fall drop inside a small elevator, and a laboratory-version earthquake. Cardiac changes were analyzed in details by measuring three distinct phases: namely, the rapid rising phase in HR, the maximum plateau phase during which HRV is greatly decreased, and the recovery phase during which HR gradually recovers to baseline values. We developed the fear resistance index based on specific cardiac response features. We demonstrated that the fear resistance index remained largely consistent across distinct fearful events in a given animal, thereby enabling us to compare and rank individual mouse's fear responsiveness among the group. We also investigated changes in HR and HRV in both short-term and long-term contextual and cued fear conditioning. We found that the HRV

reduced significantly in fear conditioning. Moreover, the time duration of the highly rhythmic phase of HRV were sensitive enough to reflect the transition from short-term to long-term fear memories and detect the fear extinction effect during the repeated tone recall. Therefore, these results suggest that the fear resistance index described here can represent a useful parameter for measuring personality traits or individual differences in stress-susceptibility in both wild-type mice and post-traumatic stress disorder (PTSD) models. In addition, HRV is a valuable physiological indicator for sensitively measuring the consolidation and expression of fear memories in mice.

Disclosures: **F. Zhao:** None. **J. Liu:** None. **W. Wei:** None. **J.Z. Tsien:** None.

Poster

093. Learning and Memory: Physiology I

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 93.14/SS9

Topic: F.02. Animal Cognition and Behavior

Support: P01 HD052112

Title: Consumption of high-energy diets induces cognitive impairment, neuroinflammation, and neurovascular damage

Authors: ***S. L. HARGRAVE**[1,4], T. L. DAVIDSON[4], W. ZHENG[2], K. P. KINZIG[3]; [2]Hlth. Sci., [3]Psychological Sci., [1]Purdue Univ., West Lafayette, IN; [4]Psychology, American Univ., Washington, DC

Abstract: Obesity and exposure to high-energy diets increase the risk for developing dementia, and short-term hyperglycemia has been shown to transiently impair memory and information processing in humans. Amnesia due to hippocampal damage has also been demonstrated to alter food intake patterns, in some cases leading to obesity. We have previously demonstrated that diet-induced obese (DIO) rats fed a high-energy (HE) diet are impaired at hippocampal-dependent tasks following 10d and 90d (but not 40d) diet access. At 90d, these rats had increased hippocampal blood-brain barrier (BBB) permeability. The present study characterized the progression of the molecular and functional chnages following HE diet exposure, and correlated these changes with cognitive ability. Compared to chow-fed rats, HE-fed animals were impaired at hippocampal-dependent spatial tasks at 10d and 90d (but not 40d), and relied on a hippocampal-independent response strategy to solve the task. Further, these animals showed

increased hippocampal and hypothalamic cytokine mRNA, decreased hippocampal and hypothalamic BDNF mRNA, and reduced hippocampal CD31 at 90d. BBB permeability to the hippocampus, dorsal striatum, hypothalamus, and hindbrain was increased following 90d access to diets. Cognitive deficits significantly correlated with increased ventral hippocampal BBB permeability. These data suggest that maintenance on HE diets can damage structures associated with both ingestive behavior and cognition. This, in turn, has the potential to increase responding to food cues. An improved understanding of the molecular and cellular processes involved in HFD-induced inflammation and neurovascular damage could yield options for the treatment or prevention of obesity and cognitive dementia

Disclosures: **S.L. Hargrave:** None. **T.L. Davidson:** None. **W. Zheng:** None. **K.P. Kinzig:** None.

Poster

093. Learning and Memory: Physiology I

Location: Halls A-C

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Program#/Poster#: 93.15/SS10

Topic: F.02. Animal Cognition and Behavior

Support: R21 MH093904

R01 MH098899

Title: The relationship between pupil diameter and neuronal activity in multiple brain areas

Authors: *S. JOSHI[1], Y. LI[1], R. M. KALWANI[2], J. I. GOLD[1];
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Abstract: The locus coeruleus (LC) is the primary source of norepinephrine (NE) in the central nervous system. The LC-NE system plays important roles in normal brain functions including arousal and sensory-motor processing. It has also been implicated in clinical disorders including attention-deficit/hyperactivity disorder (ADHD), anxiety, and schizophrenia. However, attaining a deeper understanding of this system's contributions to normal and abnormal brain function is hindered by the LC's small size and location deep in the brainstem, which make it difficult to record from or image using existing methods. It would be useful to obtain an alternative measure that reliably reflects LC activity. It has been suggested that one such measure is non-luminance-

mediated changes in pupil diameter (PD), which can be obtained relatively easily using video-based technologies. Accordingly, we have obtained the first quantitative assessment of the relationship between LC single-unit activity and PD. Our results are consistent with the hypothesis that common brain circuits affect both LC neuronal activity and PD and provide a basis for interpreting PD changes in terms of LC activity. However, LC provides NE innervation to nearly the entire brain. Thus, to accurately interpret changes in PD, it is critical to evaluate the relation between neural activity and PD in multiple brain areas. We compared the relationship between PD and neural activity in LC, superior colliculus (SC), inferior colliculus (IC), the middle temporal area (MT) of extrastriate visual cortex and the anterior cingulate cortex (ACC) of awake macaques. The monkeys performed a fixation task that allowed for reliable measurement of PD over several seconds. We found correlations between PD and neural activity in all brain areas tested over a range of timescales, including reliable spike-triggered changes in PD when averaged across spontaneously occurring spikes. For LC, SC, and IC measurements, we also used two methods to assess how PD was related to evoked changes in neuronal activity. First, we applied electrical microstimulation to the three brain regions, which in each case elicited reliable changes in PD. Second, we played a loud sound while the monkeys maintained fixation, which served as an arousing stimulus (beep trials). The beeps consistently caused phasic changes in PD, which were accompanied by phasic neural responses in LC and IC but not SC. Together, these results imply that LC-mediated arousal, assessed via changes in PD, can serve to coordinate neural activity throughout the brain.

Disclosures: **S. Joshi:** None. **J.I. Gold:** None. **Y. Li:** None. **R.M. Kalwani:** None.

Poster

093. Learning and Memory: Physiology I

Location: Halls A-C

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Program#/Poster#: 93.16/SS11

Topic: F.02. Animal Cognition and Behavior

Support: ONR MURI N00014-10-1-0198

Title: Corticostriatal plasticity with T-maze learning corresponds to the practiced turn direction and training stage, with corresponding changes in MSN excitability and dendritic remodeling

Authors: ***S. L. HAWES**[1], R. C. EVANS[5], B. A. UNRUH[2], E. A. BENKERT[1], F. GILLANI[1], N. J. ZHU[3], K. T. BLACKWELL[4];

[2]Chem., [3]Biol., [4]Mol. Neurosci., [1]George Mason Univ., Fairfax, VA; [5]NINDS, NIH, Bethesda, MD

Abstract: Learning a skill requires many repetitions of that skill, and the pattern of engagement across dorsal striatal regions changes over the course of training (Thorn and Graybeil 2014). Rats learning to navigate a T-maze for food reward initially use a *place* strategy attributed to greater dorsomedial engagement, and later switch to using a *response* strategy attributed to greater dorsolateral engagement (Packard and McGaugh 1996). Here we investigate how the transition between early- and late-training is reflected in medium spiny neurons (MSNs) in dorsomedial and dorsolateral striatal subregions. Specifically, we test whether changes in neurons' synaptic plasticity, intrinsic excitability, and morphology correspond to learning, and evaluate the distribution of these changes across striatal subregions and hemispheres relative to the practiced turn. Bidirectional plasticity was measured in field recordings and induced using either 20 Hz or theta burst stimulation. Intrinsic excitability was tested using whole cell current clamp recordings from medium spiny neurons. These same cells were biocytin-filled and reconstructed to analyze morphology. For early-trained rats, which demonstrate a mixture of *place* and *response* strategy use, dorsomedial MSN excitability is enhanced, but plasticity is reduced. Specifically, in the hemisphere contralateral to the turn, LTP is reduced, while in the hemisphere ipsilateral to the turn, LTD is reduced. There was no change in either cell excitability or plasticity in the dorsolateral striatum. For late-trained rats, which overwhelmingly demonstrate a *response* strategy, MSN excitability and LTP are restored to control levels. However, LTD is reduced dorsolaterally ipsilateral to the learned turn, and dorsomedially contralateral to the learned turn. In addition, our morphology analysis demonstrates adult dendritic remodeling across learning stages which, while recently demonstrated in mice (Cazorla et al, 2012), has not previously been linked to striatal learning. Our findings suggest that the encoding of novel information in the striatal network is facilitated by elevated intrinsic excitability in dorsomedial MSNs, and reduced proximal dendritic complexity. In our ongoing work, we are using channelrhodopsin to investigate the role of action potentials in striatal plasticity in either MSN cell class.

Disclosures: **S.L. Hawes:** None. **R.C. Evans:** None. **B.A. Unruh:** None. **E.A. Benkert:** None. **F. Gillani:** None. **N.J. Zhu:** None. **K.T. Blackwell:** None.

Poster

093. Learning and Memory: Physiology I

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Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 93.17/SS12

Topic: F.02. Animal Cognition and Behavior

Support: NIH R01 MH090188

Title: An active hippocampal network not driven by ripples

Authors: *K. KAY, M. SOSA, J. E. CHUNG, M. P. KARLSSON, I. GROSSRUBATSCHER, L. M. FRANK;
UCSF, San Francisco, CA

Abstract: The hippocampus has a central role in memory, yet we still do not know how hippocampal neural activity underlies the cognitive functions of the hippocampus. One promising lead is the hippocampal network pattern known as the sharp-wave ripple (SWR, or "ripple"), a rhythmic event (150-250 Hz) that occurs during stationary periods and during slow-wave sleep in the behaving animal. Recent findings indicate that SWRs are necessary for hippocampal function at the behavioral level, thereby qualifying a link between SWRs and higher-level hippocampal functions. At the circuit level, the SWR reflects highly synchronous hippocampal spiking, and appears to dominate the hippocampal network with strong excitation for its duration (10-100 ms). However it is not clear whether unit activation during SWRs is universal in the hippocampal network. We thereby sought to evaluate SWR spiking activity at the unit-by-unit level, using local field potential (LFP) and spike data collected from rats implanted with multitetrode arrays and performing a hippocampus-dependent memory task. SWRs in multiple running environments and from sleep sessions were detected and analyzed. Surprisingly, we have found a subpopulation of principal units in the hippocampus that are not driven by SWRs, and in some cases even consistently reduce baseline spiking during SWRs. These units are moreover highly active during behavioral epochs in which the animal is awake and engaged in the memory task. During these awake periods, SWR-driven hippocampal units are known to fire in spatial receptive fields known as place fields. We observe that non-SWR driven hippocampal units show proportionally less traditional place activity, instead often firing more when the animal is behaviorally stationary. These basic findings (1) advance our understanding of SWRs, (2) indicate that the hippocampal circuit stages an informational stream distinguishable from that associated with SWRs, (3) suggest that non-SWR activity during periods of immobility may be of special importance for hippocampal function.

Disclosures: **K. Kay:** None. **M. Sosa:** None. **J.E. Chung:** None. **M.P. Karlsson:** None. **I. Grossrubatscher:** None. **L.M. Frank:** None.

Poster

093. Learning and Memory: Physiology I

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Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant R01MH093807

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eSMCs (FP7-IST-270212)

Title: Grid cells reflect the locus of attention, even in the absence of movement

Authors: N. WILMING[1,2], P. KÖNIG[1,4], *E. A. BUFFALO[2,3];
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Abstract: The grid cell network allows for the precise decoding of the position of freely moving animals and is therefore implicated in spatial navigation. Recent work (Killian et al. 2012) has demonstrated that overt attention in the form of eye-movements is sufficient to activate the grid cell network in macaque monkeys. This suggests that grid cells may be able to support other cognitive functions besides spatial navigation. It has recently been suggested that firing patterns among grid cells may support both navigation and memory and that the neuronal algorithms underlying navigation in real and mental space are fundamentally the same (Buzsaki and Moser, 2013). A critical prerequisite for this idea is that the grid cell network can function even in the absence of physical movement through space. We examined this possibility by training a macaque monkey to covertly attend to a moving dot in the periphery while maintaining central fixation on a computer monitor. On each trial, the monkey's task was to monitor the dot and release a bar when the dot changed color after a variable amount of time (700-2200ms). Over the course of the experimental session, the dot covered the entire screen with several passes through each area of the screen. We recorded the spiking activity of 100 units in the entorhinal cortex of one macaque monkey and computed the gridness of 2D firing rate maps obtained by plotting spike trains as a function of dot position. In preliminary results, we identified entorhinal neurons that showed a significant grid-like activation with 60º rotational symmetry between the firing fields (bootstrapping procedure, $p<0.05$). Neurons were also identified that fired when the location of attention was near the borders of the screen. These findings support the hypothesis that covert attention is sufficient to activate the grid cell network, and that the primate entorhinal cortex can reflect space even in the absence of any physical movement.

Disclosures: **N. Wilming:** None. **E.A. Buffalo:** None. **P. König:** None.

Poster

093. Learning and Memory: Physiology I

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Program#/Poster#: 93.19/SS14

Topic: F.02. Animal Cognition and Behavior

Support: Spanish BFU2011-29089

Spanish BFU2011-29286

Title: Contribution of the different hippocampal synapses to classical eyeblink conditioning in mice and rabbits

Authors: *J. DELGADO-GARCIA[1], R. SÁNCHEZ-CAMPUSANO[2], A. CARRETERO-GUILLÉN[2], A. GRUART[2];
[1]Pablo Olavide Univ., Seville, Spain; [2]Univ. Pablo de Olavide, Sevilla, Spain

Abstract: Although it is generally assumed that the hippocampus is involved in associative learning tasks, the specific contribution of the different synapses present in its intrinsic circuit or comprising its input and output pathways is still poorly defined. We have addressed this important question by recording the activity-dependent changes in synaptic strength of nine hippocampal synapses (corresponding to the intrinsic hippocampal circuitry and to its main inputs and outputs) during the acquisition of a trace conditioning of eyelid responses in alert behaving mice. The evolution of the timed changes in synaptic strength of the synapses included in the study allowed us to determine their functional organization, which did not coincide with their sequential distribution according to anatomical criteria and connectivity. Results confirmed that the acquisition of a classical eyeblink conditioning is a multisynaptic process in which the contribution of each synaptic contact is different in strength, and takes place at different moments across the learning process. In a subsequent experiment, we studied the contribution of both context (environmental details) and cues (conditioned and unconditioned stimuli: CS, US) to those activity-dependent changes in synaptic strength. We recorded in rabbits the monosynaptic field excitatory post-synaptic potentials (fEPSPs) evoked at six different hippocampal synapses during the acquisition and extinction of a classical eyeblink conditioning using trace and delay paradigms, as well as during pseudoconditioning and in the absence of CS and US presentations (context). Context and pseudoconditioning training evoked early, lasting changes in synaptic strength in perforant pathway synapses in dentate gyrus (PP-DG), and hippocampal CA3 (PP-CA3) and CA1 (PP-CA1) areas. Pseudoconditioning also evoked early, non-lasting changes in strength within the intrinsic hippocampal circuit (CA3-CA1 and CA3-

cCA1 synapses). In contrast, during both trace and delay training sessions, synaptic changes in strength were mostly noticed within the intrinsic hippocampal circuit (DG-CA3, CA3-CA1; CA3-cCA1). In conclusion, the precise and timed activation of the multiple synaptic contacts in the hippocampal circuit during classical conditioning of eyelid responses evokes a specific, dynamic map of functional synaptic states in that circuit. In addition, the response of hippocampal synapses to afferent impulses seems to be modulated by both context and cues during pavlovian associative learning in behaving rabbits.

Disclosures: **J. Delgado-Garcia:** None. **R. Sánchez-Campusano:** None. **A. Carretero-Guillén:** None. **A. Gruart:** None.

Poster

093. Learning and Memory: Physiology I

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

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Topic: F.02. Animal Cognition and Behavior

Support: UoD MRI studentship to SAL

Title: Memory ontogeny in the juvenile rat

Authors: *S. A. LYON, R. F. LANGSTON;
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Abstract: Episodic memory is the recollection of unique autobiographical events, including rich detail of times, places and associated contextual information and in neurodegenerative diseases, such as Alzheimer's disease, episodic memory is greatly impaired (1). The ability to recall episodic memories is thought to develop later in childhood than other types of memory such as novelty recognition (2), however this ontogeny in animals is so far unconfirmed due to a lack of suitable behavioural tests (3). We wish to model the emergence of episodic memory for 2 reasons: firstly it will be invaluable for creating age appropriate animal models of developmental disorders (4) and secondly we wish to use memory ontogeny as a novel tool to investigate the neurobiological basis of episodic memory. We used a battery of spontaneous novelty detection tasks to assess the ontogeny of different types of memory in juvenile rats from P25 to adulthood. The episodic memory test was adapted from Eacott and Norman (5) and used alongside other control tests of recognition and associative memory adapted from Langston and Wood (6). These tasks included Novel Object Recognition, Object Context, Object Place and Object Place

Context tasks. The test battery was compressed into a 4-day protocol so as to enable acute testing of rats during restricted developmental time windows. Adult rats performed significantly above chance on all the memory tasks using this novel behavioural protocol but juvenile rats, display a differential emergence of memory types. References 1. R Swainson et al, Dement Geriatr Cogn (2001) 2. SE Gathercole, J Child Psychol Psyc (1998) 3. RGM Morris, Philos Trans R Soc Lond B Biol Sci (2001) 4. JE McCutcheon & M Marinelli, Eur J Neurosci (2009) 5. MJ Eacott &G Norman, J Neurosci (2004) 6. RF Langston& ER Wood, Hippocampus (2010)

Disclosures: **S.A. Lyon:** None. **R.F. Langston:** None.

Poster

093. Learning and Memory: Physiology I

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Topic: F.02. Animal Cognition and Behavior

Support: NIH R01 MH090188

UCOP Lab Fees Award

NSF GRFP Fellow 2012127208

Medical Scientist Training Grant from NIGMS GM07618

Title: Polymer probes allow long lasting, high-density recordings in awake, freely behaving animals

Authors: *J. E.-C. CHUNG[1], D. F. LIU[2], I. GROSSRUBATSCHER[3], V. M. TOLOSA[4], K. G. SHAH[4], A. C. TOOKER[4], S. H. FELIX[4], S. S. PANNU[4], L. M. FRANK[3];
[2]UC Berkeley - UCSF Grad. Program in Bioengineering, [3]Dept. of Physiology, Univ. of California, San Francisco, [1]Univ. of California, San Francisco, San Francisco, CA; [4]Materials Engin. Division, Lawrence Livermore Natl. Lab., Lawrence Livermore Natl. Lab., Livermore, CA

Abstract: The brain is a massively interconnected network of specialized circuits. All brain functions depend on millisecond timescale interactions across these brain networks, and there is a pressing need to develop technologies that make it possible to measure these interactions. We have therefore developed flexible multielectrode polymer probes that have the potential to yield

long-lasting recordings from single neurons across many sites in a distributed circuit. We used a novel implantation approach wherein arrays are bound to removable silicon stiffeners using polyethylene glycol, a water-soluble adhesive, allowing for insertion into the brain during implantation. Following a 10-30 minute period, the adhesive dissolves and the stiffener can be removed, leaving the probe in place. We used this method to implant a first set of high-density probes into rat pre-limbic and infra-limbic cortices. We were able to obtain high quality single units (maximum amplitude ~ 250 uV) for approximately 40 days. We are currently experimenting with changes to probe and stiffener geometry, and implantation procedures. These changes, alongside ongoing development of data-acquisition hardware and software, will further optimize recording quality, electrode stability, as well as the quantity of implanted probes and recording channels. These polymer probes provide an integral part of the path towards awake, freely behaving, high-density, chronic recording across distributed neural circuits.

Disclosures: **J.E. Chung:** None. **D.F. Liu:** None. **I. Grossrubatscher:** None. **V.M. Tolosa:** None. **K.G. Shah:** None. **A.C. Tooker:** None. **S.H. Felix:** None. **S.S. Pannu:** None. **L.M. Frank:** None.

Poster

093. Learning and Memory: Physiology I

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

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Topic: F.02. Animal Cognition and Behavior

Support: MH191180

Title: Dopaminergic modulation of lateral amygdala neuronal activity: Differential influences of D1 and D2 receptor activation on thalamic and cortical afferent inputs

Authors: *C.-H. CHANG, A. A. GRACE;
Dept. of Neurosci., Univ. of Pittsburgh, Pittsburgh, PA

Abstract: In Pavlovian auditory fear conditioning, the lateral nucleus of the amygdala (LA) integrates an acoustic conditioned stimulus (CS) with an aversive unconditioned stimulus (US). The CS reaches the LA via a direct input from auditory thalamus (medial geniculate nucleus, MGN), as well as the auditory association cortex (Te3). The thalamic input is generally believed to provide a basic version of the CS, while the cortical input provides a more processed representation of the stimulus. Dopamine (DA) is released in the LA during numerous

conditions, such as under heightened arousal of the presentation of an affective CS. However, how DA modulates the excitability of LA neurons in response to thalamic and cortical afferent inputs is poorly understood. In the current study, the effects of D1 or D2 receptor activation on LA afferent-driven neuronal firing were examined using *in vivo* extracellular single-unit recordings with local micro-iontophoretic drug application in anesthetized rats. Electrical stimulating electrodes were placed in either the MGN or Te3 (0.5 Hz, 0.25 ms pulse duration, 1 mA) in search of responsive neurons in the LA. The stimulation current was then adjusted to evoke ~50% response (baseline, 20-30 spikes in 50 trials), followed by iontophoresis of either the D1 agonist, SKF38393 (20mM in 100mM NaCl), or the D2 agonist, quinpirole (10mM in 10mM NaCl), in successively increasing doses (5, 10, 20, and 40nA). Putative LA projection neurons were categorized into "excitatory" or "inhibitory" to D1 or D2 agonists if changes in evoked responses (per 50 trials) were 1) unitary in direction, and 2) greater than 15% change relative to baseline in any of the doses applied. A total of 87 neurons were recorded from 40 rats. We found that the majority of the neurons recorded (77 out of 87) exhibited either excitatory or inhibitory response to activation of D1 or D2 receptors in both the thalamic and cortical pathways. In general, it requires significantly higher current to evoke ~50% baseline response to the cortical input compared to the thalamic input. Activation of the D1 receptor showed no difference in modulation of the inhibitory or excitatory response between the thalamic or cortical pathways. On the other hand, activation of the D2 receptor has a stronger inhibitory modulation in the cortical pathway, but a stronger excitatory modulation in the thalamic pathway. Our results suggested that there is a shift in balance favoring the thalamic pathway in response to DA acting via the D2 receptor.

Disclosures: **C. Chang:** None. **A.A. Grace:** None.

Poster

093. Learning and Memory: Physiology I

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Topic: F.02. Animal Cognition and Behavior

Support: NIH R01 MH090188

EMBO post-doctoral fellowship to GR

ELSC post-doctoral fellowship to GR

Title: Hippocampal-cortical dynamics underlying distributed memory representations

Authors: *G. ROTHSCHILD, L. Y. TIAN, I. GROSSRUBATSCHER, L. M. FRANK;
Ctr. for Integrative Neurosci., UCSF, San Francisco, CA

Abstract: Animals and humans form associations between different sensory experiences, and these memories guide future behavior. However, the systems-level neural mechanisms underlying formation of learned associations is largely unknown. Building upon the strong natural sensitivity of rodents, we studied the formation of place-sound association in rats. We examined the interplay of activity patterns across three structures: the hippocampus, which is critical for location encoding and memory formation, the auditory cortex (AC), which is a central cortical region involved in auditory processing and sound-related learning, and the prefrontal cortex (PFC), which is involved in task-relevant processing, decision making and memory processes. To do so we carried out multi-tetrode recordings of multiple single units in CA1, AC and PFC of rats learning a place-sound association task. Rats learned to associate a specific sound with a specific location on a track, and reached 80-90% performance in 6-7 days of training and recording. We found that responses of AC neurons to the target sound were behaviorally modulated, highlighting the importance of accounting for behavioral state when assessing response profiles in AC. In addition, while responses to the target sound in AC modestly increased immediately after training, the population representation did not change markedly across the week of learning. These findings suggest that AC itself is not the repository of learned sound-place associations. We therefore asked if learning could be manifested in information flow between the hippocampal CA1, AC and PFC. We focused on activity during hippocampal sharp wave ripples (SWRs), which has been identified as a key candidate mechanism of information transfer between hippocampus and cortex. During SWRs, replay of behavioral firing patterns occur, and coordination with cortical regions involved in memory processes is enhanced. Moreover, blocking SWRs during sleep or behavior impedes learning. Surprisingly, we found that spiking of many AC neurons was strongly modulated during hippocampal SWRs. For a subset of neurons, spiking activity during SWRs in sleep, when no sounds were presented, resembled responses to the target sound on the track, suggesting an offline “sound replay” mechanism. We also found that many CA1-AC pairs were significantly correlated specifically during SWRs, indicating that SWR events coordinate specific CA1 place cells and AC neurons. In addition, some neuronal pairs across PFC and AC showed enhanced correlations during SWRs. These results indicate that SWR-related activity could be a systems-level mechanism of associative memory formation.

Disclosures: **G. Rothschild:** None. **L.Y. Tian:** None. **L.M. Frank:** None. **I. Grossrubatscher:** None.

Poster

093. Learning and Memory: Physiology I

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Program#/Poster#: 93.24/SS19

Topic: F.02. Animal Cognition and Behavior

Support: CNPq

FAPERJ

Title: Endogenous levels of Lipoxin A4, a cannabinoid allosteric enhancer, impact learning and memory in mice

Authors: *L. M. LEO[1,2], C. A. CANETTI[3], O. B. AMARAL[3], F. A. BOZZA[1,2], F. A. PAMPLONA[2];
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Abstract: Lipoxin A4 (LXA4) is an anti-inflammatory molecule produced by 5-Lipoxygenase (5-LO), which also yields inflammatory leukotrienes, at the expense of arachidonic acid. In the brain, LXA4 augments anandamide signaling through its action as an allosteric enhancer on CB1 cannabinoid receptors. In light of this finding, we have recently demonstrated a role for LXA4 in mouse anxiety-like behavior (Leo et al., PLOS ONE 2014). Henceforth, we seek to identify other physiological functions regulated by the endocannabinoid system that may be skewed by LXA4 availability, such as learning and memory. Memory was assessed by the step-down inhibitory avoidance test. In this task, 5-LO knockout mice, that have reduced LXA4 levels, presented reduced step-down latency in the test session, suggesting memory impairment. In addition, these mice also presented reduced expression of CB1 receptors in the hippocampus, but not in cortex, as observed by Western Blot analysis. This finding suggests that the observed memory impairment is secondary to a down-regulated CB1-related endocannabinoid signaling in the hippocampus, as has been shown by other studies with rodents that described impaired memory in response to local infusion of a CB1 antagonist into the hippocampus. Aged Swiss mice (12 months) were also evaluated. The endogenous plasma levels of LXA4 and cysteinyl leukotrienes (cysLTs) were assessed by ELISA. Aged mice exhibited reduced LXA4 and increased levels of cysLTs, consequently presenting a lower ratio of LXA4/cysLTs. Accordingly, these mice also performed poorly in the step-down inhibitory avoidance task, compared to 3-month-old mice. These results point to an inflammatory imbalance in the brain of aged mice, with increased production of leukotrienes in detriment of LXA4. The reduction of the latter may dampen endocannabinoid signaling, contributing to the cognitive deficit observed. Our results indicate

that LXA4 may contribute to memory modulation, possibly via a CB1-related endocannabinoid mechanism.

Disclosures: L.M. Leo: None. **C.A. Canetti:** None. **O.B. Amaral:** None. **F.A. Bozza:** None. **F.A. Pamplona:** None.

Poster

093. Learning and Memory: Physiology I

Location: Halls A-C

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Topic: F.02. Animal Cognition and Behavior

Support: Hungarian Academy of Sciences

Title: Transmitters involved in the action of neuromedin S on passive avoidance learning in rats

Authors: *G. TELEGDY, SR;
Pathophysiology, Univ. of Szeged,Department of Pathophysiology, Szeged, Hungary

Abstract: The effects of different neurotransmitters was studied in the action of neuromedin S (NMS) in the memory consolidation of passive avoidance behavior by pretreating rats with receptor blockers in doses which alone did not change the test. The involvement of cholinergic, dopaminergic, adrenergic, serotonergic, opiate and GABA-ergic receptors and nitric oxide was tested. The animals were pretreated with the non selective muscarinic acetylcholine receptor antagonist, atropine, the non selective α-adrenergic receptor antagonist phenoxybenzamine**,** the β-adrenergic receptor antagonist propranolol, the D2, D3, D4 dopamine receptor antagonist haloperidol , the non selective 5-HT2 serotonergic receptor antagonist cyproheptadine, the nonselective opioid receptor antagonist naloxone,. the γ-aminobutyric acid subunit A (GABA-A) receptor antagonist bicuculline, or the nitric oxide synthase inhibitor nitro-L-arginine. Atropine, haloperidol, phenoxybenzamine, propranolol, cyproheptadine, naloxone and nitro-L-arginine prevented the effects of NMS on passive avoidance learning. Bicuculline did not change the effects of NMS. The results demonstrate that , muscarinic acetylcholine, α- and β- adrenergic, dopaminergic , 5-HT2 serotonergic and opioid receptors and nitric oxide are involved as mediators. in the action of NMS on the consolidation of passive avoidance learning. 1 2

Disclosures: G. Telegdy: None.

Poster

094. Cortical and Hippocampal Circuits: Spatial Navigation I

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 94.01/SS21

Topic: F.02. Animal Cognition and Behavior

Support: Norwegian Research Council

ENSEMBLE

Kavli Foundation

Title: Grid cells require experience with local boundaries during development

Authors: *I. U. KRUGE, T. WAAGA, T. WERNLE, E. I. MOSER, M.-B. MOSER;
NTNU, Kavli Inst. For Systems Neurosci., Trondheim, Norway

Abstract: Grid cells in the medial entorhinal cortex (MEC) represent a metric for space. In rodents, receptive fields of individual grid cells tessellate environments with regularly repeating firing fields of hexagonal spacing. Developmental studies have shown that adult-like grid cells appear during the fourth postnatal week, beginning approximately one week after pups open their eyes and actively explore their surroundings for the first time. During this developmental window it is not known whether the grid cell network relies on experience with salient spatial features in order to form an optimal connectivity, or whether the emergence of grid patterns is established by purely maturational processes. To explore this question we tested whether development of grid cells is biased by the spatial environment in which rats are raised. Rats were born and raised in a spherical environment (70-100 cm diameter) made from frosted 8 mm acrylic material, and so were deprived from experience with sharp boundaries and distal visual cues. As controls, we raised rats in frosted cubes (50 x 50 cm) with sharp vertical edges but equally limited distal visual cues. A third group was raised in enriched cages with no limitations in spatial experience. In the sphere and cube conditions all animals were handled and trained in darkness to prevent visual access to spatial features external to their home cage. Adult rats were implanted with tetrodes aimed for MEC, and putative grid cells were localized during recording in the home cage in complete darkness. When stable theta-modulated cells appeared in the MEC recordings, the rats were introduced to a lighted room and a novel square environment. Single unit activity was subsequently recorded during free foraging in normally lighted sessions over three to seven days. Grid cells with repeating firing fields were immediately present in the

spatially enriched control group. In the sphere-raised rats, a reliably regular hexagonal pattern could not be detected, although grid-like patterns started to emerge by the end of one week of recording in the new environment. In the cube-raised group, grid cells with symmetric hexagonal features developed after some days of exposure. Further experiments will determine whether the observed differences between animals raised under deprived or normal conditions persist with further training. Our results suggest that the repetitive firing of grid cells is genetically hardwired from birth, but that the animal needs experience with stable vertical reference boundaries during development in order to express perfectly hexagonal grids at adult age.

Disclosures: **I.U. Kruge:** A. Employment/Salary (full or part-time):; NTNU, Kavli Institute for Systems Neuroscience. **T. Waaga:** A. Employment/Salary (full or part-time):; NTNU, Kavli Institute for Systems Neuroscience. **T. Wernle:** A. Employment/Salary (full or part-time):; NTNU, Kavli Institute for Systems Neuroscience. **E.I. Moser:** A. Employment/Salary (full or part-time):; NTNU, Kavli Institute for Systems Neuroscience. **M. Moser:** A. Employment/Salary (full or part-time):; NTNU, Kavli Institute for Systems Neuroscience.

Poster

094. Cortical and Hippocampal Circuits: Spatial Navigation I

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 94.02/SS22

Topic: F.02. Animal Cognition and Behavior

Support: Research Council of Norway

European Research Council

Kavli Foundation

Title: A neural circuit for goal-directed spatial coding

Authors: *H. T. ITO, S.-J. ZHANG, M. P. WITTER, E. I. MOSER, M.-B. MOSER; CNC, NTNU, TRONDHEIM, Norway

Abstract: Spatial navigation to a desired location requires information about the combinatorial relationship between an animal's choices of action and subsequent spatial positions. A number of studies indicate that this relationship is represented in the activity of hippocampal CA1 neurons, which fire not only at the animal´s current spatial position but also dependent on the trajectory taken to reach a subsequent goal location. Although such trajectory-dependent activity is likely

to be a result of associations between spatial position and behavior, how the hippocampus acquires information about intended actions is still poorly understood. Here we report a neural circuit, composed of the medial prefrontal cortex (mPFC) and the midline thalamic nucleus reuniens (Re), that is required for trajectory representation in the CA1 field of the hippocampus. In a continuous alternation task on a figure-8 maze, trajectory-dependent firing was observed in both CA1 and Re cells, but significantly less in CA3 cells, which do not receive Re inputs. Trajectory-dependent firing of CA1 cells was significantly reduced by lesions or optogenetic silencing of Re cells, indicating a causal link between Re input and trajectory representation in the hippocampus. Trajectory-dependent firing was also observed in neurons in mPFC, which has reciprocal anatomical connections with Re, suggesting that mPFC shares the information with Re. The trajectory information represented in this prefrontal-to-hippocampal circuit was sufficient to predict a correct choice of trajectory, which was disrupted on error trials. Furthermore, lesions of Re led to a disruption of prospective trajectory representation in CA1, supporting the idea that Re provides information about animal's future behavior. Taken together, the results point to a functional circuit composed of mPFC, Re and CA1 that is required for the hippocampus to represent the animal's future choice of action together with spatial position.

Disclosures: **H.T. Ito:** None. **S. Zhang:** None. **M.P. Witter:** None. **E.I. Moser:** None. **M. Moser:** None.

Poster

094. Cortical and Hippocampal Circuits: Spatial Navigation I

Location: Halls A-C

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Topic: F.02. Animal Cognition and Behavior

Support: Kavli Foundation

Centre of Excellence Grant from the Research Council of Norway

European Commission's FP7 FET Proactive programme on Neuro-Bio-Inspired Systems (Grant Agreement 600725)

Title: Grid cells in non-planar environments

Authors: *H. YAMAHACHI, M.-B. MOSER, E. I. MOSER;
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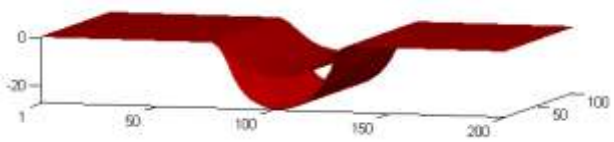
Abstract: Information about how an animal represents a complex environment in the brain is vital to understand navigation in the real world. The discovery of the grid cells in the entorhinal cortex supports the idea of an internal metric representation of space. So far, most of the experiments have been done in arenas with horizontal surfaces, and little is known about how grid cells are modulated in undulating environments. Are grid cells bound to the traversed surface? Is the map continuous or is it fragmented? In the present work, we have recorded grid cells in rats that forage in a rectangular arena (1 m x 2 m) that can be morphed from a planar to a non-planar configuration. To enable this transformation, the arena was made of three sections: both ends were flat, while the central section could be moved vertically. When the central section moved down, a rugged rubber floor created a concave curvature on the surface of the arena (Fig.). We found that firing fields of grid cells remained unaffected by the extra travelled space (7.5 to 25 cm equivalent to a curvature of maximum depth of 13 to 26 cm). Instead, their firing field positions matched exactly the ones on the planar configuration. The correspondence between planar and curved environments was present even on the first exposure to the curvature. The firing fields maintained their regularly spaced positions even where they intersected with the edges of the curvature indicating that the whole environment was represented as a single continuous map. Taken together, these data indicate that grid cells in the entorhinal cortex of the rat disregard the z-plane in undulating terrains, and instead anchor their firing fields to a horizontal x-y plane that covers the entire environment. Fig. Non-planar environments with maximum depth of 13 and 26 cm



Disclosures: **H. Yamahachi:** None. **M. Moser:** None. **E.I. Moser:** None.

Poster

094. Cortical and Hippocampal Circuits: Spatial Navigation I

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

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Topic: F.02. Animal Cognition and Behavior

Support: Kavli Foundation

Centre of Excellence Grant from the Research Council of Norway

ANPCyT (Argentina)

Title: Speed cells in the medial entorhinal cortex

Authors: *E. KROPFF CAUSA[1,2], J. E. CARMICHAEL[3,2], E. I. MOSER[2], M.-B. MOSER[2]; [1]Leloir Inst. - IIBBA - CONICET, Buenos Aires, Argentina; [2]Kavli Inst. For Systems Neurosci. and Ctr. For Neural Computation, NTNU, Trondheim, Norway; [3]Univ. of Waterloo, Waterloo, ON, Canada

Abstract: Grid cells in the medial entorhinal cortex (MEC) have spatial maps characterized by periodicity (Hafting et al, 2005). Their spatial code applies to several places within an environment as well as across environments (Fyhn et al, 2007), which suggests they could be the basis of a path-integration representation of space (McNaughton et al, 2006). A path integrator would receive a speed signal as an input and integrate it along a short window of time to obtain the displacement of the animal, using this result to update the spatial maps. Although weak correlations with theta frequency (Jeewajee et al, 2008) or grid cell firing (Sargolini et al, 2006; Wills et al, 2012) have been reported, the local availability and nature of a speed signal in MEC is still unclear. We recorded neural activity in the MEC and hippocampus of rats in a 1mx1m open field and in the Flintstone car, a task designed to control their speed. The car, which was ran by a computer along a 4m track, had no floor, constraining rats to run by their own means at an experimenter-determined speed in order to keep up and reach the end of the track, where a food reward was delivered. We report that running speed is coded in the firing rate of a large population of MEC cells, representing around 10% of the overall cell count in all layers. The firing of these speed cells is characterized by a positive-linear relationship with running speed and low spatial and head-directional information content. While other entorhinal populations (grid, head direction and border cells) present large overlaps with each other, their overlap with the population of speed cells is in all cases significantly lower than expected by chance. In agreement with the functional segregation of speed cells, when experimentally disentangling speed and position we were unable to find any modulation by speed in the grid cell population, and found only a weak modulation in hippocampal place cells. Finally, MEC speed cells exhibited prospective coding, anticipating the rats' movements around 60ms on average, although in some selected examples the anticipation reached 200-300ms. In turn, grid cells recorded in the same experiments behaved as if guided by a prospective rather than a regular path integrator, as evidenced by the anticipation of their firing position when acceleration was positive as opposed to negative, an effect that was absent in simultaneously recorded hippocampal place cells. This shift in firing position was stronger in a) layer II and b) the last portion of the theta cycle, not associated in grid cells with phase precession proper. Put together, our results point to the coordinated involvement of MEC grid cells and speed cells in path integration.

Disclosures: **E. Kropff Causa:** None. **J.E. Carmichael:** None. **E.I. Moser:** None. **M. Moser:** None.

Poster

094. Cortical and Hippocampal Circuits: Spatial Navigation I

Location: Halls A-C

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Topic: F.02. Animal Cognition and Behavior

Support: Centre of Excellence Grant from the Research Council of Norway

Kavli Foundation

European Research Council ('CIRCUIT' Advanced Investigator Grant, Grant Agreement N°232608).

Human Frontier Science Program

Title: Grid cell orientation is constrained by environmental geometry

Authors: T. STENSOLA, H. STENSOLA, *M. HAGGLUND, M.-B. MOSER, E. I. MOSER; Kavli Institute, CNC, Trondheim, Norway

Abstract: Grid cells express spatially selective firing fields which tile the environment. Earlier work has shown that grid cells form separate modules in which certain parameters are shared, such as orientation, size and ellipticity of the grid pattern. The absolute orientation of the grid axes has been assumed to be randomly distributed. Here we show that there is a striking similarity in the way grid orientation distributes in relation to the geometry of the recording environment, not only across different modules within an animal, but also across different animals in the same environment. Grid cells were recorded in two different square boxes in separate rooms. In one of the rooms, grid orientations were unimodally distributed around 7.5 degrees relative to one of the walls. In the other room, orientations took on a bimodal distribution with 7.5 degree offsets from either of the two orthogonal walls. Grid orientations near 0 or 15-degrees were almost completely absent. We next addressed possible mechanisms for the 7.5 degree orientation preference. We hypothesized that orientations were determined by the degree of asymmetry between the grid pattern and the geometry of the box. 7.5 degrees lies between the two symmetrical grid arrangements of 0 degrees (symmetry with one wall) and 15 degrees (symmetry with the diagonal) and could provide the most unambiguous representation with reference to the box geometry. To test this possibility we simulated 300 grid cell maps with uniformly distributed phases and coherent orientation. We then varied the orientation and

analyzed how well the activity between two orthogonal walls of the box correlated. The results show that 7.5 degrees is indeed the optimal solution for decorrelating border segments, and that 0 and 15 degrees are the least optimal solutions. There was a strong similarity between the correlation values in the simulated data and orientation preferences in the real data. To further investigate the importance of interactions between walls and grid orientations, we recorded grid cells from animals running in either an equilateral triangle, where offsets should be similar along all walls for a perfect hexagonal grid, or in a truncated cylinder, which had only a single straight reference wall. In both of these enclosures, the grid took on a zero degree angle in several of the experiments. These results corroborates that grid orientation is constrained by environment geometry, and suggest that grid orientation is determined by a process that optimizes disambiguation of geometrically ambiguous environments. We hypothesize that this is a result of competitive interactions imposed by the boundaries of the environment.

Disclosures: **T. Stensola:** None. **M. Hagglund:** None. **H. Stensola:** None. **M. Moser:** None. **E.I. Moser:** None.

Poster

094. Cortical and Hippocampal Circuits: Spatial Navigation I

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 94.06/SS26

Topic: F.02. Animal Cognition and Behavior

Title: Interhemispheric connections between left and right medial entorhinal cortices

Authors: ***K. ZHENG**, Ø. W. SIMONSEN, M. P. WITTER;
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Abstract: The rodent medial entorhinal cortex (MEC) contains a variety of spatially modulated neurons, including grid cells, head direction cells, border cells and speed cells. Grid cells are mostly prominent in layer II, as are stellate cells, suggesting that the stellate cell is a candidate for grid cells. We have previously reported that stellate cells are locally interconnected through fast spiking interneurons. It is well known that the left and right MEC are strongly connected and that these reciprocal connections show a strict topological organization. We studied this interhemispheric connection between the left and right MEC. We used anterograde and retrograde tracing and established that the main origin of the commissural projection is from neurons in layer III, although a low number of layer II cells contribute as well. The commissural fibers terminate in layers I/II. With the use of confocal microscopy, we observed that stellate

cells and interneurons within layer II are among the innervated targets. Using an optogenetic approach, we specifically activated axon terminals of the commissural fibers and recorded the postsynaptic responses by way of whole cell patch recordings in horizontal slices Our data corroborate the confocal findings showing that commissural fibers make excitatory synaptic contacts with stellate cells as well as interneurons in layer II of MEC. This circuit may allow the left and right grid networks to generate a unified spatial representation.

Disclosures: **K. Zheng:** A. Employment/Salary (full or part-time):; 1Kavli Institute for Systems Neuroscience and the Centre for Neural Computation, NTNU, Trondheim, Norway. **Ø.W. Simonsen:** None. **M.P. Witter:** None.

Poster

094. Cortical and Hippocampal Circuits: Spatial Navigation I

Location: Halls A-C

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Topic: F.02. Animal Cognition and Behavior

Support: Centre of Excellence Grant from the Research Council of Norway

Kavli Foundation

European Research Council ('CIRCUIT' Advanced Investigator Grant, Grant Agreement N°232608).

Human Frontier Science Program

Title: Grid cells interact with local boundaries

Authors: M. HÄGGLUND, T. STENSOLA, H. STENSOLA, M.-B. MOSER, *E. I. MOSER; Kavli Inst. Systems Neurosci, Trondheim, Norway

Abstract: Entorhinal grid cells produce a stereotyped and highly coherent triangular firing pattern when recorded in small open environments. We recently discovered that the orientation of the grid pattern is not randomly distributed, but is constrained by the geometry of the local environment. Recordings of grid cells in differently sized square boxes from 7 animals showed that grid orientation distributes with an average offset of 7.5 degrees from either wall of the box, an offset that is optimal for disambiguating geometrically similar locations within the box. The reliable offset of grid orientation suggests that grid fields anchor to environmental boundaries in

a stereotypical fashion. It is not clear, however, whether this anchoring process is global (expressed throughout the environment) or local (with different anchoring solutions at different locations). To address this question, we first recorded grid cells in large square environments, where local variations may become more visible. Grid patterns were not always coherent across the environment. Often the grid pattern would take on distinct orientations at different locations of the environment, with each local grid orientation being offset 7.5 degrees to the closest wall. When the grid anchored to two orthogonal walls, with one orientation in one part of the box and another in the rest of the environment, the grid often showed inconsistencies in hexagonality and coherence in the middle, where the two patterns merged. To further examine the effect of wall interactions on grid anchoring, we next recorded grid cells in a large triangular environment. Here the grid became highly elliptic, and took on complex forms, likely reflecting different anchoring solutions along different walls of the triangle. Orientation along one grid axis was often non-coherent, and would shift gradually across the environment. Because total area was smaller in the triangle compared to the square, despite equally long walls, reduced overall hexagonality in the pattern likely reflected a less graded merging of the locally anchored pattern fragments. Taken together, these results imply that grid cells anchor to walls locally and stitch together in open areas. The development and stabilization of grid maps in large individual environments may thus be an experience-dependent process.

Disclosures: **M. Hägglund:** None. **E.I. Moser:** None. **T. Stensola:** None. **H. Stensola:** None. **M. Moser:** None.

Poster

094. Cortical and Hippocampal Circuits: Spatial Navigation I

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 94.08/SS28

Topic: F.02. Animal Cognition and Behavior

Title: Graph analysis of the rat (para)hippocampal connectome

Authors: *N. M. VAN STRIEN[1,2,3], F. Z. M. BINICEWICZ[2], W. J. WADMAN[2], M. P. WITTER[1], M. P. VAN DEN HEUVEL[3], N. L. M. CAPPAERT[2];
[1]Kavli Inst. for Systems Neurosci. and Ctr. for Neural Computation, NTNU, Trondheim, Norway; [2]Swammerdam Inst. for Life Sci. - Ctr. for Neurosci., Univ. of Amsterdam, Amsterdam, Netherlands; [3]Dept. of Psychiatry, Univ. Med. Ctr. Utrecht, Utrecht, Netherlands

Abstract: The hippocampal formation (HF) and the parahippocampal region (PHR) contribute to encoding, consolidation and retrieval of declarative memories. Knowledge of the neuroanatomical connections of the HF and PHR will underpin our efforts to understand the network function. Connectomes are therefore indispensable, since they transparently summarize and categorize the brain's anatomical connections. From the tract tracing literature we have constructed a connectome of the 11 subregions of the rat HF and PHR (van Strien et al., 2009; Sugar et al., 2011; www.temporal-lobe.com). These subregions were further split in a three dimensional manner along the rostrocaudal, dorsoventral and laminar axis to provide unprecedented detailed information about the origin and termination of each included connection. This HF-PHR connectome is currently the most fine-grained representation of a mammalian cortical network. We have now applied graph analysis to our HF-PHR connectome in order to systematically disclose the inherent characteristics of the structural network. To obtain biologically meaningful values, we collapsed the data over each three-dimensional axis creating sub-connectomes. The network regarding the laminar dimension has a density of 19%, a path length of 2.1 and a correlation coefficient of 0.4. We tested the latter values against a set of random null models, concluding that the laminar network is regularly organized. Within this laminar network, thirteen rich club nodes were discovered, mainly situated in the PHR. Six of these rich club nodes were connector hub nodes, all six of them were positioned in the medial and lateral entorhinal cortex. This suggests that the entorhinal cortex is crucially important for information transfer in the HF-PHR network. Several modules were detected that showed a close relationship between the perirhinal and postrhinal cortex as well as between the lateral and medial entorhinal cortex. These findings are incompatible with the two stream memory model (Eichenbaum & Lipton, 2008), in which the spatial and non-spatial perceptual information streams towards the hippocampus are carried by separate routes: the postrhinal - medial entorhinal cortex 'spatial' stream versus the perirhinal - lateral entorhinal cortex 'non-spatial' stream. In our analysis, the interconnectivity between the streams is prevailing, which is supported by recent physiological evidence (van Cauter et al,2013; Deshmuk & Knierim, 2011; Hunsaker et al, 2013; Tsao et al, 2013). Although our connectome is incomplete due to lack of anatomical data in the current literature, our graph analysis nevertheless suggests that the two-stream model is not tenable.

Disclosures: **N.M. van Strien:** None. **F.Z.M. Binicewicz:** None. **W.J. Wadman:** None. **M.P. Witter:** None. **M.P. van den Heuvel:** None. **N.L.M. Cappaert:** None.

Poster

094. Cortical and Hippocampal Circuits: Spatial Navigation I

Location: Halls A-C

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Program#/Poster#: 94.09/SS29

Topic: F.02. Animal Cognition and Behavior

Support: Max Planck Society

Research Council of Norway

European Research Council

Kavli Foundation

Title: Sensory representation in lateral entorhinal cortex

Authors: *A. TSAO[1,2], P. ZMARZ[3,2], G. KELLER[3,2], M.-B. MOSER[1], M. HÜBENER[2], E. I. MOSER[1], T. BONHOEFFER[2];
[1]CNC/KAVLI, NTNU, Trondheim, Norway; [2]Max Planck Inst. for Neurobio., Martinsried, Germany; [3]FMI, Basel, Switzerland

Abstract: The lateral entorhinal cortex (LEC) is likely one of the main gateways by which sensory information reaches the hippocampus. However, it is currently unknown how the LEC represents basic sensory stimuli, and it is unclear how this representation is modified or utilized during behavior. To address these questions, we recorded activity in LEC neurons expressing the genetically encoded calcium indicator GCaMP6f while presenting a wide range of odor stimuli (including diverse single odorants, mixtures, and different concentrations). We observed sparse activation to all odor stimuli, with only a few percent of cells responding to any given odor. To test if sparse sensory representation is a general feature of LEC, we then imaged while presenting a wide range of auditory stimuli including pure tones, ultrasonic vocalizations, and birdcalls. Preliminary results indicate that representation of auditory stimuli in LEC is similarly sparse as for odor stimuli. In order to examine whether and how the sparse representation of basic odors is used during behavior, we have developed a virtual T-maze task which mice, guided by odor cues, learn consistently. We are currently imaging activity in LEC while mice perform this task to examine whether the sparse representation of odor cues changes with learning, as well as whether and how goal-oriented behavior affects representations of sensory cues. Through a better understanding of sensory processing within LEC, we may gain insight into what roles LEC plays in its interactions with the hippocampus as well as higher-order regions.

Disclosures: **A. Tsao:** None. **P. Zmarz:** None. **G. Keller:** None. **M. Moser:** None. **M. Hübener:** None. **E.I. Moser:** None. **T. Bonhoeffer:** None.

Poster

094. Cortical and Hippocampal Circuits: Spatial Navigation I

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 94.10/SS30

Topic: F.02. Animal Cognition and Behavior

Title: Grid representation for merged space

Authors: *T. WERNLE[1], E. I. MOSER[2], M.-B. MOSER[2];
[1]Kavli Institute/CNC The Fac. of Medicine, NTNU, Trondheim, Norway; [2]Kavli Inst. / CNC The Fac. of Medicine, NTNU, Trondheim, Norway

Abstract: Grid cells are part of the brain's metric for space. Their firing locations define a periodic triangular array overlaid on the entire space available to a moving animal (Hafting et al. 2005). In natural environments, space is likely represented as a mosaic of discrete grid maps that stitch together at salient landmarks such as the turning points of a maze (Derdikman et al. 2009). However, it is not clear how a global coherent map is established from discrete fragments. To address this question, we recorded from grid cells in separated and merged spatial compartments. We first trained rats in a large square environment (2 x 2 m) which was separated into two similar rectangular compartments by a central wall sharing common distal cues. Once two distinct grid maps were generated for each rectangle, we removed the wall, allowing the animal to walk instantaneously from one compartment into the other. The two distinct grid maps did not always change into a merge of the two original maps. Instead, a single global coherent map was often established from one of the two pre-existing representations, depending on the starting position of the rat. The calibration of this global map was experience-dependent and required successive visits to the different parts of the environment. Furthermore, we found that grid cells within one module operated coherently in order to establish one global map and that distinct grid modules represented merged space independently. Ongoing work is testing whether the response to merged environments is general across animals and if not, which factors determine whether a merged map is formed or not.

Disclosures: **T. Wernle:** None. **E.I. Moser:** None. **M. Moser:** None.

Poster

094. Cortical and Hippocampal Circuits: Spatial Navigation I

Location: Halls A-C

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Program#/Poster#: 94.11/SS31

Topic: F.02. Animal Cognition and Behavior

Support: Kavli Foundation

Norwegian Research Council Centre of Excellence Grant

Title: Modular organization of gamma oscillations in medial entorhinal cortex

Authors: *K. M. IGARASHI, H. STENSOLA, T. STENSOLA, M.-B. MOSER, E. I. MOSER; Kavli Inst. for Systems Neuroscience, NTNU, Trondheim, Norway

Abstract: Grid cells in the medial entorhinal cortex (MEC) provide the animal with a metric of the environment via regularly-spaced spatial firing patterns. We recently showed that grid cells are organized into discrete modules with different grid spacing (Stensola et al., 2012). Grid cells of different modules can respond independently to environmental change, whereas cells in the same module respond coherently. However, underlying mechanisms for the coherent spatial firing within modules are not clear. One possibility is that gamma oscillations promote communication between cells in the same module, and that different modules operate at different frequency bands. To test this hypothesis, we characterized gamma oscillations in local field potentials recorded from tetrodes with layer III cells belonging preferentially to a single module. Wavelet analysis was performed to characterize cross-frequency coupling of gamma oscillations against theta oscillations. The analysis showed that there are three distinct gamma bands: slow gamma (25-55 Hz), fast gamma (55-100 Hz) and very fast gamma (>100 Hz). The existence of the three gamma bands is similar to the frequency organization observed in the hippocampus (Colgin et al., 2009; Belluscio et al., 2012). We used the slow and fast gamma bands for further analyses. Preliminary analysis suggests that tetrodes predominated by cells from modules with short grid wavelengths (short spacing) exhibit fast gamma, whereas tetrodes predominated by larger-scaled modules show both slow and fast gamma oscillations. Tetrodes with large-scale modules had slow gamma oscillations that were more strongly phase-locked to theta oscillations than gamma oscillations on tetrodes with small-scale modules. Phase-locking analysis of spikes from grid cells further showed that spikes in large-scale modules are more strongly phase-locked to slow gamma oscillations than those in small-scale modules. No difference between large-scale and small-scale modules was observed for fast gamma oscillations in cross-frequency coupling and spike phase-locking. These results support the idea that modules with short spacing use only fast gamma whereas modules with larger spacing use both fast and slow gamma oscillations, and point to gamma oscillations as a mechanism for independent representation across grid modules in MEC.

Disclosures: K.M. Igarashi: None. **H. Stensola:** None. **T. Stensola:** None. **M. Moser:** None. **E.I. Moser:** None.

Poster

094. Cortical and Hippocampal Circuits: Spatial Navigation I

Location: Halls A-C

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Program#/Poster#: 94.12/SS32

Topic: F.02. Animal Cognition and Behavior

Support: EMBO long-term fellowship ALTF 246-2013

Title: 2-photon imaging of the medial entorhinal cortex in mice performing a virtual reality navigation task

Authors: *F. DONATO[1], A. TSAO[1,2], M.-B. MOSER[1], E. I. MOSER[1], T. BONHOEFFER[2]; [1]Kavli Inst. For Systems Neurosci., Trondheim, Norway; [2]Max Planck Inst. of Neurobio., Martinsried, Germany

Abstract: Navigation is thought to rely on path integration-dependent maps of the spatial environment, expressed in different forms in the hippocampus and the medial entorhinal cortex. The formation of such depends on a broad ensemble of interconnected neurons whose firing is tuned to spatial features, including place cells in the hippocampus and grid cells and border cells in the medial entorhinal cortex. Grid cells are thought to provide the metric of the spatial representation. Despite extensive characterization of the physiological properties of these cells, their organization in the entorhinal microcircuit remains elusive. In particular, conventional electrophysiological recordings have not been able to determine (i) how grid cells and other entorhinal cell types are organized in anatomical space, (ii) whether grid cells have specific molecular or morphological profiles, or (iii) how grid cells with different grid phases (firing locations) show topographical organization of any kind at a detailed level. Here, we developed a preparation that allows us to perform chronic 2-photon calcium imaging in the superficial layers of the medial entorhinal cortex of head-restrained animals navigating in a virtual reality environment. High-resolution images were obtained by imaging through a glass microprism directly placed behind the medial entorhinal cortex, therefore allowing standard calcium imaging of the deep structure while maintaining the integrity of all cortical structures surrounding MEC. Cells close to the prism face could be imaged repeatedly and reliably across several days, and their firing properties could be related to specific aspects of the navigation task. The preparation allows us to study the fine-scale anatomical distribution of spatially modulated cells as well as their molecular identity and lateral connectivity, in order to directly observe the local anatomical

organization of spatial cell types, thereby hopefully further elucidating the functional network underlying grid cells as well as other functional cell types in the medial entorhinal cortex.

Disclosures: **F. Donato:** None. **A. Tsao:** None. **M. Moser:** None. **E.I. Moser:** None. **T. Bonhoeffer:** None.

Poster

094. Cortical and Hippocampal Circuits: Spatial Navigation I

Location: Halls A-C

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Title: Towards a functional identification of stellate cells in medial entorhinal cortex layer II

Authors: *D. C. ROWLAND, E. R. SKYTØEN, C. G. KENTROS, M.-B. MOSER, E. I. MOSER;
NTNU, Trondheim, Norway

Abstract: Layer II of the medial entorhinal cortex (MECII) contains two principal cell types: pyramidal cells and stellate cells. Accumulating evidence suggests that these two cell types have distinct physiological properties and projection patterns, with the reelin-expressing stellate cells providing the main excitatory input to the dentate gyrus and CA3 region of the hippocampus. At least a subset of the pyramidal cells differ from stellate cells in that they express calbindin but not reelin, receive strong cholinergic input, and do not project directly to principal cells in the hippocampus. This calbindin-expressing pyramidal cell population forms tight clusters intermingled among stellate cells. Together, these observations hint at a fundamental difference between stellate cells and pyramidal cells but the functional identity of the two cell classes needs to be worked out. Here we investigate a transgenic mouse line (Yasuda and Mayford, 2006; Rowland et al., 2013) where the transgene is expressed almost exclusively in MECII. In the first part of the study, we sought to determine the morphological identity of the transgene-expressing

cell population. Two lines of evidence suggested that the transgenic neurons are stellate cells. First, GFP was expressed widely but with distinct circular gaps, suggesting that the transgenic neurons are outside of calbindin-expressing clusters Second, viral labelling of the transgenic cells showed that the cells have a stellate morphology and that they project to the dentate gyrus and CA3 region of the hippocampus. Molecular characterization of the cells using antibodies for calbindin and reelin is ongoing. In the second part of the study, we sought to identify the functional properties of the cells *in vivo* by expressing the optogentic silencer archaerhodopsin in the same cells and using short pulses of light to identify the transgene-expressing cells. Light-responsive cells included grid cells and border cells, but not head direction cells or interneurons. Taken together, these data suggest that the population of hippocampal-projecting stellate cells most likely includes both grid cells and border cells.

Disclosures: D.C. Rowland: None. **E.R. Skytøen:** None. **C.G. Kentros:** None. **M. Moser:** None. **E.I. Moser:** None.

Poster

094. Cortical and Hippocampal Circuits: Spatial Navigation I

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Topic: F.02. Animal Cognition and Behavior

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Centre of Excellence grant and a FRIPRO grant from the Research Council of Norway

Title: Head direction cells before the time of eye opening in rat pups

Authors: *T. L. BJERKNES[1], R. F. LANGSTON[2], I. U. KRUGE[1], E. I. MOSER[1], M.-B. MOSER[1];
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Abstract: The brain's map of space consists of several functionally specific neurons, including place cells in the hippocampus and grid cells, border cells and head direction cells in medial entorhinal cortex (MEC), pre- and parasubiculum. Head direction cells are cells that fire selectively when animals hold their head in a certain direction in the horizontal plane. The preferred firing direction of these cells can be manipulated by visual changes to the environment, for instance, the preferred direction can rotate together with visual landmarks. Head direction cells can also maintain a sharp directional representation during locomotion in the dark, but their preferred direction may drift significantly over time. In the present study we investigated the developmental origin of the head direction signal. Previous work has shown that head direction cells in rats exhibit stable directional firing from the day of eye opening at P15, well before the emergence of stable positional signals. We asked whether this directional representation is present ahead of eye opening, in the absence of visual cues. We implanted rat pups from the age of P10. Directionally modulated cells were detected in pre- and parasubiculum already at P12. Directional modulation was unstable, however, especially between trials. The stability of the head direction cells increased significantly as the rat pups opened their eyes. Head direction signals were able to rotate with visual landmarks within the first two days after eye opening. These data indicate that visual input may not be necessary for formation of directional signals in rat pups but that anchoring to visual landmarks is important for stabilizing head direction cells.

Disclosures: **T.L. Bjerknes:** None. **R.F. Langston:** None. **I.U. Kruge:** None. **E.I. Moser:** None. **M. Moser:** None.

Poster

094. Cortical and Hippocampal Circuits: Spatial Navigation I

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Program#/Poster#: 94.15/SS35

Topic: F.02. Animal Cognition and Behavior

Support: Kavli Foundation

Norwegian Research Council Center of Excellence Grant

Title: Reduced rate remapping in hippocampal subregion CA2

Authors: ***L. LU**, K. M. IGARASHI, M. P. WITTER, M.-B. MOSER, E. I. MOSER;
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Abstract: Hippocampal place-cell populations use a dual coding scheme to represent the external environment. While the animal's coordinates in space are expressed by the collective firing locations of the place cells, differential experience in the environment is often accompanied by differences in the distribution of activity among subsets of place cells with fixed firing locations. Complete transitions of firing patterns are defined as global remapping whereas transitions between rate patterns only are referred to as rate remapping. Both global remapping and rate remapping have been observed in place cells of dentate gyrus, CA3 and CA1; however, the remapping properties of CA2 place cells remain unknown. To explore the behaviour of CA2 place cells during remapping, we recorded activity of pyramidal neurons from CA2 and CA1 / CA3 simultaneously, while rats were exposed to different experiences which regularly induce global remapping and rate remapping in the hippocampus. As expected, CA2 pyramidal neurons were place cells but the spatial tuning of CA2 place cells was weaker compared to place cells in CA1 and CA3, as indicated by significantly lower spatial information content, spatial coherence and spatial stability. During random foraging in similar boxes in two different rooms, place cells recorded from all three subregions showed clear global remapping, indicated by a dramatic decrease of spatial cross correlation between different rooms. However, when the wall color of the box was reversed between trials in the same room, place cells in both CA1 and CA3 showed rate remapping, expressed by changes in rate distribution in the presence of high spatial corrrelation. The firing of the CA2 place cells, in contrast, remained almost identical across trials. Taken together, these data suggested that CA2 place cells are less sensitive to contextual input. The findings shed light on our understanding of the functional role of the CA2 subregion of the hippocampus.

Disclosures: **L. Lu:** None. **K.M. Igarashi:** None. **M.P. Witter:** None. **M. Moser:** None. **E.I. Moser:** None.

Poster

094. Cortical and Hippocampal Circuits: Spatial Navigation I

Location: Halls A-C

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Topic: F.02. Animal Cognition and Behavior

Support: NSF IOS-1149718

DGE-1144086

Title: Retrosplenial cortex and Hippocampus share modulation of firing activity by allocentric space and the Hippocampal theta rhythm

Authors: *A. ALEXANDER, B. LA, S. A. COHANTZ, M. NGUYEN, D. A. NITZ;
UCSD, San Diego, CA

Abstract: Retrosplenial cortex (RSC) neurons map position in both egocentric and external frames of reference. Egocentric mapping takes the form of neurons whose activity reflects movement to the left or right of the longitudinal axis of the rat (i.e., 'left-turn' and 'right-turn' neurons). One external frame of reference mapped by RSC neurons is the space of known routes through the environment, also known as 'route' space. Such mapping takes two main forms: 1) neurons exhibit different turn-related activity rates depending on the position of turns in a route and, 2) neurons exhibit complex but reliable changes in activity across route traversals. Critically, both forms of spatial mapping are modulated by a second external frame of reference, the 'allocentric' space, defined by the boundaries of the environment. The result is consistent with RSC anatomy, as it receives direct inputs from hippocampal (HPC) sub-region CA1 and the subiculum. RSC may influence HPC through an excitatory projection to the entorhinal cortex, the main input to the HPC. Notably, RSC inactivation disrupts navigation in the allocentric frame of reference and the reliability of place-specific firing of HPC neurons. At this time, little is known as to the forms by which RSC and HPC interact to generate spatial cognition and episodic memory processes. We examined the spatial and temporal dynamics of HPC and RSC neurons recorded while the animal performed a simple track running paradigm with the track in two different room (allocentric frame) locations on any given recording day. Correlations between positional firing rate vectors for different track locations in the room were low for both RSC and HPC neurons, indicating modulation by the allocentric spatial frame. However, some RSC neurons had similar patterns across track positions, an effect not observed for the HPC population. Mapping of position in allocentric space also took the form of 'head direction' cells whose firing followed specific orientations of the animal's head relative to the environment. Another sub-population of RSC neurons exhibited activation patterns analogous to HPC place fields. Despite the impact of allocentric position on the firing activity of RSC neurons, there was no evidence that RSC neurons exhibit 'phase precession', a phenomena wherein HPC place cell firing is organized relative to theta-frequency local field potentials. Nevertheless, a subset of RSC neurons had activation that was phase-locked to theta rhythms recorded in the HPC. The results suggest that RSC and HPC can interact in the distribution of their firing rates in both the spatial and temporal domains.

Disclosures: **A. Alexander:** None. **B. La:** None. **S.A. Cohantz:** None. **M. Nguyen:** None. **D.A. Nitz:** None.

Poster

094. Cortical and Hippocampal Circuits: Spatial Navigation I

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 94.17/SS37

Topic: F.02. Animal Cognition and Behavior

Support: NSF - 1149718

Title: Positional mapping of route space in the medial precentral cortex and superior colliculus

Authors: *J. M. OLSON, D. A. NITZ;
Dept. of Cognitive Sci., UCSD, San Diego, CA

Abstract: Neurons of the rat posterior parietal cortex (PPC) map position in both egocentric and external frames of reference. Subpopulations of PPC neurons map egocentric space by firing with relatively high regularity when movements to the left or the right are made. PPC neurons also map the external frame of reference of a well-learned route, defined by inflection points in behavior (e.g., starts, stops, turns) and the straight-run spaces that separate them. Such 'route-based', spatially-specific firing is observed irrespective of the route position in a larger environment (Nitz, Neuron, 2006). Mapping in the route-based frame of reference takes the form of: 1) neurons with left or right turn related activity that is, in turn, modulated strongly by the position of the turn in a route; and 2) neurons whose rate vectors across route space are complex, but reliable across trials. PPC efferents reach many targets, but particularly dense innervation reaches a limited number of target structures (Nitz, 2009, Neurobiology of Learning and Memory). In the present study, we address the spatial firing correlates of two such structures, the medial precentral cortex (MPC, a prefrontal cortex sub-region often referred to as M2) and the superior colliculus (SC). Published data indicate that SC neurons are sensitive to direction of movement and have correlates to locomotor behaviors (Cooper, Miya, and Mizumori, Hippocampus, 1998). Prior work (Nitz et al., Society for Neuroscience Abstracts, 2013) indicates that many MPC neurons exhibit activity tightly correlated to track positions associated with left turns, right turns, or simple forward locomotion. Additional MPC neurons exhibit 'planning' activity wherein firing rates along straight-run track sections is highly predictive of whether the animal will, at a later time and place, turn left or right. Planning activity in MPC is not limited to only one or two positions in the environment, but occurs anywhere a straight-run segment leads to a left/right-turn intersection. At issue in the present work is whether MPC neurons and/or SC neurons exhibit route-based mapping (an external frame of reference) that goes beyond activity correlates to locomotor behaviors. Sensitivity to route position has been observed in a sub-population of MPC neurons and can be readily observed, at the population level, in covariance matrices revealing that track positions associated with like turns (left versus right) yield similar,

but not indiscriminable ensemble rate vectors. The results support a model in which spatial information is gradually transformed from external to egocentric frames of references for the purpose of fluid, efficient navigation.

Disclosures: **J.M. Olson:** None. **D.A. Nitz:** None.

Poster

094. Cortical and Hippocampal Circuits: Spatial Navigation I

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 94.18/SS38

Topic: F.02. Animal Cognition and Behavior

Support: NSF I0S-1149718

Title: Tracking vertical movements and vertical space in the posterior parietal cortex

Authors: *L. E. SHELLEY, A. S. ALEXANDER, B. LA, D. A. NITZ;
UCSD, La Jolla, CA

Abstract: Neurons of both the hippocampus and posterior parietal cortex map the position of an animal in external frames of reference. Hippocampal neurons primarily map position within the space of the observable environment. In a complementary fashion, posterior parietal cortex neurons map position within the space of known routes irrespective of the position of those routes in the observable environment. In both cases, spatially-specific firing has been examined almost exclusively with respect to the horizontal dimensions. However, recently published experiments suggested that the form by which vertical space is mapped by hippocampal place cells and entorhinal cortex grid cells is fundamentally different and of lower resolution than that of horizontal space (Hayman, et al., Nature Neuroscience, 2012). Maps of route position given by the activity of some posterior parietal cortex neurons appear to derive initially from the tendency of a subset of these neurons to have activity correlates to specific locomotor behaviors such as left or right turns or simple forward locomotion. A mapping of route position is then achieved when robust and reliable modulation in the firing activity during performance of the preferred locomotor behavior occurs as a function of the location of that behavior along a full route. For other posterior parietal cortex neurons, firing patterns across route space are more complex, yet reliable from trial to trial and not related in any obvious way to specific locomotor behaviors. In the present work, we addressed the possibility that posterior parietal cortex neurons could effectively map position in the vertical dimension, which, in principle, could derive from a

tendency for a subset of its neurons to have activity correlates to locomotor behaviors that yield movement in the vertical dimension. As in previous work, posterior parietal cortex neurons recorded in rats exhibited spatially-specific activity during traversal of defined routes along tracks. Track positions combining movement in the vertical dimension with locomotion in the horizontal dimensions were associated with specific activity peaks in a sub-population of posterior parietal cortex neurons. The results suggest that posterior parietal cortex is capable of: 1) mapping specific vertical movement behaviors; and 2) generating a map of route space containing position information in the vertical dimension. Further analyses will examine the extent to which spatially-specific firing for posterior parietal cortex neurons is isotropic for the horizontal and vertical dimensions or whether, like hippocampal mapping, the representation of vertical space takes a lower-resolution form.

Disclosures: **L.E. Shelley:** None. **A.S. Alexander:** None. **B. La:** None. **D.A. Nitz:** None.

Poster

094. Cortical and Hippocampal Circuits: Spatial Navigation I

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 94.19/SS39

Topic: F.02. Animal Cognition and Behavior

Title: No movement-related oscillations in the entorhinal-hippocampal system of behaving bats despite low-frequency cellular resonance

Authors: T. ELIAV[1], M. GEVA-SAGIV[1,2], M. YARTSEV[1], A. FINKELSTEIN[1], A. RUBIN[1], L. LAS[1], *N. ULANOVSKY[1];
[1]Weizmann Inst. of Sci., Rehovot, Israel; [2]Elsc, Hebrew Univ. of Jerusalem, Jerusalem, Israel

Abstract: The hippocampal formation of rodents and bats contains several types of spatial neurons - including place cells and grid cells - which share very similar functional properties between bats and rodents. However, while spatial neurons in rodents exhibit continuous theta-rhythmic firing, bat neurons were found to exhibit very little theta rhythmicity in their spike trains. This finding created a major controversy over the role of the mammalian theta-rhythm - for example, its proposed role in generating the grid formation, as posited by 'oscillatory interference models'. Recent in-vitro experiments reported possible intrinsic membrane resonance in bat entorhinal neurons at low frequencies, indicating that the oscillatory interference model might perhaps work in bats - but at a lower, sub-theta frequency band. To test this hypothesis, we recorded several new in-vivo datasets - including extensive new data from

3D place cells in flying bats, as well as from bat hippocampal interneurons; the latter is particularly important because hippocampal interneurons in rodents exhibit very strong theta-rhythmicity, and their high firing-rate allows robust identification of oscillations, if they exist. To analyze possible rhythmicity in these new and existing datasets, we used two types of analyses: (i) spectral analysis, based on the power spectrum of the spike-train autocorrelation; and (ii) time-domain fit analysis, based on fitting an exponentially-decaying sinewave to the autocorrelation. Both types of analyses showed that grid cells, place cells and interneurons in bat entorhinal cortex and hippocampus do not exhibit in-vivo movement related oscillations in their spike trains - neither at the theta-band nor at lower frequencies - neither during 2D crawling nor in 3D flight. These findings suggest that oscillation-based models cannot account for the generation of place-fields and grid-fields across mammals - neither at theta nor at lower frequencies.

Disclosures: **T. Eliav:** None. **M. Geva-Sagiv:** None. **N. Ulanovsky:** None. **M. Yartsev:** None. **A. Finkelstein:** None. **A. Rubin:** None. **L. Las:** None.

Poster

094. Cortical and Hippocampal Circuits: Spatial Navigation I

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 94.20/SS40

Topic: F.02. Animal Cognition and Behavior

Title: Switching sensory modalities elicits remapping and sharpening of 3-D spatial codes in the hippocampus of flying bats

Authors: ***M. GEVA-SAGIV**[1,2], L. LAS[1], N. ULANOVSKY[1];
[1]Neurobio., Weizmann Inst. of Sci., Rehovot, Israel; [2]Edmond and Lily Safra Ctr. for Brain Res., Hebrew Univ., Jerusalem, Israel

Abstract: How do inputs from different sensory systems affect hippocampal spatial representations? We set out to dissociate the effects of using two long-range sensory systems - vision versus echolocation - on hippocampal neural-activity, in an animal model that allows clean dissociation between sensory modalities: the Egyptian fruit bat. We trained bats to fly back and forth along a "linear flight track" using vision without sonar - in a lit environment, or using sonar without vision - in the dark, while controlling for olfaction. We conducted in-flight tetrode recordings from hippocampal areas CA1 and Subiculum, and found that 3-D place-cells are selective to flight direction in this paradigm. Many cells exhibited “remapping” when switching

between vision and echolocation (which reverted when switching back to vision) - suggesting different cognitive maps for different sensory modalities. Surprisingly, this remapping phenomenon was observed both in pyramidal cells and in interneurons. This is the first demonstration of hippocampal remapping in a non-rodent species. Finally, we predicted that we will find more compact (sharper) hippocampal place-fields under vision versus echolocation - because visual resolution is known to be better than sonar-based sensory resolution in this species. Our preliminary results are indeed consistent with this prediction, which provides support for sensory-based models of place-field formation in the mammalian hippocampus (e.g., BVC model, View model).

Disclosures: **M. Geva-Sagiv:** None. **L. Las:** None. **N. Ulanovsky:** None.

Poster

094. Cortical and Hippocampal Circuits: Spatial Navigation I

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 94.21/SS41

Topic: F.02. Animal Cognition and Behavior

Title: Neural-network model of 3D head-direction tuning in bats

Authors: ***A. RUBIN**, N. ULANOVSKY, M. TSODYKS;
Weizmann Inst. of Sci., Rehovot, Israel

Abstract: Electrophysiological studies in Egyptian fruit bats demonstrate 3D head-direction neurons of several types: pure azimuth cells, pure elevation cells, and conjunctive cells (which are tuned to a specific combination of head azimuth and elevation). Moreover, the tuning of these cells is consistent with a toroidal representation of head-direction. Here, we set out to investigate the theoretical conditions for the co-existence of these 3 classes of cells in a neural-network model. We found that this system can be modeled using the classical ring attractor and its extension to a toroidal attractor. This model supports mixed populations of neurons tuned to two cyclical variables - in our case, head azimuth and elevation. We show that the coexistence of conjunctive and two types of pure representations is achieved over a broad parameter regime, and even in cases of unbalanced population sizes, as has been observed in the experiments. However, for certain areas of phase-space this coexistence collapses, and one population takes over - which illuminates the theoretical constraints on the co-existence of pure-variable and mixed-variable coding populations. The model can be generalized for additional brain regions that encode multiple cyclical variables, such as primary visual cortex - where neurons encode

visual orientation and spatial phase representation; or medial entorhinal cortex - where some "conjunctive grid cells" encode the spatial phase of the grid and the animal's head azimuth.

Disclosures: **A. Rubin:** None. **N. Ulanovsky:** None. **M. Tsodyks:** None.

Poster

094. Cortical and Hippocampal Circuits: Spatial Navigation I

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 94.22/SS42

Topic: F.02. Animal Cognition and Behavior

Title: 3-D grid cells in flying bats

Authors: G. GINOSAR, A. FINKELSTEIN, *L. LAS, N. ULANOVSKY;
Dept. of Neurobio., Weizmann Inst. of Sci., Rehovot, Israel

Abstract: Spatial orientation and navigation are crucial for all animals. 'Grid cells' are neurons found in the mammalian medial entorhinal cortex and nearby regions. These cells discharge when the animal passes through the vertices of a two-dimensional (2D) hexagonal grid spanning the 2D movement surface. However, although many animals navigate daily through 3D space - including squirrels, bats, dolphins, and monkeys - no studies to date have attempted to characterize the 3D volumetric firing of grid cells, in any species. To address this, we used Egyptian fruit bats to investigate whether 3D grid cells exist, and to elucidate their 3D spatial code. We have previously found 2D grid cells in bats crawling on a 2D surface, as well as volumetric 3D place cells in freely-flying bats. Here, bats were trained to fly in a large flight room (~6 x 5 x 3 m) in search of randomly-positioned food, while we wirelessly recorded single-neuron activity in several brain regions where 2D grid cells are known to exist. Preliminary results indicate the existence of grid-like structures in recorded 3D firing-rate maps, consisting of repetitive blobby firing-fields. We are now analyzing whether these 3D firing-fields are arranged in a mathematically-optimal packing arrangement, as predicted by computational models of 3D grid cells.

Disclosures: **G. Ginosar:** None. **L. Las:** None. **A. Finkelstein:** None. **N. Ulanovsky:** None.

Poster

094. Cortical and Hippocampal Circuits: Spatial Navigation I

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Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 94.23/SS43

Topic: F.02. Animal Cognition and Behavior

Title: 3D head direction cells in bats on a vertical ring and in flight

Authors: *A. FINKELSTEIN[1], D. DERDIKMAN[1,2], A. RUBIN[1], J. N. FOERSTER[1], L. LAS[1], N. ULANOVSKY[1];
[1]Weizmann Inst. of Sci., Rehovot, Israel; [2]Rappaport Fac. of Medicine, Technion – Israel Inst. of Technol., Haifa, Israel

Abstract: Head-direction cells are neurons that become active whenever the animal's head points to a specific direction (azimuth) in the horizontal plane, and were suggested to be crucial for the mammalian navigation system. Although the survival of the animal may depend on successful orientation in three-dimensional (3D) space, it is unclear how 3D head-direction is represented in the brain. Here we recorded from neurons in dorsal presubiculum of Egyptian fruit bats, and found head-direction cells tuned to azimuth, pitch or roll. Many of these cells were conjunctively tuned to multiple combinations of Euler angles. This 3D tuning was found in 3 different sets of experiments: (i) in bats crawling on an arena floor, (ii) in bats crawling on a vertical ring that allowed 360° pitch maneuvering, and (iii) in flying bats. Head-direction cells were organized according to a functional-anatomical gradient along the transverse axis of the presubiculum. When bats were held upside-down, most neurons remained directionally-tuned; surprisingly, however, their azimuth-tuning was shifted by 180° relative to the upright position - suggesting that 3D head-direction is represented on a toroidal manifold, rather than in standard spherical coordinates. The toroidal model was also supported by new preliminary findings from bats crawling on a vertical ring, in which we found that individual pitch cells were narrowly-tuned to a specific circular pitch angle within the available 360° of pitch. Taken together, these results demonstrate, for the first time, a 3D head-direction mechanism in mammals, which could support navigation in 3D space.

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Poster

094. Cortical and Hippocampal Circuits: Spatial Navigation I

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Topic: F.02. Animal Cognition and Behavior

Support: NIH 5R01MH092925

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Title: Hippocampal motifs: Intact phase precession in the absence of spatial selectivity

Authors: *Z. M. AGHAJAN[1,2,3,4], L. ACHARYA[1,2,3,5], J. CUSHMAN[1,2,3,4], J. MOORE[1,2,3,6], C. VUONG[1,2,3,4], M. MEHTA[1,2,3,4,7];
[1]W. M. Keck Ctr. for Neurophysics, [2]Integrative Ctr. for Learning and Memory, [3]Brain Res. Inst., [4]Physics and Astronomy, [5]Biomed. Engin. Interdepartmental Program, [6]Neurosci. Interdepartmental Program, [7]Neurol. and Neurobio., UCLA, Los Angeles, CA

Abstract: Hippocampal activity during spatial exploration is characterized by two distinct coding patterns: A rate code and a temporal code. The rate code refers to spatially localized firing of spikes called place fields. The temporal code refers to systematic change in local field potential (LFP) theta-phase of spikes as a function of the position of the rat within a place field, termed phase precession. Many theories and experiments suggest that the rate and temporal codes are closely linked but this has been difficult to directly test experimentally. In addition, another aspect of hippocampal activity pattern has received little attention, namely neurons fire at an elevated rate for prolonged periods (1), about 1-2 seconds, as a subject traverses the place field. To understand the link between the rate code, temporal code and sustained activity, we measured hippocampal activity while rats foraged for randomly scattered rewards in a two dimensional environment either in the real world (RW) or virtual reality (VR). We found a large reduction in spatial selectivity in VR compared to RW (see Acharya et al., SfN abstract 2014). Despite the absence of spatial selectivity, hippocampal activity was not entirely random. Specifically, instead of sporadic spikes, pyramidal neurons invariably generated approximately two-second long spike sequences. We call these hippocampal motifs. A majority of spikes were contained within motifs in both worlds, however in VR the motifs were distributed randomly over space unlike their spatially localized counterparts in RW. Motif characteristics were comparable in RW and VR including motif duration and variability at a population level. Concatenation of all motifs generated by a neuron, irrespective of their spatial location, yielded motif fields which had similar properties in RW and VR. Spikes within motifs not only showed identical theta modulation, but also comparable phase precession in both worlds. These results suggest that during spatial exploration in normal rats, sustained activation and phase precession can occur without spatial selectivity. We hypothesize that internal, network mechanisms generate

phase-precessing motifs which require coherent multisensory input for spatial localization. 1. Hahn, T. T. G., McFarland, J. M., Berberich, S., Sakmann, B. & Mehta, M. R. Spontaneous persistent activity in entorhinal cortex modulates cortico-hippocampal interaction *in vivo*. Nature Neuroscience (2012).doi:10.1038/nn.3236

Disclosures: **Z. M. Aghajan:** None. **L. Acharya:** None. **J. Cushman:** None. **J. Moore:** None. **C. Vuong:** None. **M. Mehta:** None.

Poster

094. Cortical and Hippocampal Circuits: Spatial Navigation I

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 94.25/SS45

Topic: F.02. Animal Cognition and Behavior

Support: NIH grant 5R01MH092925

W.M. Keck Foundation grant

Title: Mechanisms of persistent activity and persistent inactivity *in vivo*

Authors: ***S. BERBERICH**[1], J. M. MCFARLAND[2], T. T. G. HAHN[1], M. R. MEHTA[3,4,5,6,7]; [1]Computat. Neurosci., Central Inst. of Mental Hlth., Mannheim, Germany; [2]Dept. of Biol. and Program in Neurosci. and Cognitive Sci., University of Maryland, MD; [3]W. M. Keck Ctr. for Neurophysics, University of California, CA; [4]Dept. of Physics and Astronomy, Los Angeles, CA; [5]Brain Res. Inst., Los Angeles, CA; [6]Integrative Ctr. for Learning and Memory, Los Angeles, CA; [7]Dept. of Neurology, Dept. of Neurobio., Los Angeles, CA

Abstract: Persistent activity is thought to be crucial for learning and memory. Hence, it has been studied extensively *in vitro*1. However, few studies have investigated the mechanisms of persistent activity *in vivo*, where the network dynamics could be quite different from that *in vitro*. Further, while persistent activity has been studied *in vitro* extensively, persistent inactivity has received little attention. This is important since in a complex neural circuit, both activity and inactivity play an equally important role in governing the circuit dynamics. Recently, we showed that membrane potential of neurons in layer 3 of medial entorhinal cortex (MECL3) *in vivo* exhibit spontaneous persistent activity during Up-Down state oscillations (UDS) under anesthesia and during drug-free slow wave sleep2. This persistent activity was locked to neocortical UDS, but persisted over several cortical UDS cycles. Here we show that the MECL3

neurons engage in another form of selective decoupling from the neocortical UDS, where they remain in a persistently inactive state, skipping several neocortical Up states. As with persistent activity, these persistent inactivity occurred significantly more often in MECL3 neurons than in similar neurons in the lateral entorhinal cortex neurons in layer 3 (LECL3), and neither form of persistence was observed in recordings from neocortical neurons from several areas. The probability of MECL3 neurons generating persistent activity and inactivity was not random, and was closely related to the instantaneous properties of the afferent cortical network state. Our results demonstrate that MECL3 neurons perform a state-dependent, nonlinear transformation on afferent cortical inputs to generate both persistent activity and inactivity. Finally, as with the persistent activity, MECL3 persistent inactivity exerted a strong influence on spiking activity of the downstream hippocampal CA1 neurons to which they project. Thus, persistent activity and inactivity *in vivo* provide a mechanism for MECL3 neurons to selectively and bidirectionally control the influence of neocortical activity on hippocampal neurons. The results reveal the strong cortico-entorhinal-hippocampal interaction during slow wave sleep oscillations and reveal the contribution of nonlinear mechanisms within the medial entorhinal cortex in shaping these interactions, which could play a significant role in learning and memory and memory consolidation. References: 1.Egorov et al. 2002, Nature 420, 173-178 2.Hahn et al. 2012,. Nat. Neurosci. 15, 1531-1538

Disclosures: **S. Berberich:** None. **J.M. McFarland:** None. **T.T.G. Hahn:** None. **M.R. Mehta:** None.

Poster

094. Cortical and Hippocampal Circuits: Spatial Navigation I

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 94.26/SS46

Topic: F.02. Animal Cognition and Behavior

Support: W. M. Keck foundation

NIH 5R01MH092925

Title: Variability of hippocampal place fields on one dimensional tracks in real world and virtual reality

Authors: K. SAFARYAN[1], P. RAVASSARD[1], A. KEES[1], *M. R. MEHTA[1,2,3];
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Angeles (UCLA), Los Angeles, CA; [2]Keck center for Neurophysics, [3]Integrative Ctr. for Learning & Memory, UCLA, Los Angeles, CA

Abstract: The activity of hippocampal place cells provides a cognitive map of space. The mechanisms governing this map formation remain to be understood. It is thought that place cells are strongly influenced by the distal visual cues. However, several other variables too influence place cells' activity including internal cues such as theta rhythm, sensory cues such as olfactory and somatosensory cues; and by behavioral variables, including running speed and vestibular cues. Hence, to understand the contribution of sensory inputs in governing place cells while minimizing the behavioral variability over many trials, we measured the extracellular activity of more than 300 putative pyramidal cells from CA1 of five rats trained to run reliably on a narrow linear track in the real world (RW). Consistent with previous data, most neurons showed focused place fields, i.e. sustained level of activity in restricted region of space. All neurons did not show similar levels of spatial selectivity and there was a wide range with some neurons showing robust and focused place fields and others showing poor spatial selectivity. Could this reduced spatial selectivity arise because of the changes in non-specific sensory cues across different trials in a session in the RW maze, or are there other mechanisms governing this variable spatial tuning? To address this question we used a virtual reality system mimicking the RW experiment by reproducing similar room and track dimensions and visual cues. Thus, unlike the RW, in VR only the distal visual cues provided spatial information and other nonspecific cues did not have a systematic relationship with the distal visual cues. Remarkably, we not only found a large variation in spatial information among place cells, but also a large reduction in spatial information in VR compared with RW. The results suggest that mechanisms other than purely sensory, such as intrinsic network properties, are responsible for the variability of place cells' spatial coding.

Disclosures: **K. Safaryan:** None. **M.R. Mehta:** None. **P. Ravassard:** None. **A. Kees:** None.

Poster

094. Cortical and Hippocampal Circuits: Spatial Navigation I

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 94.27/SS47

Topic: F.02. Animal Cognition and Behavior

Support: NIH 5R01MH092925

W.M. Keck Foundation grants to MRM

Title: Impaired spatial selectivity in two-dimensional virtual reality

Authors: *L. ACHARYA[1,2,3,4], Z. M.AGHAJAN[1,2,3,5], J. CUSHMAN[6], C. VUONG[1,2,3,5], J. MOORE[1,2,3,7], M. MEHTA[1,2,3,5,8];
[1]W.M.Keck Ctr. for Neurophysics, [2]Integrative Ctr. for Learning and Memory, [3]Brain Res. Inst., [4]Biomed. Engin. Interdepartmental Program, [5]Dept. of Physics & Astronomy, [6]Psychology, [7]Neurosci. Interdepartmental Program, [8]Departments of Neurol. and Neurobio., UCLA, Los Angeles, CA

Abstract: Principal neurons in the CA1 region of the hippocampus fire in a spatially selective fashion in the real world (RW) to form an allocentric map of the environment. This is hypothesized to be primarily governed by Distal Visual Cues (DVC), but the contributions of other modalities have not been ruled out. To test this hypothesis it is necessary to eliminate cues other than DVC, which cannot be achieved in the real world. However a virtual reality (VR) environment allows us to make all other cues, apart from DVC, spatially non-informative. Virtual reality has been recently used to study spatial selectivity where clear spatially tuned activity has been reported[1,2]. However, all of these studies have used one-dimensional tracks or trajectories, and so do not constitute a cognitive map based on DVC alone since spatial selectivity on such paths could also arise due to other stimuli, such as self-motion cues or specific features of the DVC which have high correlation with spatial location. So, we studied the activity of principal neurons in hippocampal CA1 while rats foraged for randomly scattered rewards in a two dimensional VR environment where only DVC are spatially informative and other cues do not have a fixed relation with them, allowing us to unequivocally determine the contribution of DVC alone to the hippocampal cognitive map. We measured hippocampal activity during a random foraging task from three (four) male Long Evans rats in RW (VR). The two worlds had identical dimensions and DVC. While visual and somatosensory cues indicated the platform edge in RW, in VR any movement beyond the edge of the platform caused no change in the visual scene. Rats, therefore, quickly learned to avoid the edge of the platform based entirely on visual cues in VR[3]. We recorded the activity of more than 500 putative pyramidal neurons from dorsal CA1 in RW and VR. Consistent with prior studies, in RW firing of neurons was highly localized in space. However, in VR there was a large, (> 70%) reduction in all measures of spatial selectivity, including spatial information content and spatial stability of firing rate maps, with the latter being near chance level in VR. These results show that the distal visual cues alone are insufficient to generate a robust hippocampal cognitive map of space.
References: 1. Harvey, C. D., Collman, F., Dombeck, D. A. & Tank, D. W. Intracellular dynamics of hippocampal place cells during virtual navigation. *Nature* **461,** 941-946 (2009). 2. Ravassard, P. *et al.* Multisensory control of hippocampal spatiotemporal selectivity. *Science* **340,** 1342-6 (2013). 3. Cushman, J. D. *et al.* Multisensory Control of Multimodal Behavior: Do the Legs Know What the Tongue Is Doing? *PLoS One* **8,** e80465 (2013).

Disclosures: **L. Acharya:** None. **Z. M.Aghajan:** None. **J. Cushman:** None. **C. Vuong:** None. **J. Moore:** None. **M. Mehta:** None.

Poster

094. Cortical and Hippocampal Circuits: Spatial Navigation I

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 94.28/SS48

Topic: F.02. Animal Cognition and Behavior

Support: NIH 5R01MH092925

W.M. Keck. Foundation grants to MRM

Title: Behavioral modulation of neocortical dendritic spikes in freely behaving rats

Authors: *J. J. MOORE[1,2,3,4], M. R. MEHTA[1,2,3,5,6];
[1]W. M. Keck Ctr. For Neurophysics, [2]Integrative Ctr. for Learning and Memory, [3]Brain Res. Inst., [4]Neurosci. Interdepartmental Program, [5]Dept. of Physics and Astronomy, [6]Dept. of Neurology, Dept. of Neurobio., UCLA, Los Angeles, CA

Abstract: The vast majority of information about neural activity in awake, behaving animals comes from extracellular measurements of somatic spikes. However, the soma represents a small fraction of the total volume of a neuron, and neurons fire somatic spikes rarely. To understand neural dynamics, it is necessary to record sub- and supra-threshold memberane potentials not just in the soma but also in the dendrites, where the majority of excitatory synapses are made. While sharp electrodes and whole cell patch clamp can record somatic membrane potential *in vivo*, these recordings are limited in time due to injury caused to the neurons, are restricted to the soma or proximal dendrites, and must be performed in head-fixed animals. *In vitro* studies show that dendrites can support back-propagation of somatic spikes and, under certain conditions, can generate dendritic action potentials (DAP) independent of somatic spiking. It is unclear if dendritic spikes occur *in vivo* and, if they do, what their underlying mechanisms are and how they are modulated by behavior. To determine the DAP mechanisms and receptive fields it is necessary to measure dendritic membrane potential over long periods of time during unrestrained behavior. We have developed a novel technique using modified tetrodes that can achieve high amplitude dendritic recordings over long periods of time, up to several days. Using this method we have recently reported the measurement of DAP in unanesthetized rats during slow-wave sleep (SWS) and have also examined the influence of the subthreshold potential on DAP firing, also during SWS. Combining video tracking with our dendritic recordings, we have now investigated the activity of dendrites in Posterior Parietal Cortex, an area shown to encode movement in an egocentric reference frame, during free behavior. DAPs, which fire at very high

rates during SWS, also fired at high rates during locomotion. We investigated the modulation of DAP rate by several behavioral variables such as running speed, acceleration, and turning maneuvers. These variables all significantly modulated DAP firing rate. These results constitute the first measurements of dendritic spikes during drug-free, natural behavior over prolonged periods. They show that DAP do occur during natural behavior and that dendrites in Posterior Parietal Cortex have behaviorally relevant receptive fields that could shape cortical network dynamics, synaptic plasticity and learning.

Disclosures: **J.J. Moore:** None. **M.R. Mehta:** None.

Poster

094. Cortical and Hippocampal Circuits: Spatial Navigation I

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 94.29/SS49

Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant DC009318

NIH Grant NS053907

Title: Head direction cell activity in the dorsal striatum and medial precentral cortex requires intact anterodorsal thalamic nuclei

Authors: ***M. L. MEHLMAN**, S. S. WINTER, J. S. TAUBE;
Dartmouth Col., Hanover, NH

Abstract: Animals must maintain a sense of direction to effectively navigate within their environment. At the neural level, direction is represented by the activity of head direction (HD) cells. These neurons fire as a function of the animal's allocentric directional heading, operating much like a compass. While most rodent HD cells are located within the limbic system structures that form the HD circuit, small numbers are found elsewhere in the brain, including the dorsal striatum (DS) and medial precentral cortex (PrCM). Is the HD signal in these regions derived from limbic HD circuit output or is it generated independently? To examine this issue we recorded single unit activity in the DS and PrCM of freely moving rats and compared HD cell activity observed in control animals to that observed in animals with neurotoxic lesions of the anterodorsal thalamic nuclei (ADN), a manipulation known to disrupt the HD circuit. Large ADN lesions (> 85%) completely abolished the HD signal in both the DS and PrCM. Animals

with smaller lesions exhibited degraded HD cell activity in the DS; these HD cells fired over a significantly wider directional range compared to HD cells from control animals. No HD signal was identified in the PrCM of any lesioned animal. Interestingly, units modulated by the animal's angular head velocity (AHV) were found in the DS and PrCM; unlike HD cells, the activity of these units was unaffected by ADN lesions. We conclude that the HD signal is first generated by the limbic HD circuit and then projected to the DS and PrCM, possibly via the retrosplenial cortex. The AHV signal we observed in the DS and PrCM must either arise from a separate pathway, possibly a subcortical pathway involving the habenula, or be generated internally.

Disclosures: **M.L. Mehlman:** None. **S.S. Winter:** None. **J.S. Taube:** None.

Poster

094. Cortical and Hippocampal Circuits: Spatial Navigation I

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 94.30/SS50

Topic: F.02. Animal Cognition and Behavior

Support: NIH/NINDS-NS053907

Title: Anterior thalamus inactivation disrupts grid cell firing in the entorhinal cortex

Authors: *S. S. WINTER[1], B. J. CLARK[2], J. S. TAUBE[1];
[1]Psychology and Brain Sci., Dartmouth Col., Hanover, NH; [2]Univ. of New Mexico, Albuquerque, NM

Abstract: Maintaining spatial orientation is a complex process that requires the coordination of multiple brain regions. Regions involved in spatial orientation contain cells with unique patterns of spatial tuning. Head direction (HD) cells discharge as a function of the animal's heading orientation within their environment, similar to that of a compass. Theta rhythm is modulated by running speed in a freely moving rat, functioning like a speedometer. Grid cells in the medial entorhinal cortex (MEC) discharge in a repeating hexagonal grid throughout the environment. The functional role of grid cells is still unknown but they are hypothesized to play an essential role in path integration. Computational models posit that grid cell firing patterns are generated from directional information and velocity information integrated over time, and suggest an integration of HD cells and theta rhythm as a possible mechanism. Inactivation of theta rhythmicity disrupts grid cell firing characteristics; however, the role of the HD cell circuit in

grid cell generation has yet to be tested. The current study inactivated the anterior thalamus (AT), a key structure in the HD circuit that projects to regions containing grid cells, and assessed the effect upon MEC grid cells in the rat. We isolated grid cells during a baseline session in a 1.2 m square chamber. Then lidocaine, at various concentrations, was infused into the AT and an inactivation recording session was immediately conducted. Finally, we recorded a recovery session 1.5 hours following the infusion of lidocaine. During inactivation grid cell firing patterns were disrupted and the length of disruption was dependent upon the concentration of lidocaine. Inactivation had no effect on theta rhythmicity in MEC. These results provide evidence that grid cell function is dependent upon inputs from the HD cell circuit, indicating that HD cells are providing the heading information necessary for generating hexagonal grid-like firing patterns. Given that lesions to the HD circuit disrupt the ability of an animal to navigate by means of path integration; this suggests that grid cells, which are dependent upon HD cells, may play a role in path integration as well.

Disclosures: **S.S. Winter:** None. **B.J. Clark:** None. **J.S. Taube:** None.

Poster

095. Motivation and Emotion: Information Processing

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 95.01/SS51

Topic: F.03. Motivation and Emotion

Support: Air Force Research Laboratory agreement number FA9550-07-1-0537

Howard Hughes Medical Institute

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NIH Career Development Award 1K01DA036659-01

Title: Do orbitofrontal cortex neurons encode relative value during free gaze?

Authors: *V. B. MCGINTY[1], A. RANGEL[2,3], W. T. NEWSOME[1,4];
[1]Neurobio., Stanford Univ., Stanford, CA; [2]Humanities and Social Sci., [3]Computat. and Neural Systems, Caltech, Pasadena, CA; [4]Howard Hughes Med. Inst., Stanford, CA

Abstract: Does the brain encode object value on an absolute scale? Or does the value representation for a given object depend on the value of other objects nearby? In a recent human

imaging study, when subjects were shown two objects, BOLD signals in the frontal lobe were positively related to the value of the object that was the target of the subjects' gaze, and negatively related to the value of the non-fixated target (Lim et al. (2011), J Neurosci 31:13214) - suggesting a gaze-dependent, relative value code. Consistent with this, we showed that for singly presented objects, some OFC neurons expressed a value code that was strongest when the subjects fixed their gaze on the object, and weakest when they looked away (McGinty et al. (2013), SfN Abstracts Prog. No. 858.20). However, an outstanding question is how OFC neurons represent value when two objects are shown - whether their firing reflects the value of the fixated object alone, or reflects the fixated target value relative to the non-fixated as in Lim et al. Here, we address this question using Pavlovian-conditioned visual cues during free gaze. Using Pavlovian conditioning, we trained macaque monkeys to associate three visual targets with juice rewards of 0, 1 and ~3 drops. After these single-target associations were learned, we issued trials in which two randomly chosen targets were shown simultaneously, followed by random delivery (i.e. not influenced by the monkeys' behavior) of a reward associated with one of the two targets. In these trials the monkeys' gaze was unrestricted, and they usually fixated each target at least once per trial. We measured OFC neuron firing during on-target fixations, and asked whether it was driven by the fixated target value alone, or by the relative value (fixated minus non-fixated), while also controlling for other task variables. This analysis had two parts. In the first, we considered a large set of potential task-relevant variables, and used lasso regression to identify which variables most frequently explained fixation-evoked firing. In the second, we built focused linear models using only the variables identified by the lasso procedure. According to the first analysis, both relative value and fixated value alone were strongly represented in the OFC population, and according to the second analysis, fewer neurons encoded relative value compared to fixated value alone. Thus, relative value was represented in OFC, but was not the dominant gaze-based value signal. One major difference between our study and Lim et al. was the use of a Pavlovian task, rather than a choice task. Therefore, we are currently investigating value coding in OFC, and other frontal and limbic areas, during decision-making.

Disclosures: **V.B. McGinty:** None. **A. Rangel:** None. **W.T. Newsome:** None.

Poster

095. Motivation and Emotion: Information Processing

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 95.02/SS52

Topic: F.02. Animal Cognition and Behavior

Support: COFAA 20130914

COFAA20140892

Title: Local administration of FAUC 213 in the thalamic reticular nucleus of rat reduces anxiety

Authors: *M. GARCIA-RAMIREZ[1], G. AVILA[1], E. CHUC-MEZA[1], J. ACEVES[2]; [1]fisiologia, ENCB-IPN, DF, Mexico, Mexico; [2]CINVESTAV ZACATENCO, Mexico, city, Mexico

Abstract: Has been reported that bilateral loss of the dopaminergic innervation of the thalamic reticular nucleus (TRn) has anxiolytic effects (Picazo et al, 2008) whereas local administration of methamphetamine in the same nucleus produced anxiety which is reverted by haloperidol, which is D2 and D4 antagonist receptor. The TRn presented D4 dopamine receptor, so the aim of this work was show if FAUC 213 (a selective D4 antagonist receptor) could produce anxiolysis. Rats were implanted with cannulas in the TRn and infused with FAUC 213 at doses of 5 nM, anxiety was tested by the shock burying electrode test (SBT), elevated-plus maze (EPM) and motor activity in automated activity counters. Motor activity was measured after the end of EPM or SBT. In rats administered with FAUC 213 consistently produced anxiolysis viewed as increased in time in open arms, whereas infusion of Vh not reduced anxiety , motor activity was not affected. The results confirm that dopamine D4 receptor in the TRn reduces anxiety

Disclosures: **M. Garcia-Ramirez:** None. **G. Avila:** None. **E. Chuc-Meza:** None. **J. Aceves:** None.

Poster

095. Motivation and Emotion: Information Processing

Location: Halls A-C

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Topic: F.03. Motivation and Emotion

Support: NSF BCS 04-20794 to I.B

NSF BCS 05-31177 to I.B

NSF BCS 06-17699 to I.B

Title: The Neural Genesis of a Joke

Authors: O. AMIR, *I. BIEDERMAN;
Psychology/Neuroscience, USC, Los Angeles, CA

Abstract: The nature of the cognitive processes employed in humor creation has proven highly resistant to introspection. It has even been suggested that the spontaneous and unpredictable fashion in which funny ideas present themselves renders functional imaging studies of humor creation impossible (Martin, 2006). An fMRI study of 14 improv-comedians from the Groundlings troupe and the Setlist Show, who make their living coming up with funny ideas on cue and under pressure, perhaps could serve as a challenge to such pessimism. They viewed drawings of people interacting in various situations and were instructed, in one condition (HUM), to think of a humorous line for a character and, in the control condition (MUN), to think of something mundane and non-humorous. The comedians rated the funniness of their ideas after each trial. Participants showed greater activation in the HUM condition bilaterally in temporal association regions (particularly in the temporal poles, superior temporal gyrus and in the temporo-occipital junction), as well as bilaterally in the ventral striatum, a region involved in reward. Conceiving funny ideas thus involves activation in semantic association regions where remote associations are linked in a meaningful way, and greater activation in those regions results, on average, in funnier ideas. These temporal association regions are particularly dense in μ-opioid receptors and there is converging evidence that activation in such high order association areas is in itself pleasurable (Biederman & Vessel, 2006; Yue et al., 2007) and that in the case of humor appreciation a surprising burst of activity in those regions may lead to the feeling of mirth (Amir et al., 2013). We found that while approximately the same regions of neural activation are involved in humor creation vs. humor appreciation (Amir et al., 2013), the order of activation is reversed: during humor appreciation temporal association regions are activated first (where remote associations are linked meaningfully reflecting the cognitive process of "getting the joke"), followed by activation in reward regions. Conversely, during humor generation, activation in reward regions precede the activation in temporal association regions, likely reflecting an expectation of the reward of conceiving a funny idea, but likely also helping to configure/modulate the temporal association regions to facilitate humor generation. This conjecture is supported by our observation of a dose response function that within the HUM trials, higher ratings were associated with stronger early activation in the reward regions as well as the subsequent activation in temporal association regions.

Disclosures: **O. Amir:** None. **I. Biederman:** None.

Poster

095. Motivation and Emotion: Information Processing

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 95.04/SS54

Topic: F.02. Animal Cognition and Behavior

Support: NIMH grant MH099590 to RPV

Title: Serotonergic innervation of the basal, central, medial, and cortical nuclei of the amygdala

Authors: *S. B. LINLEY[1], W. B. HOOVER, III[1,2], R. P. VERTES[1];
[1]Florida Atlantic Univ., Boca Raton, FL; [2]Max Planck Florida Inst. for Neurosci., Jupiter, FL

Abstract: The neural substrates underlying affective behavior have been well studied. Both serotonin (5-HT), chemically, and the amygdala, anatomically, are two key elements underlying emotion. We analyzed the distribution of 5-HT fibers within the basal, central, medial and cortical nuclei of the amygdala using an antisera for 5-HT and for the serotonin transporter (SERT) in 5 male and 5 female rats. We observed 5-HT labeled fibers throughout the amygdala but differentially distributed across nuclei. Labeling was most pronounced in the basal nuclei of the amygdala. Of these, the basolateral nucleus (BLA) showed the strongest labeling, with an increase in density across the rostrocaudal plane, so that the posterior division (BLAp) showed the most intense labeling of the entire amygdala. Very dense fiber labeling was also present in the lateral nucleus of amygdala (LA), with the heaviest concentration of fibers in the dorsolateral division of LA. By contrast, 5-HT labeling in the central nucleus was modest. While most divisions of the central nucleus (CEA) received moderate amounts of serotonergic fibers, the lateral division (CEAl) was lightly labeled and the caudal aspect of CEAl sparsely labeled. There was a similar gradual reduction in fiber density proceeding rostrocaudally in the medial nucleus of amygdala (MEA). The anterior divisions displayed moderately dense 5-HT labeling, with only a light plexus of fibers covering the posterior division. The cortical nuclei of amygdala received strong 5-HT input. Both the anterior cortical nucleus and nucleus of the olfactory tract exhibited dense labeling homogenously distributed across laminar fields. Whereas the posterior lateral division of the cortical nucleus showed a similar pattern of labeling, 5-HT fibers were more densely concentrated in layer 1 than 2/3 in the posterior medial division. In summary, all regions of the amygdala receive significant 5-HT input, but differentially distributed across subnuclei. This further indicates that serotonin exerts pronounced actions on the amygdala thus impacting anxiety, fear, stress, and other related behaviors.

Disclosures: **S.B. Linley:** None. **W.B. Hoover:** None. **R.P. Vertes:** None.

Poster

095. Motivation and Emotion: Information Processing

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Topic: F.02. Animal Cognition and Behavior

Support: NIMH grant MH099590 to RPV

Title: Lesions of the ventral midline thalamus impair reversal learning using an odor texture discrimination task in the rat

Authors: *R. P. VERTES[1], M. M. GALLO[1], R. J. ELLIS[2], P. PINEDO[2], B. N. CLARK[2], S. B. LINLEY[1];
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Abstract: The reuniens (RE) and rhomboid nuclei (RH) of the ventral midline thalamus are positioned as a critical relay between the medial prefrontal cortex (mPFC) and the hippocampus (HF). As such, it has been suggested RE/RH play an important role in cognition and mnemonic behaviors (Vertes et al., 2014). Recent studies have not found a strong link between RE and hippocampal dependent memory, but have demonstrated a tie between behaviors dependent on both the mPFC and HF (Cassel et al., 2013). Presently, we examined the effect of electrolytic lesions of the ventral midline thalamus on attention and executive functioning using an odor texture intradimensional/extradimensional (IED) test in rats. This test measures several cognitive behaviors sensitive to prefrontal functioning including attentional set, attentional set shifting, and behavioral flexibility. Rats were tested on seven stages of olfactory and texture discriminations: a simple discrimination, compound discrimination (CD), an intradimensional shift (ID) (introduction of two novel odor pairs), an extradimensional shift (ED) (two novel odor/texture pairs where the digging medium is now attended to) and CD, ID, and ED reversal learning stages. Test measures included trials to stage completion, incorrect choices (aborted trials and errors), and latencies. We found that destruction of the RE/RH produced impairments in behavioral flexibility. Lesioned rats needed more trials to complete the CD reversal than sham controls. Behavioral inflexibility is known to be associated with orbitomedial prefrontal (PFC) functioning and is found in a number of PFC disorders such as schizophrenia and obsessive compulsive disorder. Ventral midline connections with the PFC may, in part, mediate behavioral flexibility.

Disclosures: **R.P. Vertes:** None. **M.M. Gallo:** None. **R.J. Ellis:** None. **P. Pinedo:** None. **B.N. Clark:** None. **S.B. Linley:** None.

Poster

095. Motivation and Emotion: Information Processing

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 95.06/SS56

Topic: F.02. Animal Cognition and Behavior

Support: NIH R01 DA017960

NIH R01 MH101178

Title: Anatomical investigation of projections from the noradrenergic nucleus locus coeruleus to the mediodorsal thalamic nucleus in the rat

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Abstract: The locus coeruleus (LC) provides norepinephrine to the entire central nervous system, including medial prefrontal cortex (mPFC) and mediodorsal thalamic nucleus (MD). The mPFC is involved in higher order executive functions and has dense reciprocal connections with the MD which, in turn, makes connections with other cortical and subcortical structures. A significant portion of the information conveyed to and from the mPFC is via the MD, making it a critical site of relay and integration in limbic and cognitive networks. The noradrenergic projections from the LC to the mPFC and MD maintain appropriate modulation of the diverse circuitry within those regions. Dysfunctions of the PFC and MD are implicated in several neuropsychiatric disorders including ADHD, schizophrenia and post-traumatic stress disorder (PTSD). The goal of this study is to examine the tendency for individual LC neurons to send axon collaterals to both PFC and MD. Prior studies from this laboratory have shown that functionally related structures along the same sensory pathway receive input from common pools of LC neurons. Because PFC and MD thalamus are likewise anatomically distinct yet functionally related, we predict that individual LC neurons project to both regions. Fluorescent retrograde tracers were injected unilaterally into the mPFC and MD of adult male Sprague-Dawley rats. Coronal sections through the injections sites (80μm) and brainstem including LC (40μm) were made using a sliding microtome. The injection sites were verified and the pattern of fluorescently labeled cells in LC was analyzed. It has been previously established that projections from LC to neocortical terminal fields are almost entirely ipsilateral while projections to thalamic nuclei are bilateral, but with an ipsilateral predominance. In both cases projections to these targets arise from cells scattered throughout the rostral - caudal extent of LC. Preliminary

results agree with those findings and additionally indicate that MD and mPFC receive projections from segregated populations of LC neurons. While this latter finding differs from our prediction, it raises the intriguing possibility of differential activation of target-specific LC neurons and asynchronous release of NE within cortical and sub-cortical terminal fields of the MD-PFC network. To explore that possibility, future work will examine if MD vs mPFC-projection neurons in LC show differences in their molecular and physiological properties.

Disclosures: **E.W. Prouty:** None. **B. Duffy:** None. **D.J. Chandler:** None. **B.D. Waterhouse:** None.

Poster

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Topic: F.02. Animal Cognition and Behavior

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NIH grant MH039683

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SURE Fellowships from Stonehill College

Title: GABAergic regulation of the centromedian thalamus and control of cortical gamma band oscillations in the mouse

Authors: *R. E. BROWN[1], J. T. MCKENNA[1], C. YANG[1], L. CHEN[1], M. GAMBLE[2], A. HULVERSON[2], P. WOOD[2], J. G. MCCOY[2], B. KIM[3], J. H. CHOI[3];
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Abstract: Vegetative and minimally conscious states (VS/MCS) are devastating conditions which involve severe deficits in brain arousal, occurring as a result of vascular, traumatic or metabolic insults. Improved function in VS/MCS patients following application of drugs

affecting the basal ganglia or electrical stimulation of the centromedian thalamus (CM) suggests the possibility for rational treatments. In particular, paradoxical excitation caused by the hypnotic, Zolpidem, in VS/MCS patients may be due to increased inhibition of GABAergic basal ganglia inputs to CM (Schiff, 2010). Thus, development of novel, rational treatments for VS/MCS requires a better understanding of GABAergic inputs to CM and the effects of CM on cortical activity. Accordingly, here we investigate this circuit in mice. Application of the retrograde tracer, Fluorogold (0.5-1 %), into CM in wild-type mice (n=4) confirmed that CM receives major inputs from regions containing GABAergic neurons, including a basal ganglia output nucleus, the substantia nigra pars reticulata (SNr), as well as from the thalamic reticular nucleus (TRN). Immunohistochemical staining for the calcium binding protein, parvalbumin (PV), revealed that ~40 % of the inputs from TRN and SNr were PV-positive. Whole-cell patch-clamp recordings from CM neurons showed that the GABAB receptor agonist, baclofen, reduced the frequency of spontaneous inhibitory synaptic currents to 57 % of control (n=4, p=0.05). Optogenetic stimulation of CM in isoflurane anesthetized mice expressing channelrhodopsin2 constitutively in the thalamus (Thy1-ChR2-EYFP mice) revealed increases in cortical power which were particularly pronounced in frontal regions and at gamma band frequencies (40, 50 Hz). Thus, as in humans, the CM receives GABAergic inputs from the basal ganglia as well as from TRN and increased activity of CM enhances frontal cortical activity typical of conscious states. Inhibition of GABAergic inputs to CM by the anti-spastic agent, baclofen, suggests a possible mechanism which may help explain occasional cases of improved function in patients receiving intrathecal administration of this compound.

Disclosures: **R.E. Brown:** None. **J.T. McKenna:** None. **C. Yang:** None. **L. Chen:** None. **M. Gamble:** None. **A. Hulverson:** None. **P. Wood:** None. **J.G. McCoy:** None. **B. Kim:** None. **J.H. Choi:** None.

Poster

095. Motivation and Emotion: Information Processing

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Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 95.08/SS58

Topic: F.03. Motivation and Emotion

Support: University of Kansas Doctoral Student Research Fund

University of Kansas Psychology Strategic Initiative Grant

Title: Cognitive and neural processing of facial size and valence in comorbid depression and obesity

Authors: *T. Y. PAN[1], A. C. DEMARCO[1], R. ATCHLEY[1], L. E. MARTIN[2], C. R. SAVAGE[3]; [1]Dept. of Psychology, Univ. of Kansas, Lawrence, KS; [2]Hoglund Brain Imaging Ctr., [3]Ctr. for Hlth. Behavior Neurosci., Univ. of Kansas Med. Ctr., Kansas City, KS

Abstract: Prior research suggests that obesity is a risk factor for developing depression, but not all obese individuals develop depression. It is, therefore, important to understand common factors underlying comorbid obesity and depression that may serve as markers for depression risk. To date, there has been no exploration of neural processing differences or similarities in comorbid depression and obesity. This study investigated whether attention bias differences, measured using the P300 (P3) ERP component, exist between currently obese and depressed (Dep/O, n=16, mean Age=27.13, mean BMI=37.44), currently obese and never depressed (ND/O, n=13, mean Age=19.77, mean BMI=36.01), and healthy weight and never depressed (ND/HW, n=16, mean Age=21.69, mean BMI=21.58) females when participants viewed target face pictures of varying valence (happy, sad) and weight status (healthy weight, overweight). A 3 x 2 x 2 mixed model analysis of variance (ANOVA) was implemented for P3 amplitude: 3 Group (Dep/O, ND/O, ND/HW) by 2 Stimuli Valence (happy, sad) by 2 Stimuli Weight Status (healthy weight, overweight). This analysis was run within "early" P3 (248-352 ms post stimulus onset) and "late" P3 (356-460 ms post stimulus onset) windows and with the mean difference wave averaged across a single channel (channel 62 on high-density, 128-channel Electrical Geodesic Sensor Net) with the least noise within the region corresponding to the P3 component. We found a main effect of weight in the early window [$F(1, 42)=6.23$, $p=0.01$], such that all groups responded with greater P3 amplitude to stimuli of overweight status. We also found a main effect of valence in the late window [$F(1, 42)=7.50$, $p=0.007$], such that all groups responded with greater P3 amplitude to stimuli of negative valence. In the early window, we found a two-way interaction that approached significance of group by stimuli valence, where ND/HW individuals responded with greater P3 amplitude to positive stimuli, while both ND/O and Dep/O group allocated more attention to negative stimuli [$F(2, 42)=2.54$, $p=0.08$]. The main effects of greater P3 amplitude for overweight faces and negative valence likely reflect the impact of current cultural expectations of weight and size due to decreased exposure to overweight individuals in the media and the impact of the "negativity bias", the phenomenon of heightened sensitivity to negatively valence information. The present study also provides preliminary support that neural processing of faces may be different in obese individuals versus healthy weight, non-depressed individuals and may be an important area of research in larger studies.

Disclosures: **T.Y. Pan:** None. **A.C. DeMarco:** None. **R. Atchley:** None. **L.E. Martin:** None. **C.R. Savage:** None.

Poster

095. Motivation and Emotion: Information Processing

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Program#/Poster#: 95.09/SS59

Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant DC009836

NIH Grant DC009477

Title: Thalamic encoding of dynamic audiovisual signals in the actively foraging mouse

Authors: *R. S. WILLIAMSON[1,3], K. E. HANCOCK[2,4], B. E. SHINN-CUNNINGHAM[3], D. B. POLLEY[2,4];
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Abstract: Purposeful behavior requires the dynamic integration of many sensory cues, each with a corresponding neural representation. Such representations are subject to significant modulatory effects related to the behavioral state of the animal. These include both global changes such as arousal or motivation as well as "top-down" modulation that reflects task-specific cognitive processes. Both types of modulation have been widely documented in sensory cortex, but whether such effects could be inherited from earlier stages in the sensory hierarchy is poorly understood. To address this question, we developed an audiovisual foraging task that allowed us to characterize how activity in sensory regions of the thalamus is modulated during natural search behaviors. In this task, mice attend to dynamic shifts in the interval separating pairs of visual flashes or acoustic chirps to locate the position of a randomly placed invisible target on a circular track. One modality served as the target, where successful identification triggered a water reward, while the other was a distractor that did not predict reward. This allowed us to measure how behavioral search strategies and thalamic audiovisual encoding were modulated in real time as mice accumulated sensory evidence relating to the position of a hidden target location. In addition, we were able to separate global modulation related to factors such as locomotion and motivation from "top-down" effects related to dynamic changes in the representation of task-relevant and distractor cues. Mice typically learned this task within 30 daily training sessions (~1800 trials), with additional time yielding a modest increase in both performance accuracy and decrease in trial completion time. Well-trained mice exhibited search strategies conducive to solving the task; they spent more time moving towards the target location than away from it, and also modulated their running speed whilst in the vicinity of the target

location. Chronic extracellular recording of thalamic units was achieved through implanted 32-channel silicon probes positioned to simultaneously record from the lateral and medical geniculate nuclei (LGN and MGB, respectively). Preliminary results confirm that locomotion is not associated with shifts in thalamic audiovisual encoding in a manner that has been identified in the primary visual and auditory cortex. Our ongoing analysis is focused on top-down modulation of thalamic sensory representations that accompany modality-specific changes in behavioral search strategy.

Disclosures: R.S. Williamson: None. **K.E. Hancock:** None. **B.E. Shinn-Cunningham:** None. **D.B. Polley:** None.

Poster

095. Motivation and Emotion: Information Processing

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 95.10/SS60

Topic: F.03. Motivation and Emotion

Support: Intramural Program of the National Institute of Mental Health

Title: A developmental examination of face emotion labeling

Authors: *A. H. OAKES[1], J. L. WIGGINS[1], N. E. ADLEMAN[2], P. KIM[3], M. A. BROTMAN[1], E. LEIBENLUFT[1];
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Abstract: Background: Emotional facial expressions provide information that is critical in guiding social interaction and functioning. Examining face emotion labeling in typically developing children and adults is important for understanding the trajectory of socioemotional development. The use of varying expression intensities in this study allows us to examine the subtleties of normative emotion processing. Related work has focused on infancy and early childhood, whereas this work explores late childhood and adolescence. **Method:** 21 healthy children (age 9-17, M=14.94, SD=2.43; 9F) and 21 healthy adults (age 19-47; M=29.8, SD=7.66; 11F) completed a face-emotion labeling task while participating in an fMRI study. Participants viewed images of angry, fearful, happy, and neutral expressions. Varying emotion intensities were created by morphing the 100% expression with the neutral images. Participants identified the emotion (angry, fearful, happy) for each face. A repeated-measures ANOVA on percent

correct was conducted with Emotion and Intensity as within-subjects factors and Age as a between-subjects factor to examine the Emotion (angry, fearful, happy) x Intensity (50, 75, 100) x Age (Children vs. Adults) interaction. Bonferroni-corrected post-hoc analyses were performed. **Results:** The Emotion x Intensity x Age interaction [$F(4,37) = .66$, $p = .62$] was not significant. However, the Emotion x Intensity interaction was significant [$F(4,37) = 15.69$, $p < .001$]. Post-hoc tests indicate that fewer correct answers were given on 50 intensity faces in each of the Emotions than in 75 and 100 intensity faces. 75 and 100 intensity faces did not differ from each other in any of the Emotion conditions. At 50 intensity, angry and fearful were indistinguishable from each other in terms of percent correct; at both 75 and 100 fearful and happy were indistinguishable. There were no significant Age effects. Parallel results were observed for reaction time. **Conclusions:** This study demonstrated that children and adults do not differ in ability to label facial emotions. Children and adults are equally accurate at identifying angry, fearful, and happy faces at high intensity (75, 100), and are equally inaccurate at interpreting less intense facial emotions (50). Results suggest when above a certain intensity threshold, both children and adults are able to correctly identify the emotion, regardless of age. When processing less intense emotional stimuli (50), happiness is recognized with the greatest accuracy. Future work should examine the developmental neural correlates of these behavioral findings.

Disclosures: **A.H. Oakes:** None. **J.L. Wiggins:** None. **N.E. Adleman:** None. **P. Kim:** None. **M.A. Brotman:** None. **E. Leibenluft:** None.

Poster

095. Motivation and Emotion: Information Processing

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 95.11/SS61

Topic: F.03. Motivation and Emotion

Title: Erotic visual stimulation increases cortical eeg correlations in young men during performance of the tower of hanoi task

Authors: ***C. AMEZCUA**, M. RUIZ-DÍAZ, M. HERNÁNDEZ-GONZÁLEZ, M. GUEVARA, A. SANZ-MARTIN;
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Abstract: Emotional stimuli elicit changes in the electroencephalographic activity (EEG) of several brain structures, including the prefrontal cortex (PFC). In humans, this cortical area has been associated primarily with cognitive processes and motivational processes, such as sexual

behavior. It has been reported that sexual activation generated by observing erotic visual stimuli is related to high activation of the PFC. On the other hand, the main role that the PFC plays in conjunction with the parietal and temporal cortices in the executive functions -i.e., working memory, planning, organizing, goal-setting, and decision-making, among others- is also well known. In clinical medicine and research, these cognitive processes have typically been evaluated through tasks like the Tower of Hanoi. Given that the degree of EEG correlation changes in relation to the observation of erotic stimuli and during performance of cognitive tasks, the aim of this work was to determine whether the cortical correlation patterns during performance of the Tower of Hanoi in young men is affected by previous observation of videos with sexual or aggressive content. Sixty-nine healthy heterosexual men were assigned to 3 groups. The Neutral Group was shown a video of a subject walking, taken from the movie The Long Shadow (Zsigmond); the Erotic Group watched scenes of explicit sexual interaction from The Catwoman (Leslie); and the Aggressive Group watched scenes showing aggression from Hostel (Roth). EEGs from the left and right prefrontal, temporal and parietal zones were recorded under the following conditions: at rest; during visual stimulation; and while executing the Towers of Hanoi task. A sexual arousal scale was applied to all subjects at the end of each session. Only after observation of the erotic video did subjects report a moderate state of sexual arousal, associated with an increased degree of interprefrontal and intertemporal coupling of the slow bands (delta and theta) during performance of the Tower of Hanoi. This higher synchronization in the slow bands between the prefrontal and parietal cortices could be associated with specific cognitive strategies, or with functional adaptations while the subjects were experiencing sexual arousal. The results of this study may contribute to a better understanding of the cerebral functionality that underlies the cognitive effects of emotional stimulation, particularly of an erotic nature.

Disclosures: **C. Amezcua:** None. **M. Ruiz-Díaz:** None. **M. Hernández-González:** None. **M. Guevara:** None. **A. Sanz-Martin:** None.

Poster

095. Motivation and Emotion: Information Processing

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Topic: F.02. Animal Cognition and Behavior

Support: Biomedical Research Council grant BMRC/ 10/21/19/645

Title: Modulation of structures putatively involved in anxiety, memory, pain and aversion - the ventral hippocampus, raphe nuclei, periaqueductal gray area and lateral habenula - by the nucleus incertus

Authors: *U. FAROOQ, R. RAJKUMAR, G. S. DAWE;
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Abstract: The nucleus incertus (NI), located in the brainstem, consists principally of gamma-aminobutyric acid releasing (GABAergic) projection neurons which exhibit remarkable similarity to the projections of monoamine releasing nuclei (the serotonergic raphe nuclei and the noradrenergic locus coeruleus). Furthermore, its projections release a plethora of other co-neurotransmitters: it is the main source of the neuropeptide relaxin-3 in the brain; while a small population of NI neurons is glutamate releasing (glutamatergic) in nature. Recent studies implicate this nucleus in modulation of hippocampal oscillations, anxiety and stress-mediated impairment of cognition (Ma et al., 2009; Ryan et al., 2011; Farooq et al., 2013). Based on these and other findings, it has been proposed that this nucleus might play a role in anxiety, depressive, volitional and mnemonic disorders. However, electrophysiological evidence for the effects of modulation of this heterogeneous nucleus on structures putatively involved in these functions is lacking. We studied the effects of electrical stimulation of the NI on single neuronal firing characteristics in brain regions implicated in these behaviors which receive dense afferent projections from the NI. A total of 197 single-units were isolated from the ventral hippocampus, raphe nuclei, lateral habenula and periaqueductal grey area in anaesthetized male Sprague-Dawley rats using glass microelectrodes. Various stimulation protocols at the NI revealed that the nucleus incertus significantly inhibited the majority of neurons in these structures during stimulation. The degree of inhibition varied from complete inhibition to mild reduction. A small population of neurons in all structures (except the ventral hippocampus) showed excitation during stimulation. Post-stimulation, a large proportion of neurons exhibited a significant 'rebound' increase in firing while some neurons exhibited complete inhibition for up to seconds (a subset of neurons in the ventral hippocampus and raphe nuclei). These findings indicate that the NI significantly modulates processing of neural information in structures putatively involved in anxiety, memory, pain and aversion. Furthermore, our findings corroborate and extend earlier studies which demonstrated that the projections of the NI exhibit heterogeneity (glutamatergic or GABAergic - targeting the soma or dendrite).

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Poster

095. Motivation and Emotion: Information Processing

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Program#/Poster#: 95.13/SS63

Topic: F.03. Motivation and Emotion

Title: Alpha asymmetry study in undergraduate health sciences students with hazardous alcohol consumption, but not alcohol dependence, in southeastern Mexico

Authors: *L. NUÑEZ-JARAMILLO[1], P. VEGA-PERERA[1], J. V. REYES-LÓPEZ[2], L. RAMÍREZ-LUGO[3], W. V. HERRERA-MORALES[1], E. SANTIAGO-RODRÍGUEZ[4];
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Abstract: Frontal alpha asymmetry has been proposed to reflect frontal cortical activation in an inverse relationship, a higher alpha activity reflecting lower cortical activity. In this regard, a higher frontal left activity (lower alpha) has been related with an approach behavior, while a higher right frontal activity has been related with an inhibition behavior, with an imbalance in this asymmetry being associated with a dysregulation in motivation and emotion. A higher right frontal activity has been associated with a tendency to experience depression and anxiety and has been also reported in alcohol dependent subjects, while a higher frontal left activity has been related with impulsiveness and aggression. However, inconsistent results have also been reported. Hazardous alcohol consumption is defined as a pattern of alcohol consumption that increases the risk of harmful consequences for the user or others. Since it has been found a relation between impulsiveness and hazardous alcohol consumption, it is probable that subjects with hazardous alcohol consumption present higher alpha activity in the right frontal cortex, indicating a higher left frontal activity. The incidence of hazardous alcohol consumption has been reported to be high among medicine students. We performed an alpha asymmetry study in undergraduate health sciences students (Medicine, Nursery and Pharmacy; age 18-22) who rated negative for ADHD, and suicide risk tests, while in the AUDIT test rated positive for hazardous alcohol consumption, but negative for alcohol dependence, in order to avoid the possible influence of dependence on alpha asymmetry. Interestingly, we found no difference in alpha asymmetry between controls and the hazardous alcohol consumption groups. As mentioned, while there are many reports showing a relationship between alpha asymmetry and motivation/inhibition dysregulation, inconsistent results have also been found. In different reports an influence of ethnicity has been found in qEEG studies addressing alcoholism. We suggest that results can be attributed to particular EEG features in the population under study, outlining the importance of establishing the particular characteristics of qEEG in this study population for further studies.

Disclosures: **L. Nuñez-Jaramillo:** None. **P. Vega-Perera:** None. **J.V. Reyes-López:** None. **L. Ramírez-Lugo:** None. **W.V. Herrera-Morales:** None. **E. Santiago-Rodríguez:** None.

Poster

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Topic: F.03. Motivation and Emotion

Support: SNSF grant no. 105314_140622

Title: Functional connectivity between subthalamic nucleus and orbito-frontal cortex during vocal emotion decoding

Authors: ***J. PÉRON**[1,2], S. FRÜHHOLZ[1,2], D. GRANDJEAN[1,2];
[1]Fac. of Psychology, Geneva, Switzerland; [2]Swiss Ctr. for Affective Sci., Geneva, Switzerland

Abstract: Background: Subthalamic nucleus (STN) deep brain stimulation (DBS), a neurosurgical treatment for Parkinson's disease (PD) and obsessive-compulsive disorder (OCD), has recently advanced our understanding of the apparently major role played by this structure in human emotion processing. However, the potential presence of several confounds related to the use of pathological models raises the question of how far they affect the relevance of observations regarding the physiological function of the STN itself, underscoring the crucial importance of obtaining evidences from neurologically healthy participants. Aim: In this study, we sought to probe the functional connectivity between the STN and other brain regions related to vocal emotional processing from participants without CNS disorders. Methods: To this end, we re-analyzed previous high-resolution fMRI data in N=17 healthy participants performing a vocal emotions (i.e., emotional prosody) task and using functional connectivity measures (psycho-physiological interactions, PPI) and taking the STN as seed region of these analyses. Based on Péron and colleagues (Neuroscience and Biobehavioural Reviews, 2013), we hypothesized that the STN is functionally connected to the structures known for their involvement in emotional prosody decoding, notably the OFC, and the auditory cortices as well as the amygdala and the other basal ganglia such as the caudate nucleus. Results: As expected, we showed that the STN is functionally connected to the structures known for their involvement in emotional prosody decoding, notably the OFC, the IFG and the auditory cortices as well as the other basal ganglia (pallidum) and the amygdala. More specifically we observed that the left STN showed functional connectivity to the contralateral right orbito-frontal gyrus, the right

inferior-frontal gyrus and the right temporal sulcus as well as the ipsilateral pallidum and amygdaloïd nuclei during emotional prosody processing. Conclusion: The present results confirm, in healthy participants, the major role played by the STN in human emotion and its functional connectivity with the brain network involved in vocal emotions.

Disclosures: **J. Péron:** None. **S. Frühholz:** None. **D. Grandjean:** None.

Poster

095. Motivation and Emotion: Information Processing

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Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 95.15/SS65

Topic: F.03. Motivation and Emotion

Title: Differences in quantitative EEG activity between normative database from Cuban population and age and gender matched health sciences undergraduate students in southeastern Mexico

Authors: ***P. VEGA**[1], L. NÚÑEZ-JARAMILLO[1], J. V. REYES-LÓPEZ[2], L. RAMÍREZ-LUGO[3], W. V. HERRERA-MORALES[1], E. SANTIAGO-RODRÍGUEZ[4];
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Abstract: Quantitative electroencephalography (qEEG) allows a deep and precise analysis of brain electrical activity in both patients and healthy subjects. It is important to establish basal levels of qEEG activity in order to determine possible anomalies in patients under different conditions such as cognitive or behavioral disorders. In this regard, there has been extensive work on the development of normative qEEG databases. However, even though many qEEG studies have differences have been reported in qEEG activity of different study groups under the same experimental conditions. In the present work we performed qEEG analysis to undergraduate health sciences students (Medicine, Nursery and Pharmacy). Inclusion criteria included age 18 to 22, and negative results in ADHD, AUDIT, depression and suicide risk tests. We compared, through Z transformation, the results obtained in absolute power (AP), relative power (RP) and mean frequency (MF) for delta, theta, alpha and beta frequency bands with those obtained in Cuban population. We found a statistically significant increase in AP in the delta band in FP1, F7, F8, T3, T4 and T5, and a decrease in AP for this frequency band in F3, F4, C3, C4, P3, P4, FZ, CZ and PZ. In theta band we found an increase in AP in F7, F8, T3, T4, T5 y T6,

and a decrease in F3, F4, C3, C4, P3, P4, FZ, CZ y PZ. In alpha AP we found an increase in FP1, FP2, F7, F8, T3, T4, T5 y T6 and a decrease in C3, CZ y C4, while for beta AP we found an increase in FP1, F7, F8, T3, T4, T5, T6 y O1; and a decrease in FZ, C3, C4, CZ, P3 y PZ. For RP, in the theta frequency band we found a decrease in RP in FP1, F7, F8, T3, T4 and CZ; for alpha we found an increase in RP in CZ, while for beta we found an increase in RP in F7 and F8. For MF, we found a reduction in delta MF in FP1, F7, F8, T3, T4, C3, C4, CZ, P3, P4, PZ y T6; for alpha we found an increase in MF in F7, F8 y PZ; and for beta MF an increase in F7, T3, T5, O1 and a decrease in PZ was found. Altogether, these results outline the importance of determining the basal qEEG activity in the particular study population in order to reliably study changes in qEEG activity under different conditions.

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Poster

095. Motivation and Emotion: Information Processing

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 95.16/SS66

Topic: F.03. Motivation and Emotion

Support: NIH Grant 5R01-MH-089484-02

FRSQ 25559

Title: Using information theory to quantify behavioral phenotypes in free-ranging rhesus macaques

Authors: *J.-F. GARIEPY[1,2,3], J. SUNDARARAJAN[3,2], S. MADLON-KAY[2], E. DU[2,1], D. L. XIE[2,1], L. J. N. BRENT[2,1], M. L. PLATT[1,2,3];
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Abstract: Neuropsychiatric disorders often present complex sets of behavioral impairments that most strongly manifest in dynamic, naturally-occurring social contexts like school or the workplace. Such conditions are thought to result from the interactions of genes and environment. In principle, better understanding of the causes of these disorders could be achieved by improving how researchers process information about behaviors occurring in dynamic social

contexts. Here we propose a novel approach using information theory to examine the causal influences on behaviors observed in a large, free-ranging population of rhesus macaques on Cayo Santiago Island. Our initial study focused on 266,720 behavioral events collected over 2 years from iterated focal animal samples in which individual monkeys were observed for 10 minutes and all behaviors from a 55-categories ethogram were recorded. An average of 13.3 hours of observation per adult monkey in the group (114 animals) was analyzed. Information theory allows us to find statistical links between pairs of variables without prior hypotheses about the form of the relation between these variables. Observations were grouped in 1-minute bins and mutual information was computed between behaviors at time 0 and behaviors in preceding bins up to 5 minutes. Among the multiple relations found, mounting behavior was observed significantly more often following a submissive display (bootstrap test on mutual information; significant for all time windows 1 to 5 minutes before the mount event). Conversely, fear grimacing strongly co-occurred with aggression, but it was not predictive of aggression in the following minutes (bootstrap test on mutual information). We also examined the probability of occurrence of specific behaviors in various social contexts (e.g., the presence of dominants, subordinates, females, or males). For instance, mounting was more commonly performed by dominant monkeys toward subordinates than by subordinates toward dominants. For females, subordinates were more likely to actively approach a dominant individual than dominants were to approach a subordinate. Females were also more likely to emit affiliative vocalizations (short grunts, girneys and lipsmacks) in the presence of males than in the presence of females. Despite these general tendencies, most behaviors were highly variable across individuals and thus potentially associated with genetic variation. To test this idea, the behavioral models we have developed will be compared to genetic data based on >300 single nucleotide polymorphisms (SNPs) in neuromodulatory and regulatory pathways linked to neuropsychiatric disorders.

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Poster

095. Motivation and Emotion: Information Processing

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Topic: F.03. Motivation and Emotion

Support: NIH Grant 5T34GM008395

NIH Grant GM063787

Title: Differences in facial affect processing between deaf signers and normal hearing non-signers

Authors: A. V. GONZALEZ, C. C. MORALES, J. I. RAMIREZ, S. SARKISSIANS, J. P. ABARA, *S. KANG;
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Abstract: Previous research has explored holistic processing of face recognition using Event-Related Potentials (ERPs), but little research has focused on the differences in emotion recognition between normal hearing and deaf individuals. Deaf individuals who use American Sign Language rely heavily on facial information to read emotions and to detect linguistic cues for communication. Due to this unique behavioral practice among deaf signers, it has been speculated that deaf signers might process facial information somewhat differently compared to normal hearing non-signers (Mitchell, Letourneau, & Maslin, 2013). The main purpose of this study was to investigate the difference in facial information processing by focusing on N250 using full and half faces of emotions. N250 is a component of the ERP that peaks at approximately 250 milliseconds after the stimulus has been presented. Past literature has shown that N250 is related to facial affect processing and face specific structure processing. The main hypothesis of this study was that when participants are asked to identify emotions on full faces, deaf signers would show higher amplitude of N250 than hearing non-signers, indicating that they recruit more neural resources to process facial information. The group difference in the amplitude of N250 was expected to decrease in the half face condition. In this study, the ERPs were recorded while the participants reported the emotion they perceived on the full and half faces (either top or bottom half of the face) presented on a computer screen. Six basic emotions (happy, fear, anger, disgust, surprise, sad) taken from the NimStim Face Stimulus Set (Tottenham et al., 2002) were used. The total of 11 electrodes was placed on using the 10-20 international system. The preliminary results based on 10 college students (5 congenital deaf signers and 5 hearing non-signers) from this on-going study seemed to be encouraging. In the full face condition, the mean amplitude of N250 of the deaf group was much larger (M = -5.66, SD = 4.36) than the mean of the normal hearing group (M = -2.82, SD = 2.86), although the group differences were not statistically significant, F (1, 8) = 1.47, $p > .05$. The N250 amplitude of the deaf group (M = -3.36, SD = 3.48) in the half face condition was also higher than that of the normal hearing group (M = -1.49, SD = 1.46), while the group mean difference in the amplitude decreased. The patterns of these results were consistent with the main hypotheses, implying that deaf signers' information processing for facial affect is based more on feature-based processing than holistic processing.

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Poster

095. Motivation and Emotion: Information Processing

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Support: NIH Grant R37MH57502

NIH Grant F32MH087067

NIH Grant K99MH10138

Title: Damage to the macaque anterior cingulate cortex impedes decision-making and eliminates social preference

Authors: *E. BLISS-MOREAU, G. MOADAB, D. AMARAL;
Psychiatry and Behavioral Sci., Univ. California, Davis, DAVIS, CA

Abstract: The anterior cingulate cortex (ACC) has been broadly implicated in cognitive, social, and emotional processing. In humans, ACC activity has been observed during diverse tasks ranging from the experience of detecting behavioral errors to social interactions in virtual environments to the experience of pain and discrete emotions like disgust. Despite the robust human literature, the extent to which normal ACC function is required to execute these functions is not clear. The present experiment evaluated both decision-making and preference for social information in a cohort of male rhesus macaque monkeys that received either neurotoxic lesions to the ACC (N=6) or sham operations (N=7) four years prior to testing. Testing occurred over five test days in a sound attenuated chamber using an infrared eye tracker. Subjects indicated their responses to specific stimuli by fixating on a given stimulus for a defined duration. Each test day included a maximum of 40 trials or was ended after approximately two hours. Experimental stimuli were dynamic video stimuli that either featured conspecifics engaged in species typical behaviors (i.e., “social” stimuli) or footage from nature documentaries (i.e., “nonsocial” stimuli). Each trial began when the animals fixated on a stimulus (a #) presented in the center of the computer screen. They were then presented with two visual stimuli (i.e., “choice” stimuli)-a blue square or a yellow square. For half of the subjects, fixating on the blue square activated a social video and fixating on the yellow square activated a nonsocial video. For the other subjects, the contingency was reversed. Over the five test days, control animals completed significantly more trials than the ACC-lesioned animals. Overall, control animals also

had fewer fixations on choice stimuli, and in particular nonsocial choice stimuli, indicating more efficient decision-making. Control animals selected a higher ratio of social videos, relative to nonsocial videos, than did ACC-lesioned animals. Control animals also demonstrated a preference for social information insofar as they selected social videos more frequently than chance. In contrast, animals with ACC damage did not select either social or nonsocial videos more frequently than chance, indicating no preference for social information. Implications for the ACC's role in cognitive control and social processing will be discussed.

Disclosures: **E. Bliss-Moreau:** None. **G. Moadab:** None. **D. Amaral:** None.

Poster

095. Motivation and Emotion: Information Processing

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Program#/Poster#: 95.19/TT1

Topic: F.03. Motivation and Emotion

Support: NIH RC1 MH088678

Title: Adolescent development of interoceptive pathways mediating response focused emotion regulation

Authors: *D. LI, N. L. ZUCKER, P. A. KRAGEL, V. E. COVINGTON, K. S. LABAR; Duke Univ., Durham, NC

Abstract: Adolescence is a critical period for the neural development of emotion regulation capacities, which play a key role in managing social interactions. The neural basis of emotion regulation in adolescence is relatively unknown, and the few existing studies have focused on antecedent-based strategies, such as the use of cognitive reappraisal in reinterpreting the meaning of affective stimuli in ways that alter their emotional impact. A less well-studied method of emotion regulation involves response-focused strategies such as those that alter interoceptive processes associated with emotion induction. To this end, we used functional magnetic resonance imaging (fMRI) to study developmental brain mechanisms of interoceptive regulation in healthy adolescent females. Across trials, participants were presented with virtual roller coaster movie clips (interoceptive induction phase) followed by a regulation phase, during which they were instructed to regulate their interoceptive response to the roller coaster induction either by visually monitoring their online electrogastrogram activity through a virtual thermometer (biofeedback condition), or by deep breathing exercises without visual feedback from the interoceptive

response (no-biofeedback condition). Interoceptive induction was associated with increased bradygastria that attenuated during the regulation phase and was accompanied by a compensatory increase in tachygastria. Bilateral ventrolateral prefrontal cortex and left temporoparietal junction were both activated during interoceptive regulation. The left insula showed increased activation in the no-biofeedback compared to biofeedback regulation trials, suggesting that the participants relied more on interoceptive input when external feedback was unavailable through other senses. By contrast, visually-guided biofeedback elicited more activation in the visual cortex than the no-biofeedback condition. Linear increases in age were associated with greater fMRI activation in the bilateral anterior insula during regulation but smaller structural volume, suggesting developmental pruning mechanisms that target interoceptive capacities. Across ages, the increase in tachygastria during regulation was associated with increased fMRI signal in the bilateral insula. These results suggest an increasingly important role of the insula in interoceptive regulation during adolescent development.

Disclosures: **D. Li:** None. **N.L. Zucker:** None. **P.A. Kragel:** None. **V.E. Covington:** None. **K.S. LaBar:** None.

Poster

095. Motivation and Emotion: Information Processing

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 95.20/TT2

Topic: F.03. Motivation and Emotion

Support: CONACYT

Title: Effects of emotional words on inhibitory control in adolescents

Authors: *J. RAMOS-LOYO, E. S. MARTÍNEZ-VELÁZQUEZ, L. M. SÁNCHEZ-LOYO, A. A. GONZALEZ-GARRIDO;
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Abstract: Impulsive behavior related to inhibitory control difficulties is present during adolescence, particularly when emotional stimuli are involved. The aim of the present study was to explore the differences in the effects of emotional words on the attentional control in adolescents and adults, through event related oscillations (EROs) and event related potentials (ERPs). Method: 30 male subjects, 15 adolescents and 15 adults performed 3 Stroop tasks

involving neutral, compliments or insulting words. The subjects had to press a key that corresponded to the color of the word. N2 amplitude and amplitude enhancement factor for EROs were evaluated. There were no differences between groups in behavioral measures. Adults showed lower reaction times with compliments than insults. N2 amplitude was higher in adults than adolescents in tasks involving neutral and compliment words. Adults also showed higher amplitudes than adolescents in theta for compliment words in Cz and Pz; for slow alpha in neutral words in Fz and; in fast alpha in neutral in Cz and Pz. Only the adults showed differences between conditions. Slow alpha showed lower amplitude in neutral vs compliments in Fz and; fast alpha lower in neutral vs insults. Present results suggest that even though there were no differences between adolescents and adults with respect to their behavioral measures, there were differences in the underlying brain electrical activity, that may suggest an incomplete brain maturation.

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Poster

095. Motivation and Emotion: Information Processing

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 95.21/TT3

Topic: F.02. Animal Cognition and Behavior

Title: Dynamic pupillary response to tonic and phasic patterns of Locus Coeruleus activity

Authors: *Y. LIU, E. LI, Q. WANG;
Biomed. Engin., Columbia Univ., New York, NY

Abstract: Changes in pupil size have been widely used as a measure of mental activity and changes in mental states for more than half a century. However, the underlying neurophysiological mechanism still remains poorly understood. Recent findings suggest that the locus coeruleus (LC) plays a complex role in many cognitive tasks, and it has been postulated that two distinct modes of neural activity in the LC differently modulate pupil dilation. Phasic activation of the LC would result in transient change in pupil size, whereas tonic activation of the LC would modulate average pupil size. Nevertheless, this hypothesis has not been tested yet. Here, using high-speed videography, we simultaneously measured pupillary responses of both eyes to patterned microstimulation in the LC. The location of the stimulating electrodes has been confirmed by characteristic response to tail/paw pinch of single LC neurons that were recorded

prior to the microstimulation and post-experiment histological analysis. Preliminary results demonstrate that patterned microstimulation in the LC evoked synchronous changes in pupil size for both ipsilateral and contralateral eyes, whereas changes in ipsilateral pupil size are bigger than contralateral pupils. Tonic stimulation with different frequencies (i.e. 0.5, 1, 2 and 5 Hz) evoked small changes in average pupil size and the changes increased with the stimulation frequency. In contrast, phasic stimulation elicited significantly larger and longer-lasting pupil dilation. Background tonic stimulation modulates the amplitude of pupil dilation in response to the phasic stimulation. Taken together, our results provided for the first time direct experimental evidence that different LC activity patterns have distinct effects on changes in pupil size.

Disclosures: **Y. Liu:** None. **E. Li:** None. **Q. Wang:** None.

Poster

095. Motivation and Emotion: Information Processing

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 95.22/TT4

Topic: F.03. Motivation and Emotion

Title: Emotional contexts exert a differential effect on the inhibition of a prepotent response in men and women that refer high and low behavioral regulation

Authors: *L. Á. LLAMAS, SR, J. RAMOS-LOYO, A. GONZÁLEZ-GARRIDO, J. HERNÁNDEZ-VILLALOBOS;
Inst. De Neurociencias, Guadalajara, Mexico

Abstract: The purpose of the present study was to explore sex differences in the effects of emotional contexts in response inhibition in adolescents, comparing those who refer a high behavioral regulation capacity (HRC) in social environments and those who refer a low capacity (LRC). Sixty adolescents were divided in 4 groups (n=15 each): women and men with HRC and LRC (BRIEF-A). Subjects performed 4 Go-NoGo response inhibition tasks under 4 context conditions: stimuli without context, context with neutral emotional content; pleasant and unpleasant. Subjects had to press a key when an arrow located in the middle of the screen, coincided both in direction and color with a bar presented in the left or right edges (Go) and to withhold the response when it did not match (NoGo). Women had higher number of correct inhibitions than men. LRC men showed lower number of correct inhibitions than HRC and women of both groups, in particular in emotional contexts. Women showed longer N2 latency than men. N2 amplitude was higher in LRC than HRC, both in men and women. Unpleasant

context generated higher N2 amplitude and longer P3 latency. Los hombres de BRC tienen mayores dificultades en la inhibición de sus respuestas que los de ARC y este efecto es mayor ante contextos emocionales. Results suggest that women had better inhibitory control than men, although they took more processing time. Adolescents that reported having lower abilities in their behavioral control within everyday activities, demonstrated more difficulties in response inhibition at emotional contexts than those who report higher abilities. These difficulties were related to higher N2 amplitude, which may indicate that they require recruiting more attentional and inhibitory resources in order to achieve task performance, particularly under emotional contexts. Emotional contexts attract attention making more difficult to inhibit a prepotent response than non-emotional ones, which is evidenced both in behavioral and electrophysiological measures.

Disclosures: **L.Á. Llamas:** None. **J. Ramos-Loyo:** None. **A. González-Garrido:** None. **J. Hernández-Villalobos:** None.

Poster

095. Motivation and Emotion: Information Processing

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Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant RO1-GM056398

NIH Grant R01-EB000215

Title: Brainstem stimulation increases functional connectivity of basal forebrain-paralimbic network in isoflurane-anesthetized rats

Authors: ***S. PILLAY**[1], X. LIU[1], P. BARACSKAY[2], A. G. HUDETZ[1];
[1]Anesthesiol., Med. Col. of Wisconsin, Milwaukee, WI; [2]Inst. of Biology, Lab. of Proteomics, Eötvös Loránd Univ., Budapest, Hungary

Abstract: Brain states and cognitive-behavioral functions are precisely controlled by subcortical neuromodulatory networks. Manipulating key components of the ascending arousal system (AAS) via deep brain stimulation may help facilitate global arousal in anesthetized animals. Here we test the hypothesis that electrical stimulation of the oral part of the pontine reticular nucleus (PnO) under light isoflurane anesthesia associated with loss of consciousness leads to cortical

arousal and specific changes in blood-oxygenation-level dependent (BOLD) functional connectivity (FC) of the brain. BOLD signals were acquired simultaneously with frontal epidural EEG before and after PnO stimulation. Whole-brain FC was mapped using correlation analysis with seeds in major centers of the AAS. PnO stimulation produced cortical desynchronization, a decrease in δ-and θ-band power, and an increase in approximate entropy. Significant increases in FC after PnO stimulation occurred between the left nucleus Basalis of Meynert (NBM) as seed and numerous regions of the paralimbic network. Smaller increases in FC were present between the central medial n. of thalamus and retrosplenium seeds and the left caudate putamen and NBM. The results suggest that, during light anesthesia, PnO stimulation preferentially modulates basal forebrain-paralimbic networks. We speculate that this may be a reflection of disconnected awareness.

Disclosures: **S. Pillay:** None. **X. Liu:** None. **P. Baracskay:** None. **A.G. Hudetz:** None.

Poster

095. Motivation and Emotion: Information Processing

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Program#/Poster#: 95.24/TT6

Topic: F.02. Animal Cognition and Behavior

Title: Deletion of phospholipase C beta1 in thalamic reticular nucleus lead to spontaneous absence seizures in mice

Authors: *B. CHANG[1,3], K. KIM[4], S. LEE[4], K.-S. KIM[2], E. CHEONG[3], H.-S. SHIN[4];
[1]Ctr. for Neural Seience, [2]Korea Inst. of Sci. and Technol., Seoul, Korea, Republic of; [3]Yonsei university, Seoul, Korea, Republic of; [4]institute for basic science, daejeon, Korea, Republic of

Abstract: The thalamocortical networks have long been considered to be important in control of consciousness. Absence seizures are caused by abnormal synchronized oscillations in the thalamocortical circuit, resulting in widespread spike-and-wave discharges (SWDs) in the electroencephalogram(EEG) in parallel with an impairment of consciousness. Many reports have demonstrated that thalamic reticular nucleus (TRN) and thalamocortical (TC) neurons are critical for the generation of thalamocortical oscillations during SWDs. Phospholipase C β1 (PLCβ1) knockout (KO) mice show spontaneous complex type seizures, including convulsive and absence. This spontaneous SWDs are attenuated by application of anti-absence seizure drug, ethosuximide (ETX) known as a T-type Ca2+ channel blocker. PLCβ1 is expressed only in the TRN region in the thalamus, we explored the possibility that PLCβ1 in the TRN may be critical

for the induction of spontaneous SWDs. To that end, we utilized PLCβ1 shRNA knockdown (KD) system to inhibit PLCβ1 expression in the TRN. The result showed that PLCβ1 KD mice also developed only spontaneous SWDs but no other types of seizure; indicating that the deletion of PLCβ1 in TRN leads to the absence seizure. We used γ-butyrolactone (GBL), known to induce absence seizure primarily by acting on GABAB receptors, in PLCβ1 KO mice to see further increase of SWDs. Interestingly, the number of SWDs and duration were not significantly altered by GBL injection. In contrast THIP (4,5,6,7-tetrahydroisoxazolo(5,4-c)pyridin-3-ol) a GABAa agonist which is known to induce absence seizure significantly increased the number of SWDs and duration. We conclude that PLCβ1 in the TRN plays key roles in the induction of absence seizures both in spontaneous and in drug induced seizure. Our study could provide not only understanding the thalamocortical pathway in induction of SWDs but also guide the development of therapeutic tools for absence epilepsy.

Disclosures: **B. Chang:** None. **K. Kim:** A. Employment/Salary (full or part-time):; institute for basic scence. **S. Lee:** A. Employment/Salary (full or part-time):; institute for basic scence. **K. Kim:** None. **E. Cheong:** A. Employment/Salary (full or part-time):; Yonsei University. **H. Shin:** None.

Poster

096. Genomics, Proteomics, and Systems Biology

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Topic: G.02. Genomics, Proteomics, and Systems Biology

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Title: A genome-wide association study of neonatal brain volumes

Authors: K. XIA[1], S. JHA[1], F. ZOU[2], H. ZHU[2], P. SULLIVAN[1], M. STYNER[1], J. H. GILMORE[1], *R. C. KNICKMEYER[1];
[1]Psychiatry, [2]Biostatistics, Univ. North Carolina, Chapel Hill, NC

Abstract: Brain development in the prenatal and perinatal period is critically important for later cognitive function and mental health. Genetic factors likely play a key role in determining individual variation in early brain development, but are understudied in human populations due to practical and technical challenges. The aim of this study is to use genome-wide genotyping technology combined with statistical genetics methods to facilitate the discovery of common and rare genetic variants associated with individual variation in brain structure during early postnatal development. Buccal samples from neonates were genotyped using Affymetrix Axiom Genome-Wide LAT and Exome arrays for common variants and rare variants respectively. 594 subjects (278 singletons and 316 twins/siblings) were obtained after various quality control metrics, followed by SNP imputation using reference panel of 1000 Genome Project. Structural magnetic resonance imaging (MRI) scans of neonates were acquired with a Siemens TIM Trio or Allegra 3T scanner. An automatic, atlas-moderated expectation maximization segmentation tool was used to classify brain tissue as gray matter (GM), white matter (WM), or cerebral spinal fluid (CSF). In addition to total tissue volumes, we also investigated intracranial volume (ICV) and cortical GM and WM. To account for correlation structure between twins and siblings, linear mixed effect model was used to test a total of 9.5 million genotyped and imputed SNPs against each MRI variable. An intergenic hotspot in 15q13.3 between KLF13 and OTUD7A was significantly associated with ICV (rs8030297; p=2.98 x 10-8), total WM (rs6493639, p=4.24 x 10-8), and marginally associated with GM (rs8030297; p=5.25 x 10-7 and cortical WM (rs6493639, p=1.17 x 10-7). Deletion within 15q13.3 has been associated with a range of neurodevelopmental phenotypes including developmental delay, mental retardation and seizures. KLF13 is highly expressed during murine neural development. CNVs close to KLF13 can result in microcephaly, macrocephaly, intellectual disability (ID), and psychosis. A number of marginally significant hotspots were also identified for all phenotypes. Our results suggest that common genetic variants are associated with brain volumes in neonates and they are located in/near biologically plausible genes.

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Poster

096. Genomics, Proteomics, and Systems Biology

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Topic: G.02. Genomics, Proteomics, and Systems Biology

Support: NIH grant NS039518

Title: Whole proteome analysis of peripheral nerve injury via Mass Spectrometry

Authors: *A. S. YEKKIRALA, H. CHEN, K. HEMPEL, J. A. STEEN, C. J. WOOLF; Harvard Med. School/children's Hosp. Boston, Boston, MA

Abstract: It is an intriguing fact that peripheral nerves can regenerate well after injury while injury to the CNS is often debilitating, as CNS neurons do not exhibit such regenerative capacity. To identify novel proteins involved in peripheral nerve regeneration we, for the first time, utilized the Q-exactive MS/MS proteomics platform. Initial analysis of whole DRG lysates yielded a proteome of ~5000 proteins. TMT-labeling performed to identify protein differential expression in DRGs after a 5-day sciatic nerve crush injury (SNI) yielded 129 novel proteins that have not been implicated in regeneration. However, we were unable to identify many transcription factors (TFs), as they are not abundantly expressed. Efforts to improve proteome coverage helped identify >9000 proteins with numerous TFs and surface receptors and these methods are currently being used for a new multiplex experiment utilizing whole DRGs and sciatic nerve fragments 5 days after SNI. The results provide the first insights into whole proteome networks regulating the regenerative process in the PNS.

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Poster

096. Genomics, Proteomics, and Systems Biology

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Topic: G.02. Genomics, Proteomics, and Systems Biology

Title: Single-cell transcriptomic analysis identifies molecularly distinct subclasses of excitatory and inhibitory neurons in mouse visual cortex

Authors: *V. MENON, B. TASIC, T.-N. NGUYEN, C. LEE, T. KIM, N. SHAPOVALOVA, B. LEVI, J. GOLDY, D. BERTAGNOLLI, S. PARRY, K. SMITH, S. M. SUNKIN, M. HAWRYLYCZ, H. ZENG;
Allen Inst. For Brain Sci., Seattle, WA

Abstract: The mammalian nervous system comprises numerous neuronal populations, some of which can be distinguished based on various properties or molecular signatures. As part of the Mouse Cell Types program at the Allen Institute for Brain Science, we have characterized and categorized neurons in the mouse visual system using single-cell transcriptomic profiling methods. Having developed procedures to reliably isolate individual neurons labeled by specific transgenic Cre lines, we used single-cell RNA-sequencing methods to obtain transcriptional readouts of hundreds of individual excitatory and inhibitory cells from multiple cortical layers. Unsupervised clustering of the data reveals that excitatory neurons from the same cortical layer tend to have similar expression profiles, whereas interneurons cluster by known marker types. However, this analysis also identifies subtypes of excitatory neurons within a single layer, as well as among interneurons expressing the same canonical marker. Based on these data-driven clusters, we extract a set of well-known and lesser-studied genes to generate combinatorial codes of transcription factors, cell adhesion molecules, and ion channels to distinguish each of these neuron types. Finally, we also examine differential exon usage within single cells belonging to the same and different types to address the extent to which alternative splicing helps refine neuronal taxonomy.

Disclosures: **V. Menon:** None. **B. Tasic:** None. **T. Nguyen:** None. **C. Lee:** None. **T. Kim:** None. **N. Shapovalova:** None. **B. Levi:** None. **J. Goldy:** None. **D. Bertagnolli:** None. **S. Parry:** None. **K. Smith:** None. **S.M. Sunkin:** None. **M. Hawrylycz:** None. **H. Zeng:** None.

Poster

096. Genomics, Proteomics, and Systems Biology

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 96.04/TT10

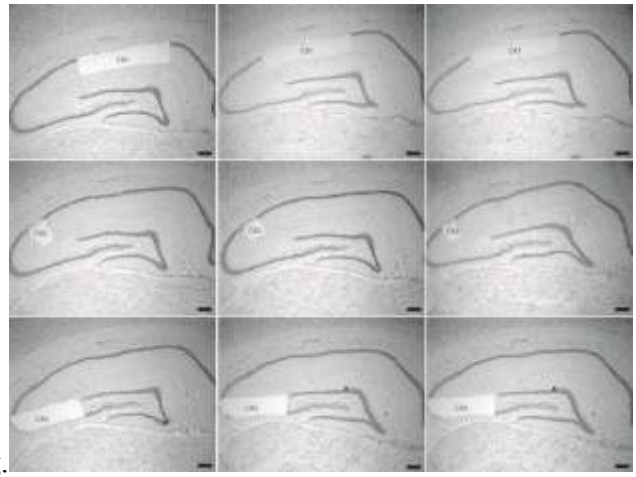
Topic: G.02. Genomics, Proteomics, and Systems Biology

Support: NIMH 5R44MH091909 (SLK)

Title: Brainpix - Rodent brain region-specific RNA bank

Authors: A. ZAVALA, Z. MA, *L. C. KUDO, S. KARSTEN;
NeuroInDx, Inc., Signal Hill, CA

Abstract: RNA isolation from specific subanatomical regions is a challenging task requiring high cost laser assisted microdissection instrument or flow sorting instruments. This complicates carrying out numerous pilot studies when information for a specific regional gene regulation has to be obtained in order to generate or support a hypothesis. To facilitate access for the scientific community to brain region specific RNA, we constructed a BrainPix bank of RNA isolated from rodent brain subanatomical regions. A recently developed low-cost cell and tissue acquisition system, KuiqpicK v.1.0, was used to acquire region specific samples from fresh frozen rat and mouse brain tissues. Representative collection of rat CA1, CA2 and CA3 regions of hippocampus are shown below. Total RNA was isolated from KuiqpicK dissected brain tissues using time proven protocols to yield highly pure, intact RNA. Every RNA sample underwent through rigorous quality controls that included analysis using Agilent 2100 Bioanalyzer. Currently RNA isolated from over twenty brain subanatomical areas is provided in RNase-free storage solution at convenient concentrations from 10 to 100 ng/µl, depending on the initial size of the dissected region (www.neuroindx.com/brainpix/). Purified RNA is DNase treated and ready to use in any downstream applications. It is ideal for pilot gene expression studies including microarray based analysis and high throughput sequencing.



Disclosures: **A. Zavala:** A. Employment/Salary (full or part-time):; Employment/Salary. **Z. Ma:** A. Employment/Salary (full or part-time):; Employment/Salary. **L.C. Kudo:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Ownership Interest. **S. Karsten:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Ownership Interest.

Poster

096. Genomics, Proteomics, and Systems Biology

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 96.05/TT11

Topic: G.02. Genomics, Proteomics, and Systems Biology

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Title: Protein Microarray Characterization of the S-Nitrosoproteome

Authors: Y.-I. LEE[1], H. KANG[3], *Y. LEE[4], J. JEONG[5], M. GHASEMI[6], S.-C. CHO[2], S.-C. PARK[2], V. DAWSON[6], T. DAWSON[6];
[1]Well Aging Res. Center,, Samsung Advanced Inst. of Technol. (SAIT), Seoul, Korea, Republic of; [2]Well Aging Res. Ctr., Samsung Advanced Inst. of Technol. (SAIT), Yongin-si, Korea, Republic of; [3]Dept. of Physiol., Ajou Univ. Sch. of Med., Suwon, Korea, Republic of; [4]Johns Hopkins Med., BALTIMORE, MD; [5]Dept. of Pharmacol. and Mol. Science,, Johns Hopkins Univ. Sch. of Med., baltimore, MD; [6]Neuroregeneration Program, Inst. for Cell Engin., Johns Hopkins Univ. Sch. of Med., Baltimore, MD

Abstract: Nitric oxide (NO) mediates a substantial part of its physiologic functions via S-nitrosylation, however the cellular substrates for NO-mediated S-nitrosylation are largely unknown. Here we describe the S-nitrosoproteome using a high-density protein microarray chip containing 16,368 unique human proteins. We identified 834 potentially S-nitrosylated human proteins. Using a unique and highly specific labeling and affinity capture of S-nitrosylated proteins, 138 cysteine residues on 131 peptides in 95 proteins were determined, defining critical

sites of NO's actions. Of these cysteine residues 113 are novel sites of S-nitrosylation. A consensus sequence motif from these 834 proteins for S-nitrosylation was identified, suggesting that the residues flanking the S-nitrosylated cysteine are likely to be the critical determinant of whether the cysteine is S-nitrosylated. We identify eight ubiquitin E3 ligases, RNF10, RNF11, RNF41, RNF141, RNF181, RNF208, WWP2, and UBE3A, whose activities are modulated by S-nitrosylation, providing a unique regulatory mechanism of the ubiquitin proteasome system. These results define a new and extensive set of proteins that are susceptible to NO regulation via S-nitrosylation. Similar approaches could be used to identify other post-translational modification proteomes.

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Poster

096. Genomics, Proteomics, and Systems Biology

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 96.06/TT12

Topic: G.02. Genomics, Proteomics, and Systems Biology

Title: A fast and simple preparation method for whole-genome bisulfite sequencing library preparation from ultra-low dna input

Authors: X. SUN[1], K. GIANG[1], T. CHUNG[1], *L. CUI[2], X.-Y. JIA[1];
[1]Zymo Res. Corp, Irvine, CA; [2]Zymo Res. Corp., Irvine, CA

Abstract: The distribution of 5-methylcytosine (5-mC) in DNA within the eukaryotic genome is known to greatly affect gene regulation and is currently a major topic of research. Studies on DNA methylation have been aided by advancements in bisulfite conversion and next-gen sequencing technologies which, when coupled, provide single-base resolution of 5-mC in the whole genome. Many whole-genome bisulfite sequencing (WGBS) library preparation protocols designed to analyze 5-mC distribution in the whole genome employ bisulfite to convert unmethylated cytosine bases to uracil after the library preparation. While these protocols produce reliable results, degradation of DNA is inherent to bisulfite conversion. As such, a large proportion of the adapterized library is fragmented and can no longer be amplified, which requires these protocols to call for large amounts of starting input DNA that is often times impossible to obtain. By rearranging the order of library preparation and bisulfite conversion, we developed a streamlined protocol that reveals whole-genome methylation patterns at single-base

resolution. The work-flow leads with the degradation inherent to bisulfite conversion to randomly fragment the DNA prior to the library preparation, which allows the protocol to accommodate for pico-gram quantities of starting input, making it ideal for analysis in precious and limited samples. Comparisons of sequencing data from this WGBS library preparation method with the established Reduced Representation Bisulfite Sequencing (RRBS) method using human DNA showed a correlation coefficient of 0.95 for CpG sites with more than 10X coverage. With slight modifications, this protocol is versatile in its ability to prepare libraries for ChIP-seq and RNA-seq.

Disclosures: **X. Sun:** None. **K. Giang:** None. **L. Cui:** None. **T. Chung:** None. **X. Jia:** None.

Poster

096. Genomics, Proteomics, and Systems Biology

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Program#/Poster#: 96.07/TT13

Topic: G.02. Genomics, Proteomics, and Systems Biology

Support: NIH Grant NS031609

NIH Grant P30DA018310

Title: D-amino acid-containing peptides in the mammalian nervous system

Authors: *H.-C. TAI, I. LIVNAT, E. T. JANSSON, S. S. RUBAKHIN, J. V. SWEEDLER; Univ. of Illinois at Urbana-Champaign, Urbana, IL

Abstract: Neuropeptides undergo a variety of post-translational modifications (PTMs) that are often critical for their cell-to-cell signaling properties. Peptide isomerization is a PTM in which an L-amino acid in the peptide chain is enzymatically converted into a D-amino acid, leading to important changes in the three-dimensional structure and bioactivity of the resulting D-amino acid-containing peptide (DAACP). Due to the lack of a molecular mass shift upon isomerization, this PTM is difficult to characterize in current mass spectrometry based peptidomics [1]. To address this challenge, we have developed a multi-stage method for the discovery of DAACPs in complex biological samples. Our method begins with screening for potential DAACPs based on resistance to degradation by aminopeptidase M (APM), an enzyme that selectively cleaves off L-amino acids but stops at a D-amino acid in the peptide. A common feature of DAACPs found in vertebrates is that L-to-D isomerization occurs at the second residue from the N-terminus,

making them poor substrates for an aminopeptidase. Therefore, we use APM to assay mixtures of endogenous neuropeptides, and peptides that degrade slowly on exposure to APM become candidate DAACPs. When a resistant peptide is found, we use liquid chromatography to purify greater quantities of the peptide and acid-hydrolyze it. The resulting amino acids are derivatized with Marfey's reagent (1-fluoro-2,4-dinitrophenyl-5-L-alanine amide) to enhance chiral separation, and then analyzed with a triple quadrupole mass spectrometer to determine the presence of specific D-amino acids. While initial experiments have used the well-defined *Aplysia* central nervous system (CNS), we are adapting these approaches to neuropeptides extracted from the CNS of the rat. We have already discovered several candidate DAACPs in preliminary screening of the rat pituitary. Several peptides have been found that are resistant to APM degradation. As one example, endogenously isolated little SAAS (amino acid sequence SLASAASAPLAETSTPLRL), is resistant to APM degradation while a synthetic all-L-amino acid version of little SAAS was easily degraded, suggesting a structural difference between the two. We are currently confirming the presence of this modification in little SAAS and several other mammalian peptides. 1. Bai et al., Analysis of endogenous D-amino acid-containing peptides in Metazoa, Bioanal Rev (2009) 1:7-24.

Disclosures: **H. Tai:** None. **I. Livnat:** None. **E.T. Jansson:** None. **S.S. Rubakhin:** None. **J.V. Sweedler:** None.

Poster

096. Genomics, Proteomics, and Systems Biology

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Program#/Poster#: 96.08/TT14

Topic: G.02. Genomics, Proteomics, and Systems Biology

Support: NIH R01 NS031609

NIH P30 DA018310

Title: Discovery of D-amino acid containing peptides in rodent islets of Langerhans

Authors: E. T. JANSSON, I. LIVNAT, H.-C. TAI, E. V. ROMANOVA, S. S. RUBAKHIN, *J. V. SWEEDLER;
Dept Chem., Univ. Illinois, Urbana, IL

Abstract: As the number of new drug approvals by the FDA has steadily declined since the mid-1990s, one may need to turn to more exotic and understudied peptide modifications in the search of novel drug candidates. The epimerization of a single amino acid in an endogenous peptide may occur as a post-translational modification (PTM) through *in vivo* enzymatic activity, recently shown in the mouse heart. However, the specific enzyme identity and its resulting endogenous rodent D-amino acid containing peptides (DAACPs) remain to date unknown. Quite a few biologics have been developed to treat various diseases based on peptides such as insulin, oxytocin, and C-peptides. Changing the three-dimensional structure of a peptide often changes its interaction with a receptor, and may hence affect its bioactivity and degradability. Given the presence of enzymatic activity and information on this modification in an increasing number of animals, the question we address here is whether there are endogenous mammalian DAACP hormones. Here, we are developing methods for performing studies of DAACP content in the peptidergic islets of Langerhans isolated from rat pancreas. While PTMs that do not induce any mass nor charge changes are inherently challenging to study with mass spectrometry (MS), they often impose changes in other physicochemical properties of the peptide which may be utilized for detection and characterization with MS. In liquid chromatography separations, DAACPs and their all-L-form counterparts can be separated due to their different interactions with the stationary phase. Also, DAACPs are more slowly digested than all-L-peptides by Aminopeptidase M, a digestive enzyme which can be utilized to highlight DAACPs from a complex biological sample. Further, in tandem MS, the intensity of the fragments of L- and D-form peptides upon collision induced dissociation can be different. Using a combination of these analytical state-of-the-art techniques, we investigated the presence of putative DAACPs in rat islets of Langerhans. Peptide extractions with a synthetic DAACP (NdWFa) added as an internal standard, were subjected to digestion with Aminopeptidase M, and the following MS-analysis uncovered peptides which remain intact - analogously as the DAACP-standard - while other peptides were readily degraded. Our results indicate that DAACPs are present in the islets of Langerhans. We also confirmed that the major cell-to-cell signaling molecules, e.g. the insulin C-peptide, synthesized by islets in healthy animals do not possess D-amino acids. Further development of our methodology will allow us to identify the exact site of epimerization in the peptide backbone.

Disclosures: **E.T. Jansson:** None. **I. Livnat:** None. **H. Tai:** None. **E.V. Romanova:** None. **S.S. Rubakhin:** None. **J.V. Sweedler:** None.

Poster

096. Genomics, Proteomics, and Systems Biology

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Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 96.09/TT15

Topic: G.02. Genomics, Proteomics, and Systems Biology

Support: NIH Grant GM076468

Title: Genetics of hippocampal gene expression in Diversity Outbred mice

Authors: *E. J. CHESLER[1], N. RAGHUPATHY[1], R. F. ROBLEDO[1], D. M. GATTI[1], S. C. MUNGER[1], C. PHILLIPS[2], J. NDUKUM[1], T. WILCOX[1], J. GRABER[1], M. HIBBS[1], G. A. CHURCHILL[1], M. LANGSTON[2];
[1]The Jackson Lab., Bar Harbor, ME; [2]The Univ. of Tennessee, Knoxville, TN

Abstract: Genetic analysis of gene expression in laboratory mice has enabled the discovery of regulatory variation and covariation among transcripts and behavioral traits. Early studies typically relied on microarray analysis in two-progenitor populations for the detection of quantitative trait loci (QTL) and genetic correlation. Recent advances in genetic mapping populations and RNA quantitation have improved the utility, information content, precision and power of these studies tremendously. RNA sequencing now enables genetic analysis of more precisely characterized isoforms, allelic variants, and imprinted loci. The Diversity Outbred (DO) mouse population, derived from an intercross of the five common and three wild-derived inbred mouse founders strains of the Collaborative Cross (CC) has over 45 million segregating SNPs, high recombinational precision and high behavioral diversity. We sequenced the transcriptome from the hippocampus of >290 DO mice of both sexes. A genome-wide expression QTL analysis using 17,539 genes against 7700 SNP genotype markers revealed 10,341 significant associations at 1% FDR threshold (11,863 significant eQTLs at 5% FDR threshold). Among the significant associations, over 85% of the associations are within 2MB of the transcription start site of the gene. The remaining 15% of the significant associations are away from the start site, and 4.2% are on different chromosomes. Allelic effects of each of the eight founder haplotypes are estimated for every transcript, and through the integration of publicly available inbred strain sequence data, several of these allelic effects are attributable to specific polymorphic variants. Combinatorial, genome wide gene co-expression analysis of genes co-expressed at $|r| > .7$ reveals ~100 precise, functionally coherent co-expression networks. For example, a network consisting only of seven co-expressed genes in the AP-1 transcriptional network was extracted in an unsupervised analysis. Genetic analysis of expression correlation to behavior and convergent functional genomic analyses in the GeneWeaver system enable interpretation of the functional role of co-expression values in the context of other functional genomic experimental results. The availability of these data will enable the interpretation and refinement of new and existing QTL analyses of brain and behavior.

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Poster

096. Genomics, Proteomics, and Systems Biology

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Program#/Poster#: 96.10/TT16

Topic: G.02. Genomics, Proteomics, and Systems Biology

Support: NIH RO1 NS50465

Title: Gene network strategy to elucidate the complexity of brain trauma and related neurological disorders

Authors: *Z. YING[1], Q. MENG[1], R. AGRAWAL[1], X. YANG[1], F. GOMEZ-PINILLA[2]; [1]Dept of Integrative Biol. and Physiol., [2]Dept of Neurosurgery, and Integrative Biol. and Physiology,, UCLA, LOS ANGELES, CA

Abstract: Traumatic brain injury (TBI) affects a large variety of cellular and molecular processes ultimately compromising brain function and cognitive abilities. The complexity of the pathology of TBI that involves multiple components seems to pose crucial challenges for development of effective cures. Indeed, current pharmacological approaches focusing on single events have yielded poor results on the prevention of the short- or long-term consequences of TBI. The lack of a comprehensive mechanistic understanding of the complexity of TBI pathology likely explains the poor outcomes of the current therapeutic approaches. We carried out a systems biology study to address these challenges by using state-of-the art methodologies that can capture the tremendous genomic variability inherent to TBI. The unique aspect of our approach is to determine the effects of TBI on the interaction of genes within a genome-wide scale to grasp the whole dimension of the TBI pathology. We used next generation sequencing and integrative genomics analyses to determine how TBI affects networks of genes that could characterize main events in the TBI pathology. We report that moderate fluid percussion injury (FPI) engages the action of master genes such as Anxa2 and Ogn to coordinate the function of hundreds of genes in the network. Increasing evidence indicates that TBI poses risks for neurological disorders such as Alzheimer's disease, and psychiatric disorders. We report that gene network reorganization in our rodent model of TBI overlaps with existing human libraries of gene-wide association studies (GWAS) for brain disorders such as Alzheimer's disease, bipolar disorder, autism, etc. These results reveal mechanistic information how TBI impacts specific gene networks which may confer vulnerability to neuropsychiatric disorders. Our ongoing studies suggest that the broad spectrum of action of dietary docosahexaenoic acid

(DHA) is instrumental to counteract TBI pathology by restoring gene network reorganization. These studies may set basis for development of network-based medicine, a new line of therapeutic strategy, to improve TBI outcome and prevent TBI-associated brain disorders.

Disclosures: **Z. Ying:** None. **Q. Meng:** None. **R. Agrawal:** None. **X. Yang:** None. **F. Gomez-Pinilla:** None.

Poster

096. Genomics, Proteomics, and Systems Biology

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Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 96.11/TT17

Topic: G.02. Genomics, Proteomics, and Systems Biology

Support: IRSC Grant

Title: Application of translational profiling method for the molecular characterization of post-ischemic inflammatory response

Authors: ***H. BOUTEJ**, L.-C. BÉLAND, M. LALANCETTE-HÉBERT, J. KRIZ; CRIUSMQ, Le Ctr. De Recherche De L'Institut Univ., Québec, QC, Canada

Abstract: Microglial cells are the resident macrophage-like cells of the brain and act as the first line defense in the central nervous system. Activation of this primary immune effector can be observed in almost all CNS pathologies, including stroke. Brain damage following transient or permanent ischemia results from a series of pathophysiological events that evolve in time and space. They include acute necrosis in the centre of infarction, followed by peri-infarct (the penumbra) depolarizations, glutamate-mediated excitotoxicity and oxidative stress. Although there is evidence that inflammation in ischemic injury is orchestrated by microglia, their dual role (neurotoxicity/neuroprotection) still remains ambiguous. To gain a better understanding of the molecular mechanisms involved in the post-ischemic inflammatory response, we used a translational profiling approach. This elegant method, developed in recent years, first elucidate the biological properties of distinct neuronal populations (Doyle et al., 2008; Heiman et al., 2008). Basically, the translating ribosome affinity purification (TRAP) allows the opportunity to capture gene expression after genetic alteration, disease or pharmacological perturbations. For this purpose, we generated a BV2 cell-line that stably expresses a double epitope-tagged form of the large subunit ribosomal protein L10a: Flag-EGFP-RPL10a. Cells were treated with lipopolysaccharide (LPS) (1ug/ml) or with glutamate (100μM). Cells lysates were

immunoprecipitated with anti-Flag resin. Bound proteins were eluted with EDTA-elution buffer and tryptic fragments of ribosomes-associated proteins were sequenced by mass spectrometry. Scaffold was used to validate MS/MS based peptide and proteins identifications. The TRAP analysis resulted in detection of several proteins that belong to different functional categories: cytoskeletal proteins, metabolic enzymes, chaperones, kinases, proteases and proteins involved in signalling and in proteins degradation. Interestingly, we found that some of these proteins are enriched in LPS or glutamate treated cells compared to untreated cells. This differential proteomic analysis enabled us to characterize gene expression pattern, *in vitro*. Furthermore, we have established a transgenic mouse that expresses the large subunit ribosomal protein L10a: Flag-EGFP-RPL10a under the control of the CD11B promoter. The resulting mouse line will help us to draw up an, *in vivo*, translational profile. Finally, these observations may contribute to decipher the molecular mechanisms involved in microglial cells activation and inflammatory response *in vitro* and *in vivo*.

Disclosures: **H. Boutej:** None. **L. Béland:** None. **M. Lalancette-Hébert:** None. **J. Kriz:** None.

Poster

096. Genomics, Proteomics, and Systems Biology

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 96.12/TT18

Topic: G.02. Genomics, Proteomics, and Systems Biology

Title: High-throughput functional analysis of human brain enhancers

Authors: ***A. R. PFENNING**[1], M. HEMBERG[2], T. A. NGUYEN[3], M. FRIESE[3], M. KELLIS[1], J. M. GRAY[3];
[1]CSAIL, MIT, Cambridge, MA; [2]Dept. of Ophthalmology, Boston Children's Hospital, Boston, Boston, MA; [3]Dept. of Genet., Harvard Med. Sch., Boston, MA

Abstract: The gene expression programs necessary for development, learning, and memory in neurons are coordinated by genomic enhancers. The locations of these enhancers within the mouse and human genomes are now known, thanks to the ENCODE Project and related studies. However, the specific nucleotides within enhancer sequences that are necessary for gene regulation remain to be identified. It therefore remains difficult to predict the effects of human genetic variation on enhancer activity, neural development, and neural function. We analyzed the sequences of neural enhancers and identified enriched transcription factor binding site sequences,

8 to 15 nucleotides long, likely to be important for neural enhancer function. Then, we tested our computational predictions of important sequences using a Massively Parallel Reporter Assay (MPRA) that we adapted to primary neurons. In MPRA experiments, we infect cortical neurons with a library consisting of thousands of synthetically produced enhancers that control the expression of unique barcodes. We have identified hundreds of enhancers that are activated by neural activity, as well as many predicted transcription factor binding sites required for their function.

Disclosures: **A.R. Pfenning:** None. **M. Hemberg:** None. **T.A. Nguyen:** None. **M. Friese:** None. **M. Kellis:** None. **J.M. Gray:** None.

Poster

096. Genomics, Proteomics, and Systems Biology

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JSPS KAKENHI Grant Number 25560428

JSPS KAKENHI Grant Number 26280110

JST Acceleration Utilization of University IP Program

RIKEN Neuroinformatics Japan Center

Title: Transcriptome tomography: Mapping genes onto 3D brain structures

Authors: *Y. OKAMURA-OHO[1,2], K. SHIMOKAWA[3], S. NAKAMURA[1], Y. TSUJIMURA[1], M. NISHIMURA[1], S. TAKEMOTO[1], M. MORITA[1], T. IJIRI[1], T. TAWARA[1], H. YOKOTA[1]; [1]RIKEN Ctr. For Advanced Photonics, Wako-Shi, Saitama, Japan; [2]Brain Res. Network (BReNt), Zushi-shi, Japan; [3]Tohoku Med. Megabank Organization, Tohoku Univ., Sendai-shi, Japan

Abstract: Expression-anatomy association is crucial for understanding molecular functions of, in particular, novel coding and non-coding genes. We have invented a framework for comprehensive gene expression density mapping on the whole three dimensional (3D)

anatomical context, Transcriptome Tomography (PLoS One 2012; 7, e45373, Video http://www.youtube.com/watch?v=Td4rGRQIZuY&list=UUIGmhpdcVev1Wc0YK7FHIig). Expression densities measured with high-throughput methods are usable for their mapping onto the 3D brain structure detected with other modalities such as MRI. Also they are usable directly for gene-by-gene correlation analysis of co-expression. This framework comprehensively assesses co-expression patterns that are latent within expression maps. Expression maps and co-expression search results in the mouse brain can be browsed in our website, ViBrism-DB (http://vibrism.neuroinf.jp/). In this presentation we would focus on previously uncharacterized mouse-specific genes that were co-expressed with gene groups encoding transcription factors and related molecules. The genes were expressed in areas associated with specific brain functions and the previously uncharacterized non-coding genes were located in a co-expression network position linking the groups. This linkage suggests characteristics of the non-coding genes that may coordinate multiple gene groups and create mouse-specific neural designs. A part of this work was supported by members in RIKEN Neuroinformatics Japan Center and conducted within the WHS and DAI Task Forces of the INCF Program on Digital Brain Atlasing.

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Poster

096. Genomics, Proteomics, and Systems Biology

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Topic: G.02. Genomics, Proteomics, and Systems Biology

Support: NIH R15NS061303

NIH R15NS070746

NIH COBRE P20 RR15583

Whitehall Foundation

Title: Compartmentalization and collaboration in neuroblastoma tyrosine kinase signaling networks

Authors: *M. L. GRIMES[1], J. PALACIOS-MORENO[2], A. GUO[3], M. STOKES[3], M. J. COMB[3], E. KUEHN[4];
[1]Div. of Biol. Sci., [2]Neurosci. Grad. Program, Univ. of Montana, MISSOULA, MT; [3]Cell Signaling Technology, Inc., Danvers, MA; [4]Dept. of Neurosci., Johns Hopkins Univ. Sch. of Med., Baltimore, MD

Abstract: Phosphorylation of proteins on tyrosine residues, and the recognition of these modifications by specific binding domains, play central roles in creating a highly dynamic network of interacting proteins that reads and responds to signals from growth factors in the cellular microenvironment. Mutations that cause aberrant activation of receptor tyrosine kinases cause tumors, for example point mutations in anaplastic lymphoma kinase (ALK) are a major contributor to familial neuroblastoma. We found that more than half of all the receptor tyrosine kinases in the human genome were activated in a large scale study that examined phosphorylated proteins in neuroblastoma cell lines and cell fractions including endosomes and detergent-resistant membranes. To understand tyrosine kinase signaling mechanisms, new computational and bioinformatics approaches were developed. Neuroblastoma phosphoproteomic data analyzed using pattern recognition algorithms revealed clusters of interacting phosphorylated proteins that are likely to represent functional signaling pathways. Statistical patterns of phosphorylation in the same samples, combined with protein interaction data, suggest that receptor tyrosine kinases are functionally compartmentalized into distinct collaborative groups. Signaling components were also compartmentalized within cells. The SRC-family kinases, FYN and LYN, responded differently to different RTKs, and, together with the scaffold protein, PAG1, were phosphorylated and localized in endosomes and lipid rafts differently under conditions where different receptor tyrosine kinases are activated. These results suggest that dynamic activation and intracellular localization of SFKs provide a mechanism to distinguish signaling responses to different receptors that govern cell differentiation and cancer, and that tyrosine kinase signaling plays a major role in developmental pathways that govern the behavior of neural crest, which gives rise to neuroblastoma.

Disclosures: **M.L. Grimes:** None. **J. Palacios-Moreno:** None. **A. Guo:** A. Employment/Salary (full or part-time):; Cell Signaling Technology, Inc. **M. Stokes:** A. Employment/Salary (full or part-time):; Cell Signaling Technology, Inc. **M.J. Comb:** A. Employment/Salary (full or part-time):; Cell Signaling Technology, Inc.. **E. Kuehn:** None.

Poster

096. Genomics, Proteomics, and Systems Biology

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Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

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Grants from SENSHIN Medical Research Foundation to Dr Miyawaki

Title: Genetic analysis of RNF213 c.14576G>A variant in various phenotypes of intracranial major artery stenosis/occlusion

Authors: *S. MIYAWAKI[1], H. IMAI[1], M. SHIMIZU[2], S. YAGI[2], H. ONO[1], A. MUKASA[1], H. NAKATOMI[1], T. SHIMIZU[2], N. SAITO[1];
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Abstract: (Background and Purpose) The c.14576G>A variant in ring finger protein 213 (RNF213) was recently identified as a susceptibility gene variant for moyamoya disease (MMD). We assumed that the c.14576G>A variant could be the cause of a wide spectrum of phenotypes of intracranial major artery stenosis/occlusion (ICASO) including MMD, and so some cases of ICASO originally considered to originate from atherosclerosis might be associated with the c.14576G>A variant in RNF213. In this study, the occurrence of c.14576G>A variant was evaluated in patients with ICASO without signs of MMD (non-MMD ICASO), as well as in patients with MMD and other cerebrovascular diseases as controls. (Methods) Study participants were recruited from The University of Tokyo Hospital and Kanto Neurosurgical Hospital. The occurrence rate of c.14576G>A variant was investigated in 519 patients, 64 with definite MMD, 14 with unilateral MMD, 125 with non MMD-ICASO, 55 with extracranial carotid atherosclerosis, 105 with cerebral aneurysm, 21 with intracerebral hemorrhage, and 135 control subjects. (Results) RNF213 c.14576G>A variant was found in 82.8% (53/64) of the definite MMD group, and 57.1% (8/14) of the unilateral MMD group. Moreover, the variant was found in 23.2% (29/125) of the non-MMD ICASO group. RNF213 c.14576G>A variant had significant associations with definite MMD ($P<0.0001$; odds ratio, 320.4; 95% confidence interval, 68.6-1494.4), unilateral MMD ($P<0.0001$; odds ratio, 88.6; 95% confidence interval, 15.3-511.3), and also with non-MMD ICASO ($P<0.0001$; odds ratio, 20.0; 95% confidence interval, 4.68-86.2). There was no significant association with extracranial carotid atherosclerosis, cerebral aneurysm, or intracerebral hemorrhage. (Conclusions) A particular subset of patients with various phenotypes of ICASO has a common genetic variant, RNF213 c.14576G>A, indicating that RNF213 c.14576G>A variant is a high-risk allele for ICASO (Miyawaki et al Stroke 2012, Miyawaki et al Stroke 2013).

Disclosures: **S. Miyawaki:** None. **H. Imai:** None. **M. Shimizu:** None. **S. Yagi:** None. **H. Ono:** None. **A. Mukasa:** None. **H. Nakatomi:** None. **T. Shimizu:** None. **N. Saito:** None.

Poster

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HHMI

Title: Using Single-cell RNA-seq to classify retinal cell types

Authors: ***Y.-R. PENG**[1,2], Q. MU[1,2], X. ZHANG[1,3], A. K. SHALEK[3,4,5], B. HAAS[5,6], H. PARK[1,3,4], A. REGEV[5,6], J. R. SANES[1,2];
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Abstract: The mouse retina contains ~100 types of cells that process visual information gathered by photoreceptors and send it to the brain via retinal ganglion cell (RGC) axons. Understanding how visual information is processed requires classifying cells so that they can be identified, marked and manipulated. However, classification is currently incomplete and markers are unavailable for many types that have been distinguished morphologically. Single-cell RNAseq (scRNAseq) has the potential to circumvent these limitations by providing transcriptome data without a priori identification of the cell type. However, retinal cells are small, so establishing an unbiased amplification method is critical. Here, we asked whether newly developed scRNAseq methods could be used for this purpose. As an initial step, we compared two very different classes of retinal cells, photoreceptors and RGCs. We then compared two different types of RGCs: alpha-cells and on-off directional selective RGCs (ooDSGCs). Finally, we compared two very closely related subtypes: ooDSGCs that respond to ventral or nasal motion (DRD4-RGCs, Huberman et al., Neuron, 2009; HB9-RGCs, Trenholm et al., Neuron, 2011). In each case, we used fluorescence-activated cell sorting (FACS) to isolate individual cells from transgenic lines in which specific cell types express a fluorescent protein (Kay et al., Nature, 2012). We then amplified transcripts using either the Multiple Annealing and Looping-Based Amplification

Cycles (MALBAC) or SMART-Seq2 method and performed scRNAseq on multiple cells of each type. In cone photoreceptors, we detected expression of cone-specific genes (opn1sw, opn1mw, arr3), but not genes from other retinal cells types, such as rhodopsin (rods), Vsx (bipolar cells), GFAP (glial cells), or Thy1 (RGCs). Conversely, only RGC-specific genes were detected in RGCs. Using principal component analysis and gene-expression distance analysis, we could easily separate cones from RGCs and alpha-RGCs from ooDSGCs. Strikingly, these methods even separated HB9-RGCs from DRD4-RGCs, indicating the ability to distinguish very similar subtypes. Ongoing studies are aimed at identifying new subtypes in mixed populations, and assessing the degree of heterogeneity within nominally homogeneous subtypes.

Disclosures: **Y. Peng:** None. **Q. Mu:** None. **X. Zhang:** None. **A.K. Shalek:** None. **B. Haas:** None. **H. Park:** None. **A. Regev:** None. **J.R. Sanes:** None.

Poster

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Support: Japan Society for the Promotion of Science Fellows (12J00633)

Ministry of Education, Culture, Sports, Science and Technology (MEXT) for the Body and Mind Integrated Sports Sciences (BAMIS) Project of Japan

Title: Down-regulation as the dominant hippocampal gene expression profile with voluntary resistance wheel running by DNA microarray analysis

Authors: *M. LEE[1,2], R. RAKWAL[3], J. SHIBATO[1], K. INOUE[1], H. SOYA[1];
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Abstract: BACKGROUND: Physical exercise has beneficial effects on our brawn and brain, not only with regard to muscular adaptations, but also with respect to brain function enhancing neuronal plasticity and cognitive enhancement. We have recently demonstrated that voluntary resistance wheel running (RWR) exercise can increase neurogenesis and spatial memory associated with hippocampal brain-derived neurotrophic factor (BDNF) signaling. Despite these

new evidence, underlying molecular mechanisms for RWR-induced improvement of hippocampal function remained to be clarified. Here we have utilized the high-throughput DNA microarray approach to gain deep insight into molecular mechanisms underlying. These changes could be novel targets of RWR-induced hippocampal plasticity. METHODS: 10-week-old adult male Wistar rats were assigned randomly to sedentary control (Sed), wheel running with no resistance (WR), and resistance wheel running to 30% of body weight (RWR) groups for 4 weeks. Whole genome (4x44K) high-density oligonucleotide microarrays were used to monitor the expression level of gene transcripts in the hippocampus of rats voluntary running for 4 weeks in comparison with sedentary animals. RESULTS: Our results showed that a significant decrease in average running distances was observed although average work levels increased immensely by 12-fold in the RWR compared to the WR group, resulting in muscular adaptation for the fast-twitch plantaris muscle without any negative stress effects. Additionally, transcriptome data on the hippocampi revealed that 128 (sedentary x WR) and 169 (sedentary x RWR) genes were up-regulated (> 1.5-fold change) as compared with 97 (sedentary x WR) and 468 (sedentary x RWR) down-regulated genes (< 0.75-fold change). Functional categorization using pathway- or specific disease states-focused gene classifications and Ingenuity Pathway Analysis (IPA) revealed expression pattern changes in the major categories of disease and disorders, molecular functions, and physiological system development and function. Among the RWR specifically regulated genes, we found highly down-regulated SYCP3, PRL, PDK4 genes, inflammatory cytokines (IL1B, IL10, IL2RA, and TNF) and chemokines (CXCL1, CXCL9, CXCL10, CCL2, CCL13, and CCR4), which might help to counteract neuronal dysfunction and vulnerability. CONCLUSION: Our findings indicate that this is a first study presenting not only new information on the voluntary RWR influenced transcriptome in the rat hippocampus, but also these gene candidates are suggested to play a critical role in hippocampal plasticity.

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Poster

096. Genomics, Proteomics, and Systems Biology

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Topic: G.02. Genomics, Proteomics, and Systems Biology

Support: MH094445

Title: Examining the serine/threonine kinome in complex mental illness

Authors: *J. L. MCGUIRE[1], S. MARWAHA[2], A. A. FUNK[1], E. A. CAREY[1], J. H. HAMMOND[3], V. HAROUTUNIAN[4], H. R. EGHBALNIA[2], R. E. MCCULLUMSMITH[1]; [1]Psychiatry, [2]Mol. and Cell. Physiol., Univ. of Cincinnati, Cincinnati, OH; [3]Psychiatry and Behavioral Neurobio., Univ. of Alabama, Birmingham, Birmingham, AL; [4]Psychiatry, Mount Sinai Sch. of Med., New York, NY

Abstract: A kinome represents the full complement of protein kinase activity in a cell or tissue. High throughput peptide array screening was recently developed to identify altered kinase activity in tumor cells. These have been instrumental in the identification of new drug targets and therapies for multiple cancer types. Here we applied kinome array technology to investigate patterns of serine/threonine kinase activity in schizophrenia. We used complimentary approaches to more fully elucidate putative abnormalities in signaling cascades in schizophrenia. First, we analyzed peptides differentially phosphorylated between schizophrenia and control groups based on fold-change. We anticipated that small changes in kinase activity within related networks could profoundly effect cellular functioning without the gross abnormalities seen in cancer. 19 substrates were differentially phosphorylated +/- 1.15-fold. We used Ingenuity pathway analysis (IPA) to further analyze the dataset (dataset 1). IPA determines the probability (with associated p-values) that identified peptides act within particular known pathways. Functions and pathways with p-values less than .05 and containing > 5 peptides were considered most relevant. Second, we analyzed the kinome array using an algorithm calculating significance based on consistency rather than magnitude of signal changes. From this analysis we took the 10 substrates with the lowest p-values (dataset 2). These substrates partially overlapped with dataset 1. Dataset 2 was also analyzed using IPA. Finally, principal component analysis (PCA) was used to attempt to extract patterns in kinase activity differentiating schizophrenia and control subjects. PCA identified 10 components explaining 90% of the variance in the dataset. Only 1 component, containing a single substrate, differentiated schizophrenia from control. Separate IPA analyses of datasets 1 and 2 identified leukocyte development and differentiation, cytoskeletal remodeling, molecular transport, ion homeostasis, and Ca2+ mobilization as probable functions and pathways. Additionally, direct and indirect targets of protein kinase B (AKT) and the ERK and JNK mitogen-activated protein kinase pathways were overrepresented in both datasets. Total ERK1/2 and JNK1/2 protein was increased in schizophrenia however AKT protein was equivalent to the control samples. These analyses implicate relatively small changes across multiple components in key interrelated signaling cascades in schizophrenia and demonstrate the utility of this technology in investigating new avenues for drug development and targeted therapies in complex mental illness.

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Poster

096. Genomics, Proteomics, and Systems Biology

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Topic: G.02. Genomics, Proteomics, and Systems Biology

Title: Identification of cell type-specific gene expression using genome-wide databases

Authors: H.-J. KIM[1], B.-S. KANG[3], M.-H. KIM[2], T.-Y. JEONG[1], *J.-H. KIM[1], D.-H. HWANG[3,2];
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Abstract: Various types of neurons have been classified in accordance to morphology, biochemical and electrophysiological features. Recently, pinpointing of genes enriched in a given brain area as well as discovery of selective marker proteins for subtypes of neurons may allow for development of noble animal models in which certain animal behavior would be controlled and the underlying neural circuits could be elucidated in the system level. Hence, gene expression profiles provide an informative modality to define cellular diversity in the brain. Despite functional importance and practical applicability, genome-wide histological data sets have not be systematically produced and analyzed to be translated to easily accessible formats, yet. As a result, a limited set of established molecular markers have been utilized thus far whereas a vast majority of enriched genes at the select areas remain currently uncharacterized. Allen brain Atlas (ABA) offers huge expression data of mouse brain in public domains including ***in situ*** hybridization images. Here, we attempted to search noble candidate marker protein in a systematic way, from the ABA database and were able to successfully provide candidate genes for distinct neuronal subtypes in the mouse ventral tegmental area, which substantiates the usage of protocol and algorism for future studies.

Disclosures: **H. Kim:** None. **T. Jeong:** None. **J. Kim:** None. **D. Hwang:** None. **M. Kim:** None. **B. Kang:** None.

Poster

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Topic: G.02. Genomics, Proteomics, and Systems Biology

Support: NIMH 5R44MH091909 (SLK)

Title: Noninvasive region- and cell-specific RNA acquisition from fixed and fresh frozen brain tissues using KuiqpicK

Authors: *S. L. KARSTEN, A. ZAVALA, Z. MA, L. C. KUDO;
NeuroInDx, Inc., Signal Hill, CA

Abstract: Current methods of region-specific RNA isolation involve either microdissection or fluorescence assisted cell sorting. Both methods are time-consuming, require expensive instrumentation, and result in the destruction/loss of the original and often rare tissue material that is under study. In addition, RNA isolation from the collected cells must be performed, thereby increasing the time and cost of such studies. We have developed a novel noninvasive approach for region-specific RNA acquisition from fresh frozen and fixed brain tissues that does not require microdissection or flow sorting procedures. RNA can be collected directly from the desired subanatomical regions, and used immediately for gene expression studies, including transcriptome analysis. Briefly, the tissue is evenly covered with a RNA capturing solution forming a gel like resin on the surface of a tissue section. Then taking advantage of a recently developed cell and tissue acquisition system, KuiqpicK (www.neuroindx.com/kuiqpick/), resin samples are collected from the corresponding region of a specific area, leaving the tissue intact. RNA contained in the collected resin is extracted using standard protocols and may be used directly for a range of downstream enzymatic reactions. Using this approach region specific RNA (e.g. dentate gyrus, CA1, 2, 3 areas of hippocampus) was collected from fresh frozen, sucrose treated and fixed brain tissues. Evaluation of isolated RNA for yield, degradation rates and suitability for cDNA synthesis and labeling protocols were performed. This protocol eliminates the necessity for tissue microdissection for acquisition of region-specific RNA and preserves potentially valuable tissue samples for further immuno-based studies. In addition to being rapid and simple, KuiqpicK provides near cellular resolution, which is critical for many downstream gene expression studies. Patent pending

Disclosures: **S.L. Karsten:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Ownership Interest. **A. Zavala:** A. Employment/Salary (full or part-time):; Employment/Salary. **Z. Ma:** A. Employment/Salary (full or part-time):; Employment/Salary. **L.C. Kudo:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Ownership Interest.

Poster

096. Genomics, Proteomics, and Systems Biology

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Support: NIDA Grant DA018343

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Title: Characterization of post-synpatic density protein enrichment using targeted quantitative mass spectrometry methods

Authors: ***F. SAKAUE**[1], C. M. COLANGELO[2], L. M. CHUNG[3], T. B. ABBOTT[2], R. R. KITCHEN[1], A. C. NAIRN[1];
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Abstract: The postsynaptic density (PSD) is a specialized protein complex at the synaptic junction of glutamatergic excitatory synapses. The protein components of the PSD, including neurotransmitter receptors, cytoskeletal proteins, and signaling molecules, can be altered by synaptic activity and drug exposure. Therefore, methodologies to quantify the changes in the abundance of PSD proteins should help our understanding of the molecular basis of synaptic plasticity. In this study, 112 proteins in PSD fractions prepared from rat brain were initially selected for analysis using multiple reaction monitoring (MRM) mass spectrometry based on the number of peptides detected, peak distribution and signal/noise ratio. However considerable variation in the levels of a sub-set of proteins was observed that was dependent on sample preparation. To produce more consistent data, we applied fraction-enrichment analysis and

analyzed the levels of a larger number of proteins than initially targeted by the MRM approach. Crude synaptoneurosome fraction (P2) and PSD fractions were prepared systematically and analyzed by SWATH LC-MS/MS, a novel data-independent acquisition technique. We examined the levels of ~1,700 proteins by SWATH that were differentially enriched in PSD compared to the P2 fraction. Bioinformatic analysis revealed classes of proteins that were enriched or excluded from the PSD fraction compared to the P2 fraction, and identified factors that contributed to higher levels of technical and biological variance for identified PSD proteins. The results from these studies will be helpful in defining proteins that exhibit robust association with the PSD, and that can be reproducibly analyzed by targeted mass spectrometric methods.

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Poster

096. Genomics, Proteomics, and Systems Biology

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Topic: G.02. Genomics, Proteomics, and Systems Biology

Support: NIH Grant RO1 NS050465

Title: Genomics and brain pathophysiology: Omega-3 fatty acid reprograms gene networks in the brain underlying the behavioral consequences of metabolic syndrome

Authors: *F. GOMEZ-PINILLA[1,2], Q. MENG[1], R. AGRAWAL[1], Z. YING[1], X. YANG[1]; [1]Integrative Biol. and Physiol., UCLA, LOS ANGELES, CA; [2]Dept of Neurosurgery,, UCLA, Los Angeles, CA

Abstract: Diet-induced metabolic syndrome (MetS) is becoming a major threat for neurological and psychiatric disorders. We previously found that rats treated with high fructose (15% in drinking water) developed peripheral signs of MetS and disturbances in learning and memory abilities (Agrawal et al., J Physiolo 2012). We profiled the transcriptome of the hypothalamus and hippocampus - brain regions with known functions in cell metabolism and cognition - of these rats by RNA sequencing. We discovered large-scale perturbations in gene expression in the hypothalamus and hippocampus with 581 and 146 genes showing differential expression and 99 and 53 genes with alternative splicing, respectively. We found that the dietary supplementation of docosahexaenoic acid (DHA), which ameliorates the unfavorable effects of fructose on the

brain, altered the expression of 654 and 462 genes, and exon splicing of 156 and 81 genes in the hypothalamus and hippocampus, respectively. Strikingly, among the hundreds of genes with expression changes in response to both fructose and DHA, 100% hippocampal genes and 98% hypothalamic genes demonstrated complete reversal of the directionality in gene expression. By integrating brain gene regulatory networks constructed from multiple rodent populations, we derived hippocampus and hypothalamus gene networks that entail the interactions among the genes affected by high fructose and DHA. We also identified shared key network regulators including Bgn, and Fmod which demonstrated opposite expression patterns under the two treatment conditions, suggesting dietary DHA normalizes the gene networks affected by fructose-induced MetS by affecting these key regulators. In summary, our study provides the much needed information on how MetS affects the interaction among multiple genes in brain regions involved in cognitive processing. Our results evoke a powerful strategy to target key aspects of the gene network organization with dietary agents such as, DHA to promote broad changes in the multifactorial spectrum of complex neurological disorders.

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Poster

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Topic: G.02. Genomics, Proteomics, and Systems Biology

Title: Antigen and peptide microarrays reveal autoantibody targets in vaccine-induced narcolepsy

Authors: ***A. HÄGGMARK**[1], A. ZANDIAN[1], B. FORSSTRÖM[1], T. OLSSON[2], M. PARTINEN[3], M. UHLÉN[1], J. M. SCHWENK[1], P. NILSSON[1];
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Abstract: Narcolepsy is regarded as a rare sleeping disorder with yet unknown cause, however, the specific loss of hypocretin producing neurons together with a strong HLA association have lead to the hypothesis that autoimmune components are involved. In several countries, the incidence of narcolepsy was dramatically increased after vaccination campaigns against the

pandemic H1N1 influenza in 2009. Children and adolescents were affected and symptoms were characterized by a higher prevalence of cataplexy as compared to narcolepsy patients with disease onset unrelated to vaccination. We have performed a broad scaled screening for novel autoimmunity targets by investigating the specificities of antibodies present in blood through antigen and peptide microarrays. In an initial screening stage, a Finnish cohort with 58 serum samples was analyzed for reactivity against 11500 antigens using planar microarrays with human protein fragments produced within the Human Protein Atlas project. Additionally, autoantibodies in narcolepsy samples were analyzed on ultra high density peptide microarrays containing 175 000 12-mer peptides with an overlap of 11 amino acids. Based on observations in the screening, 244 antigens and 40 peptides were selected and reactivity towards these were further investigated in an independent Swedish cohort including 179 narcolepsy patients and controls. This was performed in a suspension bead array setup where also previously published suggestions of autoimmune targets were included for profiling. The results from the massive screening and second stage validation revealed several potential autoantibody targets. The majority of them represented either of the following protein categories; enzymes, secreted proteins and membrane proteins. The identification of this set of autoimmunity targets, where the myelin protein zero-like 1 (MPZL1), plexin A3 (PLXNA3) and anoctamin 8 (ANO8) are among the most interesting, will after further exploration, have the potential to enable increased understanding and could finally shed some light on the disease mechanism behind vaccine-induced narcolepsy.

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Poster

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Polish Ministry of Science Grant Mobility Plus DPN/MOB109/II/2012

Title: RNA-sequencing implicates HMGN1 and HMGN2 in the modulation of transcriptional profiles of prefrontal cortex and hippocampus in mice

Authors: *P. LISOWSKI[1], S. ZHANG[2], T. DENG[2], T. FURUSAWA[2], M. BUSTIN[2];
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Abstract: High mobility group N (HMGN) family members are nucleosome-binding proteins that affect chromatin structure and modulate the fidelity of the cellular transcription profile. HMGN variants are expressed in most brain regions; however, their effect on the cellular transcription profile and on neurological functions is not known. Here, we study the role of this protein family in in the hippocampus (HP) and the prefrontal cortex (PFC)regions,by transcription profiling of genetically altered mice that either lack, or overexpress functional HMGN variants. Transcription profiles of 3 months-old wild-type (WT), HMGN1/2 double knockout (HMGN1/2 DKO), and HMGN1 overexpressing (HMGN1 OE) mice were analyzed by paired-end, deep RNA-sequencing, in triplicates, using IlluminaGAIIx. The sequence reads that passed quality filters were analyzed at the transcript isoform level with ANOVA and TopHat followed by Cufflinks. Approximately 100 of the transcripts showed differential expression in the WT vs. HMGN1/2 DKO and WT vs. HMGN1 OE, both in the hippocampus (HP) and the prefrontal cortex (PFC), with a fold change ≥ 2 and p-value <0.05. Functional annotation analysis revealed that loss or overexpression of HMGNs result in deregulation of genes involved in neurological system processes such as perception and cognition in the PFC while in the HP transcription of genes associated with neuronal signaling, RNA metabolic processes, transcription regulator activity, apoptosis, cell death, and cell homeostasis was affected. Thus, epigenetic factors such as HMGNs could affect the proper functioning of CNS by modulating the transcriptional profiles in adult brain. This preliminary study posits a potential role of HMGNs in neuropsychiatric disorders, and suggests targets for hypothesis generation on the role of HMGNs in adult brain.

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Poster

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Topic: G.02. Genomics, Proteomics, and Systems Biology

Support: EY021716

Title: Molecular analysis of retinal neuronal mitochondria: Genome copy number and heteroplasmy

Authors: *D. R. MASSER[1], D. STANFORD[2], B. WRONOWSKI[2], W. M. FREEMAN[2]; [1]Physiol., The Univ. of Oklahoma Hlth. Sci. Ctr., Oklahoma City, OK; [2]Univ. of Oklahoma Hlth. Sci. Ctr., Oklahoma City, OK

Abstract: Diabetic retinopathy (DR), a secondary complication of metabolic dysregulation, remains the leading cause of blindness among working age adults. Although the pathophysiology of DR is not known, early deficits in retinal neuronal structure, function and gene expression occur in diabetic patients and these deficits have been recapitulated in experimental rat models. Mitochondria, the cellular organelle responsible for ATP generation through oxidative phosphorylation as well as calcium regulation, are implicated in neural deficiencies with DR in the retina. Mitochondria contain multiple circular 16.5 kb genomes (mtDNA), which encode necessary subunits of the respiratory chain. Maintenance of the mtDNA is important for mitochondrial health and function. We hypothesize that mtDNA copy number and maintenance are decreased with DR in retinal neuronal cells. In order to test this hypothesis, we have developed assays to quantify mtDNA copy numbers and assess mtDNA mutations and deletions, or degree of heteroplasmy, in retinal tissue from rat animal models. Absolute mtDNA copy number quantitation was achieved by using digital PCR (dPCR) with multiplexed custom TaqMan probe sets specific for mtDNA and nuclear DNA. Heteroplasmy was measured through mtDNA enrichment, rapid transposome-mediated library generation and next-generation sequencing. Sequencing reads were aligned to a mtDNA reference genome, and probabilistic variant detection and sliding window read depth analysis were carried out to identify mutations and deletions, respectively. We have applied these assays to retinal mtDNA isolated from the neural retina of non-diabetic Sprague-Dawley (SD) rats streptozotocin-induced diabetic SD rats, to test the effect of diabetes on mtDNA maintenance in the retina. Additionally, diabetic rats with systemic insulin treatment groups of differing durations were assessed to determine the effects of insulin replacement of mtDNA maintenance. These methods provide a powerful assessment of mtDNA maintenance in neuronal cell populations, which can be applied to any tissue or cell type, with metabolic dysregulation seen in diabetes.

Disclosures: **D.R. Masser:** None. **D. Stanford:** None. **B. Wronowski:** None. **W.M. Freeman:** None.

Poster

096. Genomics, Proteomics, and Systems Biology

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 96.26/TT32

Topic: G.02. Genomics, Proteomics, and Systems Biology

Support: IUCAM MEXT

Title: Three-dimensional (3-D) behavior analysis for mice using the commercial depth sensor

Authors: *A. TOYODA, T. GOTO, T. OKAYAMA;
Ibaraki Univ., Inashiki Ibaraki, Japan

Abstract: Behavioral tests of animals have been widely used for various studies of life sciences. Animal behaviors are often observed and evaluated by human eyes. Because the observations and assessments by humans are practically subjective and time consuming, many automated analysis systems for animal behaviors, especially for mouse behaviors have been developed and used for researches. Automated systems mainly use two-dimensional (2-D) images, while evaluating 2-D information has some difficulties in 3-D behaviors in mice. Therefore, we have been developing the analysis system for mouse behaviors using the commercial 3-D depth sensor, and will report the results of behavioral analysis for nest building and social interaction in mice at this annual meeting. Seven-week-old male C57BL/6J (B6) and retired ICR were used in this study. Mice were housed at room temperature (22 ± 1 °C) with exposure to light from 7:00 to 19:00 and ad libitum access to food and water. A cage was made with acrylic transparent boards which transmit near infrared light. A 3-D depth sensor (Xtion LIVE PRO, ASUS) was placed above the cage, and mouse behaviors were measured by the sensor following the data analyzes. Nest building and resident intruder tests were performed as the previous reports (Nature Protocols, 2006, Nature Neuroscience, 2006). All the experimental procedures followed the guidelines of the Animal Care and Use Committee of Ibaraki University. In the nest building test, we could observe the building processes of the nest using the 3-D depth sensor and finally evaluate the volume and shape of the nest. In the resident intruder test, B6 were repeatedly attacked by ICR and finally showed submissive postures as described previously. Using the depth sensor, we could evaluate and analyze the counts of biting and jumping behaviors in the tests. In the future, we try to apply this analysis system to other behaviors in mice and those in larger animals including farm animals.

Disclosures: **A. Toyoda:** None. **T. Goto:** None. **T. Okayama:** None.

Poster

096. Genomics, Proteomics, and Systems Biology

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 96.27/TT33

Topic: G.02. Genomics, Proteomics, and Systems Biology

Title: The calcium-activated chloride channel ANO2 as an autoimmunity target in multiple sclerosis

Authors: *P. NILSSON[1], B. AYOGLU[2], A. HÄGGMARK[2], J. M. SCHWENK[2], M. UHLÉN[2], N. MITSIOS[3], J. MULDER[3], L. ALFREDSSON[4], I. SKELTON KOCKUM[4], M. KHADEMI[4], T. OLSSON[4];
[2]SciLifeLab, [1]KTH - Royal Inst. of Technol., Solna, Sweden; [3]SciLifeLab, [4]Dept. of Clin. Neurosci., Karolinska Institutet, Stockholm, Sweden

Abstract: Affinity proteomic strategies for broad and unbiased exploration of the autoimmunity repertoire hold a great promise towards identification and confirmation of novel antigenic targets in multiple sclerosis (MS). We have previously profiled IgG reactivity in a MS-related plasma sample cohort on protein microarrays with 11,520 protein fragments (representing 7,500 unique human proteins), which were generated within the Human Protein Atlas. Out of these, 51 were recognized differentially across the MS subtypes and non-diseased controls (Ayoglu B. et al., Mol Cell Proteomics, 2013). Here, we aimed to verify the significance of reactivities against these 51 targets, as well as other targets suggested from literature (e.g. KIR4.1), using plasma samples from a larger and independent cohort. Following the use of planar protein microarrays for the initial discovery stage, we here utilized suspension bead arrays for a multiplex profiling of IgG reactivity in 2,210 plasma samples against a set of 384 antigens while consuming less than a microliter of neat sample per analysis. Comparison of IgG reactivity in plasma from 1,106 MS patients and 1,104 non-diseased controls revealed anoctamin 2 (ANO2), a calcium-activated chloride channel also known as transmembrane protein 16B (TMEM16B), as a strong autoimmune target, which originated from our unbiased discovery approach. A statistically significant difference in positive reactivity between MS cases and non-diseased controls was found in particular for the intracellular N-terminal region of ANO2 (FDR-adjusted Fisher´s exact p-value=2x10-21). Further studies with available ANO2 antibodies in human post-mortem MS brain tissue revealed moderate staining in neuronal cell bodies and more prominent staining in reactive astrocyte-like structures from various parts of the tissue. A proteomic-based screening approach facilitated the discovery of anoctamin 2 (ANO2) as a novel autoimmune target candidate in multiple sclerosis, which was here verified in an independent set of 2,210 plasma samples. Subsequent efforts will reveal the contribution of the identified target to disease pathogenesis.

Disclosures: **P. Nilsson:** None. **B. Ayoglu:** None. **A. Häggmark:** None. **J.M. Schwenk:** None. **M. Uhlén:** None. **I. Skelton Kockum:** None. **T. Olsson:** None. **M. Khademi:** None. **L. Alfredsson:** None. **N. Mitsios:** None. **J. Mulder:** None.

Poster

096. Genomics, Proteomics, and Systems Biology

Location: Halls A-C

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Program#/Poster#: 96.28/TT34

Topic: G.02. Genomics, Proteomics, and Systems Biology

Support: NIH Grant R01EY022306

NIH Grant RC2EY020678

Title: Screening gene variants for disruption of microRNA binding activity and application to optic nerve degeneration in glaucoma

Authors: *A. N. DUBINSKY[1], A. R. LA SPADA[2,3], T. GAASTERLAND[4,5,6];
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Abstract: We aim to identify microRNA activity in glaucomatous optic nerves that may confer risk or progression in glaucoma, the second leading cause of irreversible blindness affecting over 60 million people worldwide. Primary open angle glaucoma (POAG) is the most common subtype of glaucoma and has complex genetics. Visual field loss is due to loss of axons in the optic nerve through apoptosis of retinal ganglion cells. Several genes were connected to glaucoma through genome-wide association, linkage and family studies. Our exome sequencing of 295 POAG patients identified potential causal variants in 90 genes, including 12 with variant sites in 3' untranslated regions (UTR). Such variants may significantly alter binding sites for microRNAs. Technologies are needed for accurate and efficient screening of microRNA binding site disruption by genome variants, and for tissue-specific microRNA regulation of genes implicated in disease through exome sequencing. Available algorithms to computationally predict binding sites have limitations that hinder their application to assess binding site differences due to genome variation. Existing binding site predictions require substantial computation and, in some methods, simulation of binding to generate predictive scores. For example, many databases that serve computed sites neglect the second arm of microRNA hairpins, which have been observed to be loaded preferentially into the microRNA RISC complex under tissue- or time-specific conditions. To address this shortcoming and analyze microRNA effects in glaucoma, we developed a novel, efficient process called ZoomMiR which allows detection of seed sites and ranks microRNAs for experimental screens. To validate

ZoomMiR output, we developed a novel method to assay disrupted seed sites in a reporter gene system. This dual luciferase reporter system permits the quantitation of binding of, and hence regulation by, a computationally ranked miRNA. We applied this method to variations found in POAG patients. Importantly, we identified a 3'UTR glaucoma risk SNP in CDKN2B and demonstrated it disrupted binding of a specific miRNA. Further, next-gen sequencing of microRNA from optic nerve and retina identified optic nerve-specific microRNA which ZoomMiR predicts bind several of the 90 genes associated with POAG through our exome sequencing. Together, ZoomMiR and our novel experimental approach provide critical screening functionality to identify genome variants that disrupt microRNA binding sites. We used the system to establish a molecular mechanism for a glaucoma risk SNP found through GWAS.

Disclosures: **A.N. Dubinsky:** None. **A.R. La Spada:** None. **T. Gaasterland:** None.

Poster

096. Genomics, Proteomics, and Systems Biology

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 96.29/TT35

Topic: G.02. Genomics, Proteomics, and Systems Biology

Title: Transcriptional profiling of *Drosophila* optic lobe neurons

Authors: *K. KAPURALIN[1], M. WERNET[1], C. DESPLAN[1,2], A. DEL VALLE RODRIGUEZ[1];
[1]New York Univ. Abu Dhabi, Abu Dhabi, United Arab Emirates; [2]Dept. of Biol., New York Univ., New York, NY

Abstract: The optic lobe development and function depends on the precise regulation of gene expression. However, our understanding of the complexity and dynamics of the transcriptome of neurons in the *Drosophila* brain is very limited. The main aim of our project is to generate a transcriptional profile for *Drosophila*'s optic lobe neurons and to correlate this transcriptome with their different defining anatomical and functional characteristics. Next generation RNA sequencing technology will allow us systematic characterization of the complete gene-expression profile of individual neuron types. Applying this technique to specific cell types requires a method for the isolation of specific subpopulations of cells, a laser based flow cytometry fluorescence-activated cell sorting. We have been screening unique database of *Drosophila* mutants in which transcriptional activator GAL4 is placed under the control of endogenous regulatory elements. Each GAL4 line is crossed to a UAS-GFP reporter line placing the

expression of GFP under the positive control of GAL4 in the progenies. Analysis of whole mount brain preparations is used for identifying and characterizing the morphology of neuronal populations. Flip-out technique followed by neuroimaging technique of confocal microscopy is used to confirm the single-cell specificity of GFP expression in the cell types of choice. GAL4 driver lines restricted to one neuron type in a specific neuropil of the optic lobe are selected for further studies. Adult optic lobes from those animals are dissected, tissue is dissociated into single cell suspension and individual neuronal types are separated according to their differences in GFP intensity and cell size by FACS sorting. Cell populations that are over 98% pure are further used for gene expression analysis. Transcriptional profiling of isolated single-cell types has the power to identify network of genes, neurotransmitters, receptors, peptides and transcription factors expressed by the individual neurons and to characterize neuronal diversity respectively. By combining molecular biology screening techniques with computational methods, we expect to uncover genetic programs responsible for controlling a neuron location, shape, synaptic connections and neurotransmitter synthesis. Understanding the dynamics of transcriptome is essential for studying the complexity of transcriptional regulation and its impact on phenotype and regulatory mechanisms that control differentiation.

Disclosures: **K. Kapuralin:** None. **M. Wernet:** None. **C. Desplan:** None. **A. del Valle Rodriguez:** None.

Poster

097. Imaging Advances: Cell Biology

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 97.01/TT36

Topic: G.03. Staining, Tracing, and Imaging Techniques

Support: NSF 1040462 MRI

NSF 08-46660 CAREER

NSF 0939511 EBICS

Title: Label-free 3D imaging of live neurons

Authors: ***G. POPESCU**[1], T. KIM[2], C. LIU[2], M. U. GILLETTE[2];
[1]Univ. of Illinois At Urbana-Champaign, Urbana, IL; [2]Univ. of Illinois at Urbana-Champaign, Urbana, IL

Abstract: Many of traditional investigations on neuronal culture are based on imaging methods, such as phase contrast of fluorescence microscopy, which are limited to 2D plane measurements. However, in reality, neurons from a 3D network, which eventually run throughout the entire living organism. Therefore, it is very important to investigate neurons using 3D imaging modalities. With the advances in biochemical markers and laser technology, fluorescence confocal microscopy has become one of the main methods to investigate live cells or a system of cells, such as embryonic body, in 3D with a high resolution. However, fluorescence confocal microscopy requires fluorescence markers that ultimately modify the live samples. Furthermore, the high-power laser illumination of the imaging system causes photo-toxicity which adversely affects live cells. Therefore, this 3D imaging technique is not optimal for live cell imaging in 3D. White-light diffraction tomography (WDT) is a recently developed tomographic imaging technique based on quantitative phase imaging (QPI) (Kim et al., Nat Photon, 8, 2014). This technique is based on a commercial phase contrast microscope upgraded with a QPI module, therefore, uses low-irradiance white-light illumination and also the environmental control to keep the specimen unaffected by the external stress. Based on a QPI method, this technique provides a 3D map of the refractive index distribution of the sample, which relates to the 3D distribution of the non-aqueous content within the cell. The sub-micron resolution of this technique is suitable for single cell imaging and the sub-cellular structures can be resolved in all three dimensions. Using this technique, both hippocampal neurons and dorsal root ganglion (DRG) neurons have been imaged in 3D. Throughout all of the imaging process, the neuronal samples are kept unmodified and live. Structures, such as nucleoli and nuclear membrane, within the cell body have been resolved in 3D. Furthermore, the 3D structure of neuronal processes has also been imaged. Dendritic connections made between two neurons have also been imaged. These results reveal that these connections are in fact made in 3D, connecting neurons at different heights from the bottom of the culture dish. Further optimization of this technique can reveal new and unique insights into the dynamics of the formation of neuronal circuits.

Disclosures: **G. Popescu:** None. **T. Kim:** None. **C. Liu:** None. **M.U. Gillette:** None.

Poster

097. Imaging Advances: Cell Biology

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 97.02/TT37

Topic: G.03. Staining, Tracing, and Imaging Techniques

Title: Imaging Applications to Study Cells *in vivo* and *in vitro*

Authors: T. HUHTALA[1], V. JANKOVIC[2], *T. LAITINEN[1], K. LEHTIMÄKI[1], U. HERZBERG[2], A. NURMI[1];
[1]Charles River Discovery Res. Services Finland, Kuopio, Finland; [2]Celgene Cell. Therapeut., Warren, NJ

Abstract: Different cell types are currently under evaluation to be used as a novel methodology to treat various diseases and acute injuries. Before proceeding to clinical studies with cell administrations to patients, several properties need to be studied *in vivo* and *in vitro*. Imaging modalities like SPECT/CT and MRI offer effective tools to study e.g. ADME properties or local concentration of cells *in vivo*. Due to physical properties of these imaging technologies, SPECT/CT is suitable for ADME studies since whole-body imaging can be easily performed in rodents using small animal scanners. Also, high sensitivity of radiolabeled cells makes it optimal methodology to compare cell distribution from various areas and excretion from several time points within an individual. Also, possible effects of administration route can be easily compared. However, the anatomical resolution in SPECT is not optimal and differs from MRI's resolution, which especially in soft tissue is excellent. This makes MRI an effective tool for more detailed study e.g. cell concentration in specific tissue or organ. However, to track cells with MRI, cells need to be labeled for visualization. In our studies we have used Indium-oxine (^{111}In-ox) for SPECT/CT and ultrasmall superparamagnetic iron oxide (USPIO) in MRI to track and quantify cells *in vivo* and *in vitro*. Labeling efficiency with In-ox is high (ca. 80 %) with no effect on cell viability. After labeling, distribution of cells has been studied from various time points using SPECT/CT. More precise quantification of ^{111}In-ox labeled cells can be done using gamma counter analysis *post mortem*. Since the number of administered cells and total injected dose are known at the time of injection, results can be converted to cell number in organs at the time of termination. To measure sensitivity of MRI and the lowest number of detected cells, a titration curve of cells internalized with USPIO was generated using absolute T2 weighted MRI. Briefly, a titration curve consisting of 0 - 1e^6 cells in Matrigel was generated to determine the smallest measurable number of cells *in vitro*. In summary, current imaging modalities such as SPECT/CT and MRI offer extensive tools to study various properties of cells *in vivo* and *in vitro*.

Disclosures: **T. Huhtala:** None. **T. Laitinen:** None. **K. Lehtimäki:** None. **A. Nurmi:** None. **V. Jankovic:** None. **U. Herzberg:** None.

Poster

097. Imaging Advances: Cell Biology

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 97.03/TT38

Topic: G.03. Staining, Tracing, and Imaging Techniques

Support: NIH Grant ES012241

Title: Exposures to cholinesterase inhibitors leads to persistent impairments of axonal transport *in vitro*

Authors: *J. GAO[1], J. MAGRANE[2], C. HERNANDEZ[1], A. TERRY[1];
[1]Pharmacol. and Toxicology, Georgia Regents Univ., Augusta, GA; [2]Dept. of Neurol. and Neurosci., Weill Med. Col. of Cornell Univ., New York, NY

Abstract: Axonal transport is a critical process in neurons that is responsible for the movement of mitochondria, lipids, synaptic vesicles, receptors and other key macromolecules to and from the neuron's cell body. It plays an essential role in neuronal function, neuronal maintenance and integrity, as well as synaptic transmission. Impairment of axonal transport has recently emerged as a common feature in several neurodegenerative disorders such as Alzheimer's disease, Parkinson's disease and Huntington's disease, and there is now substantial evidence that exposures to environmental toxicants may be a contributing factor. Previous studies in our laboratory indicate that organophosphate (OP)-based cholinesterase inhibitors can lead to protracted deficits in cognition in rodent models and that impairments in axonal transport (as indicated by deficits of vesicle movements in peripheral nerves *ex vivo*, mitochondrial movements in primary neuronal cultures *in vitro*, etc.), may represent a potential underlying mechanism of the cognitive dysfunction. In order to evaluate the effects of OP exposure on fast axonal transport of membrane bound organelles (MBOs), we transfected the amyloid precursor protein that has been tagged with a fluorescent protein Dendra (APPdendra) into primary cortical neurons and then treated them with the (OP)-based cholinesterase inhibitor, diisopropyl fluorophosphate (DFP). The percentage of stationary and mobile (anterograde and retrograde) MBOs and their velocities (μm/sec) was determined by a time-lapse imaging technique. Compared to vehicle-treated control axons (where the majority of APP movement was in the anterograde direction) DFP exposure resulted in more retrograde movements, a decrease in velocity, and a significant increase in the number of stationary MBOs. These preliminary results indicate that DFP can alter the fast axonal transport of MBOs that contain APP and provide further evidence that OPs can impair the neuronal trafficking of important macromolecules.

Disclosures: **J. Gao:** None. **J. Magrane:** None. **C. Hernandez:** None. **A. Terry:** None.

Poster

097. Imaging Advances: Cell Biology

Location: Halls A-C

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Topic: G.03. Staining, Tracing, and Imaging Techniques

Support: NIH Grant ES012241

DOD Grant W81XWH-12-1-0536

Title: Repeated exposures to cholinesterase inhibitors leads to persistent impairments of axonal transport *in vivo*

Authors: *C. M. HERNANDEZ[1], W. D. BECK[1], S. X. NAUGHTON[1], I. PODDAR[1], B.-L. ADAM[1], N. YANASAK[2], C. MIDDLETON[3], A. V. TERRY, Jr.[1];
[1]Pharmacol. and Toxicology, [2]Radiology, [3]Cancer Res. Ctr., Georgia Regents Univ., Augusta, GA

Abstract: Axonal transport is a fundamental process in neurons that is responsible for the movement of important macromolecules to and from a neuron's cell body through the cytoplasm of its axon. There is increasing evidence that impairments in axonal transport may contribute to the pathology of some neurological illnesses (e.g., Alzheimer's disease) especially those where cognitive function is impaired, and further, that exposures to environmental toxicants may be a contributing factor. The results of our animal experiments to date with the insecticide, chlorpyrifos and the nerve agent, diisopropylfluorophosphate, have established that subthreshold exposures (operationally defined as doses not associated with acute toxicity) to organophosphate (OP)-based cholinesterase inhibitors can lead to protracted deficits in cognition and that impairments in axonal transport may represent a potential underlying mechanism, as determined in primary neuronal cultures (*in vitro*), peripheral nerve preparations (*ex vivo*) and brain. The objective of the work described here is continue this line of research to evaluate the effects of subthreshold OP exposures on axonal transport *in vivo* (i.e., in the living mammalian brain). We took advantage of manganese (Mn)-enhanced magnetic resonance imaging (MEMRI) to image and tract-trace Mn-enhancement/s along a neural pathway connecting the retina to the superior colliculus and lateral geniculate nucleus. We hypothesized that exposing animals to the same OP-treatment regimens previously associated with cognitive dysfunction would also result in impaired Mn transport in brain. Adult male Wistar rats were administered a single, unilateral Mn chloride injection (intravitreal, 40 micromole/rat) then MEMRI was conducted 6 and 24 hr later in order to compare the Mn-enhancement between eye and optic nerve projections. OPs were administered subcutaneously (sc) and subjects were divided into two groups based on exposure period. In the acute exposure group, animals were co-administered OP (sc) and Mn (intravitreal). In the chronic exposure group, animals were administered OP (sc) for 14 consecutive days. To

detect persistent axonal transport disruptions in the chronic exposure groups, MEMRIs were acquired for each animal at baseline (pre-OP exposure) and compared to a short (24-hr) and extended (30-day) washout period timed from the last injection. To date, using the same dosing paradigm previously associated with cognitive dysfunction, our data indicate that axonal transport is impaired by OPs in the living rodent brain in a dose-dependent manner and that these impairments can persist up to 30 days after washout.

Disclosures: **C.M. Hernandez:** None. **W.D. Beck:** None. **S.X. Naughton:** None. **I. Poddar:** None. **B. Adam:** None. **N. Yanasak:** None. **C. Middleton:** None. **A.V. Terry:** None.

Poster

097. Imaging Advances: Cell Biology

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 97.05/TT40

Topic: G.03. Staining, Tracing, and Imaging Techniques

Title: A multicompartment microfluidic culture system for extended long-term fluidic isolation and study of axonal neurobiology

Authors: *T. SARMA, H. H. CAICEDO, G. PIGINO, S. BRADY;
Univ. Illinois Chicago, CHICAGO, IL

Abstract: Current *in vitro* compartmentalized culture methods for studying central nervous system axonal biology preclude relevant biological experimentation. We have developed and optimized an open-top multicompartmentalized microfluidic neuronal culture chamber that not only allows isolation of different neuronal subcellular compartments (i.e., either somatodendritic or axonal domains) into separate and precisely defined biochemical microenvironments but also allows extended long-term fluidic isolation between different compartments, efficient on-chip immunocytochemistry, in-situ cell transfection and optimum live cell imaging studies primary neurons and axons. Additionally, to allow targeting of proximal axons without affecting cell bodies, we engineered a central channel to facilitate fluid flow without the need of external pumps and tubing. We used our culture platform to study trafficking of p38-mCherry expressing-synaptic vesicles by fast axonal transport in isolated axons, using genetic and pharmacological manipulations. The results presented here document an optimized compartmentalized culture system that facilitates relevant biological experimentation for studying CNS neurons and subcellular compartments.

Disclosures: **T. Sarma:** None. **H.H. Caicedo:** None. **G. Pigino:** None. **S. Brady:** None.

Poster

097. Imaging Advances: Cell Biology

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Program#/Poster#: 97.06/TT41

Topic: G.03. Staining, Tracing, and Imaging Techniques

Support: JST PRESTO

KAKENHI 23111532

KAKENHI 24650163

Title: Riesz transform-assisted differential interference contrast imaging: Application to three-dimensional kinematic analysis of the growth cone motility

Authors: *A. TAMADA[1,2,3], M. IGARASHI[1,2];
[1]Niigata Univ. Grad. Sch. of Med. and Dent. Sci., Niigata, Japan; [2]Ctr. for Transdisciplinary Research, Niigata Univ., Niigata, Japan; [3]PRESTO, Japan Sci. and Technol. Agency, Kawaguchi, Japan

Abstract: Differential interference contrast (DIC) microscopy has been frequently used for the high-contrast live-cell imaging without phototoxic fluorescent labeling. However, the shadow-cast appearance of DIC images along the shear axis has hampered three-dimensional (3D) visualization by the reconstruction of z-scanned images and an application of quantitative intensity-based image processing. To overcome these defects, we devised a novel technique that converts the non-linear shadow-cast DIC images into the linearized self-luminous images using the Riesz transform, known as a multidimensional extension of the one-dimensional Hilbert transform. The transform is a combination of the first-order Riesz transform along the shear axis that shifts the phase by 90 degrees, and the second-order transform along the orthogonal axis that reverses the phase. This transform succeeded in efficiently removing the shadow of DIC images and converting them to the images whose intensity reflected the optical depth of objects. We applied this novel Riesz transform-assisted DIC (RT-DIC) imaging technique to the visualization and quantitative analysis of the 3D growth cone motility in 3D collagen gel cultures. Reconstruction of the time-lapsed 3D RT-DIC images revealed a complex 3D motility of filopodia. These filopodia exhibit overall retraction, tip extension and right-screw rotation

(Tamada et al., J Cell Biol 188, 429 [‘10]), which were driven by the retrograde actin flow, actin polymerization and left helical actomyosin interaction, respectively. We further developed the automated, mathematical methods for analyzing the growth cone motility and structure. The structural feature was estimated by “the structure tensor”, which is defined as the second-moment matrix of image gradients. The filopodia were visualized as fiber-like structures estimated by the fractional anisotropy, calculated from the eigenvalues of the structure tensor. The direction of filopodia was estimated by the minor eigenvector. Motion parameters including acceleration, jerk and angular velocity were calculated from the velocity by the Frenet-Serret formulas. We concluded that we succeeded in full kinematic description of growth cone motility using these 3D RT-DIC images, suggesting that the methods described here will provide a precise kinematic analysis with a conventional label-free DIC microscopy.

Disclosures: **A. Tamada:** None. **M. Igarashi:** None.

Poster

097. Imaging Advances: Cell Biology

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Topic: G.03. Staining, Tracing, and Imaging Techniques

Support: NSF SBIR grant IIP-1248820

Title: Gfp+ human stem cell derived neurons amenable to high content neurite outgrowth assays

Authors: J. LE[1], *S. L. STICE[2];
[1]ArunA Biomed. Inc., ATHENS, GA; [2]Dept Animal & Dairy Sci., Univ. Georgia, ATHENS, GA

Abstract: Adverse outcome pathways (AOP) are conceptual frameworks that portray existing knowledge concerning the linkage between a molecular initiating event and an adverse outcome. High throughput screening (HTS) and high content screen (HCS) approaches seek to gain a greater perspective of the AOP, leading to more efficient and reliable identification of adverse outcomes of compounds and chemicals. However, HCS assays often utilize primary or stem cell sources which are not amenable to large scale screening and can require extensive cell culture, fixation and permeation, and immunocyotochemistry prior to imaging. Thus, major costs are associated with 96-well and 384-well based high content neurotoxin screening. In addition, preserving or fixative steps can alter cell architecture and a new plate of cells is required for each time point, thus introducing variability to data. These GFP+ human stem cell derived neurons

were developed to provide a scalable solution to the previously labor intensive, single-end-point, and hence limited nature of neurotox assays, while preserving the breadth and quality of data. These neurons are differentiated from embryonic stem cell derived human neural progenitors that have been genetically modified with a non-viral vector encoding a Green Fluorescent Protein (GFP) reporter gene driven by a ubiquitous promoter. A method was developed to demonstrate their ability to form clonal colonies capable of proliferation and neural differentiation. Our previous hN2™ human neuronal cells faithfully reproduced early brain development, providing a cell model that is > 90% positive for β-III tubulin and >60% positive for MAP2. A new differentiation method has been developed which increases MAP2 expression to >90%, resulting in more mature neurons that exhibit significantly longer neurite length post-thaw. These GFP+ neurons maintain an adherent monolayer that is amenable to both high content imaging as well as high throughput screening. They have successfully been used in a neurite outgrowth assay to measure the effects of the neurotoxin bisindolylmaleimide 1 on neurite length. Our previous hN2™ cells demonstrated higher sensitivity to neural toxins than mouse cortical neurons, and our new GFP+ neurons are equally sensitive. By eliminating the need for fixing and staining cells, these GFP+ human neurons provide a scalable means to analyze neurite outgrowth in live cells spanning the course of hours to days following exposure to test compounds.

Disclosures: **J. Le:** A. Employment/Salary (full or part-time):; Employee- ArunA Biomedical Inc.. 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.; NSF SBIR Grant IIP-1248820. **S.L. Stice:** A. Employment/Salary (full or part-time):; ArunA Biomedical Inc.. 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.; NSF SBIR IIP-1248820.

Poster

097. Imaging Advances: Cell Biology

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 97.08/TT43

Topic: G.03. Staining, Tracing, and Imaging Techniques

Support: Medical Research Council funded PhD (ASD)

Wellcome Trust Career Development Fellowship (MSG)

Title: Live imaging of the axon initial segment

Authors: *A. S. DUMITRESCU, M. P. MEYER, M. S. GRUBB;
Kings Col. London, London, United Kingdom

Abstract: The axon initial segment (AIS) is a specialised neuronal compartment involved in the maintenance of axo-dendritic polarity and in the generation of action potentials. Recent work has uncovered a great deal concerning the molecular composition of the AIS and its development. The AIS has also been shown to be a novel site for neuronal plasticity, as changes in ongoing activity levels *in vitro* and *in vivo* result in significant alterations in the structure's position or size. However, all findings described so far have been based on experiments carried out on fixed samples offering only a snapshot view. We are now pursuing live imaging experiments in order to follow the AIS as it undergoes plastic changes in real time, making use of two systems: (1) *in vitro* rat hippocampal dissociated cultures and (2) *in vivo* zebrafish. For the *in vitro* part of the project we have successfully live-labelled AISs with two separate plasmid constructs: a fusion protein of 270kD ankyrinG and GFP (AnkG-GFP), and a YFP-tagged AIS targeting sequence used by voltage-gated sodium channels (NavII-III-YFP). We have used them to follow rapid structural changes at the AIS in cultured dentate granule cells (DGCs), which undergo activity-dependent AIS shortening when chronically depolarised for only a few hours. The larval zebrafish is our model of choice for AIS *in vivo* imaging; however at the current time there is almost nothing known regarding the AIS in this animal model. We have started to address this significant gap in knowledge by developing tools to visualise the AIS in both fixed samples and live larvae. In an initial set of experiments on 5-7dpf zebrafish wholemount fixed tissue, we labelled individual AIS-like structures in retinal ganglion cells (RGCs), tectal and cerebellar neurons using antibodies against voltage gated sodium channels (Pan-Nav), the cell adhesion molecule neurofascin, and a custom-made zebrafish-specific antibody against AnkG. In parallel we have started to create zebrafish-specific fluorescently-tagged AIS live imaging probes. We are now testing the expression pattern of two constructs for this purpose: GFP-tagged fusion proteins of a beta sodium channel subunit (Navβ4-GFP) and neurofascin (NF-GFP). Once able to image AISs in both fixed and live zebrafish, we will exploit the power of this model organism to watch the structure develop and adapt, live and *in vivo*, during controlled manipulations of sensory experience.

Disclosures: **A.S. Dumitrescu:** None. **M.S. Grubb:** None. **M.P. Meyer:** None.

Poster

097. Imaging Advances: Cell Biology

Location: Halls A-C

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Topic: G.03. Staining, Tracing, and Imaging Techniques

Support: NIH Grant R01 NS062842

NIH Grant R21 NS064403

NIH Grant R21 EB006301

Title: Thermal noise driven bio-nanomachine distinguishes between apoptotic and necrotic cell engulfment by macrophages

Authors: *V. V. DIDENKO, C. L. MINCHEW;
Neurosurg., Baylor Col. of Med., HOUSTON, TX

Abstract: We describe a new type of bio-nanomachine which runs on thermal noise. The machine is solely powered by the random motion of water molecules in its environment. The construct can rapidly detect DNA damage produced during phagocytic engulfment of dying cells in cell suspensions. Such suspensions, ranging from cultured cells to clinical biopsy specimens and blood test samples, are widely used in cell biology and biomedical research. The construct distinguishes the macrophages engulfing apoptotic cells from the macrophages digesting necrotic cells. The reaction takes 3 minutes. The positive detection is signaled by fluorescence at 525 nm. By being both a nanodevice and a biological probe, the new construct bridges the fields of bio-nanotechnology and cell biology. It can be useful in cell death studies as a real-time sensor. Analysis of its operation principles can be instrumental for the development of future molecular-scale appliances for biomedicine.

Disclosures: **V.V. Didenko:** None. **C.L. Minchew:** None.

Poster

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Program#/Poster#: 97.10/TT45

Topic: G.03. Staining, Tracing, and Imaging Techniques

Support: Intramural program at NIDA/NIH

Title: Neuronal phagocytosis in SH-SY5Y cell culture and in rat brain

Authors: *V. WALLACE, L. FORTUNO, J. HINKLE, B. WARREN, R. MADANGOPAL, M. HENDERSON, B. HARVEY, B. HOPE;
NIH/NIDA, Baltimore, MD

Abstract: Endocytosis plays a central role in regulating intracellular homeostasis and extracellular interactions in the brain and includes the uptake of material into cells via pinocytosis (particles smaller than 0.5um) and phagocytosis (particles larger than 0.5um). While microglia are generally considered the resident phagocytes of the central nervous system, neurons can also participate in phagocytosis. Yet little is known about the phagocytic capacity of neurons and the role of neuronal phagocytosis in both normal neuron function and pathological states. Our objective is to assess the phagocytic capacity of neurons in cell culture and in brains of behaving rats. *In vitro* experimentation will elucidate the effect of particle concentration on passive endocytosis, cell viability, and intracellular particle localization. We are currently investigating the capacity of undifferentiated SH-SY5Y neuroblastoma cells to endocytose large particles (0.5-1.5um fluorescent microspheres) with different surface charges (positive, negative, and neutral). Fluorescence and confocal microscopy are used to assess particle uptake and cell viability. Subcellular distribution of particles in SH-SY5Y cells will be examined by immunolabeling cellular organelles. We are also undertaking *in vivo* studies to assess the extent of neuronal phagocytosis in different brain regions. Rats will be injected with different concentrations of positive, negative and neutral fluorescent microspheres, and brains will be examined at different time points following injections. We will use histochemistry of formaldehyde-fixed tissue to assess uptake into different cell types, including neurons, microglia, astrocytes, and oligodendrocytes. Fluorescently labeled cells will be further analyzed by flow cytometry to quantitate particle uptake into the various cell types. We hypothesize that different neuronal cell types in the highly heterogeneous brain will exhibit different capacities for phagocytosis.

Disclosures: V. Wallace: None. **L. Fortuno:** None. **J. Hinkle:** None. **B. Warren:** None. **R. Madangopal:** None. **M. Henderson:** None. **B. Harvey:** None. **B. Hope:** None.

Poster

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Support: DFG Grant SFB 1089-B2

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BonnFOR Instrument1

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DFG Grant DI853-3

Title: Minimally disruptive quantification of endogenous calcium binding using fluorescent lifetime imaging (FLIM)

Authors: *E. A. MATTHEWS, D. DIETRICH;
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Abstract: While whole-cell patch clamp recordings have provided a wealth of information about the activity and function of neurons, one draw-back of the method is the dialysis of cellular components during the recording period. When using a patch pipette to infuse calcium dyes into the cell, endogenous calcium binding proteins - such as Calbindin - are at least partially removed, thus perturbing the natural calcium binding environment by adding an exogenous binding species while simultaneously removing or diluting the endogenous species. This problem can be mitigated by single cell electroporation of calcium dyes; however, with electroporation the exact concentration of added dye is not exactly known, which may limit the precise quantitative analysis of the resulting calcium transients. We have developed a 2-P laser scanning based approach to derive highly precise quantitative information about the resting calcium, activity induced increases in free calcium, and dye concentration using time correlated single-photon counting fluorescent lifetime imaging (FLIM) and single cell electroporation in dentate granule (DG) cells. Two calibrations are required for this approach: 1) calibration of the fast and slow fluorescent lifetime decay constants with free calcium concentration, and 2) calibration of the dye concentration in the cell based on a bead ratio. For the first calibration, we performed an *in vitro* determination of the effective K_d (151.8 nM), and the fast and slow fluorescent lifetime decay time constants of OGB-1 ($\tau_{fast} = 3.08 \times 10^{-10}$s; $\tau_{slow} = 2.83 \times 10^{-9}$s). For the second calibration, we lowered a pipette containing a standardized fluorescent bead next to neurons whole-cell patch clamped with known dye concentrations. After adjusting the somatic fluorescence for free calcium concentration, the ratio of the bead brightness to the cell soma at different dye concentrations yielded a calibration curve. These standardized beads are used in all subsequent experiments to control for electroporation efficiency. DG cells from P22-P30 mice were electroporated with OGB-1. In control experiments, patching the cells after electroporation revealed that cells were healthy and capable of firing action potentials. A stimulating pipette in the hilus provided antidromic stimulation to induce dendritic calcium transients from back

propagating action potentials. 2-P FLIM line scans (4-10 ms) from the dendrites were acquired during antidromic stimulation, and resting calcium concentration and free calcium concentration during stimulation were analyzed by biexponential reconvolution fitting. Dye concentration in each cell was determined by the bead ratio at the soma.

Disclosures: **E.A. Matthews:** None. **D. Dietrich:** None.

Poster

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Program#/Poster#: 97.12/TT47

Topic: G.03. Staining, Tracing, and Imaging Techniques

Support: US NIH Grants DC005259

Korea NRF Grant WCI 2009-003

Title: Improving signal dynamics of fluorescent protein voltage sensors by optimizing FRET interactions

Authors: ***U. SUNG**[1], M. ALLAHVERDIZADEH[1], L. JIN[2], T. HUGHES[3], L. B. COHEN[1,2], B. J. BAKER[1];
[1]Ctr. for Functional Connectomics, Korea Inst. of Sci. and Technol., Seoul, Korea, Republic of; [2]Yale Univ., New Haven, CT; [3]Montana State Univ., Bozeman, MT

Abstract: FRET (Fluorescence resonance energy transfer)-based voltage sensors can be especially useful for monitoring neuronal activity *in vivo* because the ratio of signals between the donor and acceptor pair removes common source noise. We aimed at improving the performance of protein voltage sensors by optimizing FRET interactions between donor and acceptor fluorescent proteins located in the voltage sensitive domain of the *Ciona intestinalis* voltage sensitive phosphatase. We designed a series of constructs with insertions of the two fluorescent proteins at different locations in the voltage sensitive domain. We engineered a total of 39 different combinatorial constructs to evaluate 6 different locations in the N-terminus of the voltage sensitive domain for one fluorescent protein and 8 different locations at the C terminus of the domain for the other fluorescent protein. Evaluation of voltage sensitive optical responses identified amino acid residues or combinatorial positions in the voltage sensitive domain linked to voltage responses with large signals, fast kinetics, and low membrane potential at half

maximum signal change. We found voltage sensors, named “Nabi”, with improved signal size (up to 16% ΔF/F) and faster time constant (~2 msec) compared to previously reported FRET based voltage probes. Nabi2 probes expressed well in cultured neurons and had easily detectable responses to individual action potentials. The Nabi2 responses were substantially faster than those of the fluorescent voltage sensor, ArcLight.

Disclosures: **U. Sung:** None. **M. Allahverdizadeh:** None. **L. Jin:** None. **T. Hughes:** None. **L.B. Cohen:** None. **B.J. Baker:** None.

Poster

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Topic: G.03. Staining, Tracing, and Imaging Techniques

Support: NIGMS grant GM48677

Title: The discovery of a novel conopeptide that is active in the mammalian nervous system from the worm-hunting Conus virgo

Authors: *S. S. ESPINO[1], J. IMPERIAL[2], M. AGUILAR[4], J. GAJEWIAK[2], M. WATKINS[3], R. TEICHERT[2], B. OLIVERA[2];
[1]Biol., Dept. of Biology, Univ. of Utah, Salt Lake City, UT; [2]Biol., [3]Pathology, Univ. of Utah, Salt Lake City, UT; [4]Lab. de Neurofarmacologia Marina, Dept. de Neurobiologia Celulary Molecular,, Univ. Nacional Autonoma de Mexico, Juriquilla, Queretaro, Mexico

Abstract: The cone snails have proven to be a vast resource of biologically active natural products. These compounds known as conotoxins/conopeptides are especially interesting because they exhibit a very high degree of selectivity and potency towards their molecular targets. This research aims to discover novel conopeptides that are active in the mammalian nervous system from Conus virgo, a member of a clade of worm-hunting cone snail. This clade has not been previously explored for conopeptides that target the mammalian nervous system. Bioactivity guided purification using calcium imaging of dissociated mouse dorsal root ganglion (DRG) neurons led to the discovery of a conopeptide that inhibits the increase in intracellular calcium concentration upon depolarization with 20 mM potassium and 20 μM veratridine. Veratridine is a compound that delays the inactivation of voltage-gated sodium channels (VGSCs). This peptide is 32 amino acids long, cross linked by three disulfide bridges and is

provisionally named VrVIA. Analysis of the VrVIA precursor showed a very high degree of sequence similarity with the precursor sequence of omega-conotoxin GVIA (ω-GVIA) and omega-conotoxin MVIIA (ω-MVIIA). ω-GVIA and ω-MVIIA are potent antagonists of the N-type calcium channel (Cav2.2). Intracerebroventricular (ICV) injection of 1.0 nmol VrVIA into mice elicited a distinct behavioral phenotype characterized by hyperactivity and excessive grooming. This is different from the shaking phenotype elicited by ICV injection of ω-GVIA and ω-MVIIA. The differences in behavioral phenotype suggest that VrVIA may not target the N-type calcium channel. The inhibition of the increase in intracellular calcium concentration by VrVIA under the previously described depolarizing condition was observed in small, medium and large diameter neurons. Three types of inhibitory effects were observed. The first type was a rapidly reversible inhibition seen in a majority of the affected neurons. A small population of affected neurons exhibited a slowly reversible inhibition while another small population was irreversibly inhibited. Depolarizing the cells using 40 mM potassium occluded the inhibitory effect of VrVIA. In contrast, a modest inhibition by VrVIA was observed when cells were depolarized by the application of 20 mM potassium. The inhibitory effect was strongest when the inactivation of VGSCs was delayed by veratridine, suggesting that VGSCs are the probable target of VrVIA. The identification of the molecular target of VrVIA is currently being pursued.

Disclosures: **S.S. Espino:** None. **J. Imperial:** None. **M. Aguilar:** None. **J. Gajewiak:** None. **M. Watkins:** None. **R. Teichert:** None. **B. Olivera:** None.

Poster

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Program#/Poster#: 97.14/TT49

Topic: G.03. Staining, Tracing, and Imaging Techniques

Title: Live-cell imaging of individual endocytosis of AMPA receptor around postsynaptic membrane

Authors: *S. FUJII, H. TANAKA, T. HIRANO;
Dept. Biophys.,Grad. Sch. Sci. Kyoto Univ., Kyoto, Japan

Abstract: In the central nervous system, AMPA receptor (AMPAR) plays a major role in excitatory synaptic transmission. The efficacy of transmission changes depending on the neuronal activity, which is called synaptic plasticity and regarded as a basal mechanism of learning and memory. During expression of synaptic plasticity, the number of AMPAR is

dynamically controlled. AMPARs show substantial mobility such as lateral diffusion, exocytosis and endocytosis, which seem to change during synaptic plasticity. In the last decade, researchers have attempted to reveal trafficking of AMPARs during synaptic plasticity, and advances of imaging techniques have made it possible to observe lateral diffusion of AMPAR using quantum dot, and discrete exocytosis event using Total Internal Reflection Fluorescence Microscopy (TIRF). However, it has been difficult to observe individual endocytosis event of AMPAR. Here, we report visualization of individual endocytosis event of AMPAR using the pH-sensitive GFP variant, super ecliptc pHluorin(SEP)-tagged AMPAR and TIRF, both of which have been commonly used for observation of exocytosis. In addition, we changed extracellular pH intermittently to visualize vesicles containing AMPAR, which have been internalized just before the pH change to 6. At pH6, the fluorescent signal from SEP fused to AMPAR on the cell surface became undetectable, and only signals from SEP in intracellular vesicles with neutral pH were detected. Further in this study, we formed postsynaptic-like membrane (PSLM) on a glass surface using neurexin-coated glass (Tanaka et al., 2014 Nature Protocols), and succeeded in detecting endocytosis around PSLMs. Using this experimental method, we observed stimulus-triggered endocytosis of AMPAR. Bath application of NMDA enhanced endocytosis of AMPAR around PSLM, which might be related to long-term depression. Thus, a new methods to record individual endocytosis event of postsynaptic AMPAR has been established, which will contribute to study regulation mechanisms of the number of postsynaptic receptors both in a basal condition and during synaptic plasticity.

Disclosures: **S. Fujii:** None. **H. Tanaka:** None. **T. Hirano:** None.

Poster

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Topic: G.03. Staining, Tracing, and Imaging Techniques

Support: Burroughs Wellcome Fund

University of Cincinnati

Title: Simultaneous voltage-sensitive dye (VSD) imaging of both surfaces of leech ganglia

Authors: ***A. STOWASSER**, D. A. WAGENAAR;
Biol. Sci., Univ. of Cincinnati, Cincinnati, OH

Abstract: One major goal for studying how information is processed by a nervous system is the development of techniques that allow recording the activity of a large number of neurons at once. In this context, the voltage sensitive dye VF2.1.Cl has been proven promising because the dye is very fast, sensitive, and non-toxic. Already, this dye has allowed the simultaneous observation of the activity of most of the neurons on one side of a leech ganglion. To take full advantage of this technique, we now developed a method that allows the simultaneous observation of both surfaces of a ganglion. With our customized double microscope we imaged simultaneously neural activity of both (dorsal and ventral) surfaces of isolated leech ganglia. Optical crosstalk between the layers was substantial, but could be effectively suppressed algorithmically. Stereotyped electrical activity associated with local bending in response to P-cell stimulation could be successfully reconstructed from VSD image sequences from both surfaces simultaneously. The technique should extend readily to other situations in which the activity of two distinct layers of neurons needs to be monitored.

Disclosures: **A. Stowasser:** None. **D.A. Wagenaar:** None.

Poster

097. Imaging Advances: Cell Biology

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Topic: G.03. Staining, Tracing, and Imaging Techniques

Support: MEST WCI 2009-003

Title: Bongoori is a genetically-encoded fluorescent protein voltage sensor that resolves action potentials at 60 hz in neurons

Authors: H. PIAO[1], B. KANG[1], D. RAJAKUMAR[1], A. JUNG[1,2], *B. J. BAKER[1];
[1]Ctr. for Functional Connectomics, Korea Inst. of Sci. and Technol., Seoul, Korea, Republic of;
[2]Col. of Life Sci. and Biotechnology, Korea Univ., Seoul, Korea, Republic of

Abstract: Genetically-encoded fluorescent sensors of membrane potential fuse a fluorescent protein to a voltage-sensing domain. Using cassette mutagenesis to alter conserved charged and polar residues in the voltage-sensing domain changed the speed of the optical response, the size of the optical response, and the voltage-sensitivity of the optical response. Comparison of the voltage-sensing phosphatase from Ciona to other species identified several conserved charged and polar amino acids residing in the transmembrane domains. Mutations in the S2 and S4

transmembrane domains had the largest effect on voltage-sensitivity and signal speed. One mutation in the S1 transmembrane domain yielded an omega current. A combination of three mutations in the voltage-sensing domain and the tweaking of the linker sequence between the voltage-sensing domain and the fluorescent protein, super ecliptic pHlorin A227D yielded a new probe, Bongwoori, capable of resolving action potentials in primary cultured hippocampal neurons at 60 hz.

Disclosures: **H. Piao:** None. **B.J. Baker:** None. **B. Kang:** None. **D. Rajakumar:** None. **A. Jung:** None.

Poster

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Topic: G.03. Staining, Tracing, and Imaging Techniques

Support: CIHR

Title: *In vivo* quantification of amygdala subnuclei using 4.7t fast spin echo imaging

Authors: ***A. AGHAMOHAMMADI SERESHKI**[1], F. OLSEN[2], N. V. MALYKHIN[3]; [1]Ctr. for neuroscience, Univ. of Alberta, Edmonton, AB, Canada; [2]Biomed. Engin., [3]Ctr. for Neuroscience, Biomed. Engin., Univ. of Alebrta, Edmonton, AB, Canada

Abstract: Introduction: The amygdala an almond-shaped structure in the medial temporal lobe which is involved in the processing of emotions and memory. Changes in the amygdala have often been implicated to the pathophysiology of many neurological disorders. The amygdala consists of an evolutionarily primitive subdivision associated with the olfactory system (the cortico-medial region) and an evolutionarily recent subdivision associated with the neocortex (the basolateral region). The cortico-medial region includes the cortical, medial, and central nuclei, while the basolateral region consists of the lateral, basal and accessory basal nuclei. Over the past decade, interest in the human amygdala has grown considerably, spurred on by the progress in animal studies and the development of neuroimaging methods. However, the current information about function in the human amygdala has mainly been at the level of whole amygdala structure. The goal of the present study was to develop a comprehensive volumetric Magnetic Resonance Imaging (MRI) protocol which enables quantification of the amygdala subnuclei *in vivo*, to best correspond with its anatomical subnuclei. Method: Sixteen healthy

volunteers (8 males and 8 females, aged 18-47 years) with no prior history of neurological disorders were recruited. Images were acquired on a 4.7 T Varian Inova scanner with T2-weighted 2D FSE and whole brain T1-weighted 3D MPRAGE sequences. Our detailed volumetric protocol for the amygdala has been previously reported. In addition, we manually segmented the amygdala into five subnuclei (lateral, basal, accessory basal, cortical, and centromedial group) using DISPLAY Software (MNI, Montreal, QC), defined by the internal anatomy of the amygdala and neighbouring structures. Results: Intra-rater ICCs (intraclass correlations coefficient) for amygdala and its subregions were as follows: 0.93 for the amygdala, 0.97 for the lateral nucleus, 0.98 for basal nucleus, 0.98 for accessory basal nucleus, 0.92 for cortical nucleus and 0.88 for the centromedial subregion. Amygdala subnuclei volumes were consistent between hemispheres and showed distributions within the amygdala that were consistent with histological data. Conclusion: We developed a reliable MRI volumetric protocol for segmentation of the amygdala subnuclei *in vivo* for the first time. Future applications of this technique to psychiatric and neurological disorders may reveal new information about vulnerability of amygdala subnuclei to specific pathology.

Disclosures: **A. Aghamohammadi Sereshki:** None. **F. Olsen:** None. **N.V. Malykhin:** None.

Poster

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Topic: G.03. Staining, Tracing, and Imaging Techniques

Support: Ministry of Health and Welfare Grant HN13C0078

Title: Lensed fiber optic stimulator for single neuron

Authors: *J. LEE[1], J. JANG[2], H. KIM[1], N. JEON[2], W. JUNG[1];
[1]biomedical engineering, Ulsan Inst. of Sci. and Technol., Ulsan, Korea, Republic of; [2]Seoul Natl. Univ., seoul, Korea, Republic of

Abstract: Optical stimulation has advantages of being noninvasive, high spatial resolution in two or three dimensions, and large-scale patterned stimulation over the existing electrode methods. To apply intrinsic characteristics of optical stimulation to *in vitro* and *in vivo* experiments, many fiber optic probes have been developed such as fiber optic light guides with uncaged neurotransmitters and two-photon optogenetic stimulation (TPOS). However, it has

known that the conventional probes has a restriction on focusing the light due to the property of light diffraction, so that it has difficulty of simulating the single neuron. In this study, we develop a optical stimulator based on lensed fiber for single neuron stimulation. We tried different fibers such as single mode fiber, photonic crystal fiber (PCF), band-gap fiber varying wavelength, pulsed width, and fiber length to explore characteristic of light delivery. Ball lensed fiber made it possible to focus light with the advantage of high coupling efficiency, strong focus power, and simplicity of fabrication. We also investigated the numerical analysis of fiber optic stimulator to derive optimal design. Numerical results reported optical performance of probe in terms of working distance (>2mm) and beam diameter (<10μm). In addition, photolysis technique of uncaged neurotransmitters was used with guided light. We explored the degree of uncaging dependent on duration, intensity and wavelength (350 ~800 nm) of light. To demonstrate the feasibility of fiber optic stimulation, cortical neurons in rat brain slice were optically activated. Evoked regular action potentials were recorded by extracellular electrodes and then numerous spikes in single cell were sorted out from the intricated electrical signals. This new type of optical stimulator is expected to become a new experimental method for investigating neural network in brain slice non-invasively.

Disclosures: **J. Lee:** None. **H. Kim:** None. **J. Jang:** None. **W. Jung:** None. **N. Jeon:** None.

Poster

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Topic: G.03. Staining, Tracing, and Imaging Techniques

Support: Wellcome Trust

Gatsby Foundation

ERC

EMBO

People Programme (Marie Curie Actions, FP7/2007-2013, Grant 328048)

Title: All-optical manipulation and recording of neural circuit activity *in vivo*

Authors: *A. M. PACKER, H. W. DALGLEISH, M. HAUSSER;
Wolfson Inst. for Biomed. Res., Univ. Col. London, London, United Kingdom

Abstract: Probing neural circuits at the spatial and temporal resolution at which they function is crucial to understanding how populations of neurons work together to drive behavior. Optical approaches provide a means to directly address these questions in a minimally invasive manner. We have developed an all-optical strategy for activating and recording the same neurons with cellular resolution in mice *in vivo* using a dual two-photon optogenetic and calcium imaging approach. The strategy relies on viral coexpression of a genetically encoded activity sensor (GCaMP6s) in conjunction with a red-shifted opsin (C1V1). A spatial light modulator enables targeting tens of user-selected neurons for temporally and spatially precise, simultaneous optogenetic activation using 1064 nm excitation. In parallel, simultaneous fast calcium imaging with single action potential resolution provides readout of the manipulation as well as its effect on hundreds of other neurons. We calibrated the reliability and temporal precision of both activation and readout of activity using cell-attached targeted patch clamp recordings from identified neurons. The combination of indicator sensitivity and lack of spectral overlap between the optogenetic excitation and GCaMP6s emission wavelengths minimizes the stimulation artifact. Thus, even for a single spike, the observed GCaMP fluorescence transient is larger than the optical artifact. The optical nature of the approach combined with a chronic window preparation provides the flexibility to select individual neurons for stimulation while the animal is awake and behaving under the microscope over weeks to months, and the ability to target activation of neurons which are normally active during a behavioral task. We demonstrate proof-of-principle experiments by activating groups of layer 2/3 pyramidal neurons in mouse barrel cortex in defined spatiotemporal patterns during behavior. Our method enables high-throughput, flexible, and long-term optical interrogation of neural circuits in the mammalian brain *in vivo*.

Disclosures: **A.M. Packer:** None. **H.W. Dalgleish:** None. **M. Hausser:** None.

Poster

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Program#/Poster#: 97.20/TT55

Topic: G.03. Staining, Tracing, and Imaging Techniques

Title: *In vivo* optical and electrophysiological simultaneous recordings of auditory responses in mouse's inferior colliculus using a micro-endoscope

Authors: *H. YASHIRO[1], I. NAKAHARA[4,5], K. I. KOBAYASI[2,3], K. FUNABIKI[4,5,1], H. RIQUIMAROUX[1,2,3];
[1]Grad. Sch. of Life and Med. Sci., [2]Dept. of Biomed. Information, Fac. of Life and Med. Sci., [3]Neurosensing and Bionavigation Res. Ctr., Doshisha Univ., Kyoto, Japan; [4]Grad. school of Biostudies, Kyoto Univ., Kyoto, Japan; [5]Dept. of Systems Biol., Osaka Biosci. Inst., Osaka, Japan

Abstract: The *in vivo* imaging is a powerful method to reveal neural circuits of interest. Many studies have been conducted with confocal microscopes and fluorescence microscopes. Those microscopes could get images which had very high spatial resolution. On the other hand, it has been difficult to observe circuits located deep in the brain below cortex. Recently, penetrable endoscopes using an optical fiber bundle or GRIN lens were reported to solve those problems. However, those methods utilizing microscopes used to have very low temporal resolution compared to electrophysiological recording methods. We, therefore, developed a micro-endoscope system which enables us to record optical fluorescence and electrical neural activities simultaneously. The micro-endoscope was fabricated from a fiber bundle in which six-thousands of single mode fibers were bundled. The probe tip was gold-coated and further coated with enamel for insulation to be used as an electrode. The tip of fiber was beveled as pencil-tip-like shape for minimum invasion. Usually the impedance of electrodes was around 200 kΩ. Using our micro-endoscopic system, we could successfully in record fluorescence intensity change of calcium ion indicator dye (Oregon green 488 BAPTA-1 AM, Invitrogen), local field potentials (LFPs), and unit activities through endoscope tip at the same time from an identical recording site in the mouse's inferior colliculus (IC). In optical recording, calcium responses (ΔF/F) with sound stimuli (50 ms white noise burst or tone burst) were in the range of 3 ~ 6 %. By systematically changing the sound frequencies (5 ~ 60 kHz) and sound pressure levels (15 ~ 95 dB SPL, re: 20 μPa), we could measure frequency tuning of the recording sites which are above the observable range of conventional confocal microscope (for example, from at the depth of 380, 500, and 1500 μm from brain surface). Furthermore, there were several different activated areas activated by different sound stimuli in the same view field. In electrophysiological recording, neural activities were recorded in the range of 1 Hz ~ 10 kHz with 20 kHz sampling. In PC, we separated this electrical signal into LFPs (4 - 50 Hz) and unit activities (300 - 5000 Hz) by using a digital filter in Matlab (MathWorks). Thus, our micro-endoscopic system allows us to record calcium responses and electrical neural activities in the same area. This new system will be useful in analyzing several neural circuits, including the auditory system.

Disclosures: **H. Yashiro:** None. **I. Nakahara:** None. **K.I. Kobayasi:** None. **K. Funabiki:** None. **H. Riquimaroux:** None.

Poster

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Program#/Poster#: 97.21/TT56

Topic: G.03. Staining, Tracing, and Imaging Techniques

Title: Simultaneous Imaging of EB3 and ATP reveals the mechanism of cell shape control in HeLa cells

Authors: *R. SUZUKI, K. HOTTA, K. OKA;
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Abstract: Although adenosine triphosphate (ATP) is a major energy source of cells, only a little is known about cytosolic ATP dynamics because of its analytical difficulty. Recent research showed that sponge's choanocyte, which engages in flagella's movement, has high level of ATP (Bilela et al., 2011). We, therefore, guessed that cytosolic ATP concentration changes during cytoskeletal movement or cell morphological change, especially at the peripheral region of the cell because of their high motility and limited volume. We used two fluorescent proteins: ATeam (ATP indicator based on Epsilon subunit for analytical measurements, Imamura et al., 2009) and EB3-mCherry (End binding protein 3 fused to mCherry). We can estimate ATP level by calculating the FRET ratio of ATeam. EB3 binds to the plus ends of microtubules, and fluorescent labeled EB3 is used for an indicator of microtubule dynamics. Moreover, EB3 probe also diffuses within cells, so we can detect the form of lamella, which consists of microtubule, and is an approximate form of the cell. We first observed HeLa cell expressing these two proteins under the physiological condition. Then, the fluorescent images were processed using hand-made computer software. Next, we choose some regions in the cell edge, and estimated relative ATP concentration, EB3 density, and the area of newly-generated cell edges. By calculating cross correlation among time-course of these parameters, we showed that EB3 accumulation induces lamella form's change, and this change involves rise of ATP concentration in these specific regions. Cell edge also has structure named lamellipodia or filopodia. These structures consist of actin filaments, and can be visualized by using FM4-64 which is a fluorescent dye visualized for cell membrane. Simultaneous observation of EB3-Venus and FM4-64 showed that there is a correlation between morphological change of lamella and that of filopofdia or lamellipodia. We also observed spatiotemporal ATP behavior before and after application of microtubule polymerization inhibitor, Taxol. Before the inhibition, cytosolic ATP is especially high at the peripheral area. After the inhibition, high ATP concentration area is

expands to all over the cytosol. These results indicate a possible : 1) ATP concentration of peripheral area is always maintained high. 2) EB3 accumulation at the edge of the cell induces lamella form's change and local ATP concentration increase. 3) lamella form's change causes lamellipodia or filopodia form's change, and the ATP is consumed during morphological changes of filopodia or lamellipoidia.

Disclosures: **R. Suzuki:** None. **K. Hotta:** None. **K. Oka:** None.

Poster

097. Imaging Advances: Cell Biology

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 97.22/TT57

Topic: G.03. Staining, Tracing, and Imaging Techniques

Support: VR 2010-5938

VR 2010-4270

VR 2013-6041

Title: Functional consequences of Na,K-ATPase topology in dendritic spines revealed by superresolution microscopy and 3D finite element modelling

Authors: ***H. B. BRISMAR**[1,2,3], T. LIEBMANN[2,3], O. MANNEBERG[1,3], A. APERIA[2,3], H. BLOM[1,3];
[1]KTH, Royal Inst. of Technol., Stockholm, Sweden; [2]Women's and Children's Hlth., Karolinska Institutet, Stockholm, Sweden; [3]Sci. for Life Lab., Stockholm, Sweden

Abstract: The Na,K-ATPase (NKA) plays an essential role for ion homeostasis in all mammalian cells, including neurons. It is one of the most abundant proteins in the brain and also the protein that consumes the largest fraction of energy in the brain. NKA exists as a heterotrimeric α/β/γ protein complex, where α is the catalytic subunit. Neurons express two α isoforms: the ubiquitous α1 (ATP1a1) and the neuron-specific α3 (ATP1a3). ATP1a3 has a lower sodium affinity and it is suggested that it is important for the efficient restoration of intracellular sodium following excitatory synaptic activity. Despite the enormous importance of NKA there is as yet little known about the isoform specific dynamic distribution and regulation in neurons. We have used super-resolution microscopy to dissect the spatial distribution of the neuron specific α3 and the ubiquitous α1 isoforms. The microscopic findings of dendrite and

spine localization of the protein have been used to create 3D finite element models for analysis of intracellular sodium maintenance in different models of synaptic activity. Super-resolution STED microscopy of antibody labelled NKA revealed a discrete distribution of proteins in both dendrites and spines. The findings were supported by PALM super-resolution microscopy based on PAGFP and mEos modified NKA isoforms. For quantification of protein density, the STED measurements were used as a lower estimate and the PALM measurements as an upper estimate. Mathematical modelling of an isolated dendritic spine with a discrete distribution of NKA based on the nanoscopic findings suggest that a discrete localization of NKA in spines lead to an efficient isolation of intra-spine Na+. Topologically discrete concentration of NKA in the spine neck contributes to an energy efficient restoration of intra-spine Na+. Based on findings from super resolved imaging and 3D finite element modelling we suggest that a compartmentalized distribution may have implications for the generation and regulation of intra-cellular sodium gradients.

Disclosures: **H.B. Brismar:** None. **H. Blom:** None. **O. Manneberg:** None. **T. Liebmann:** None. **A. Aperia:** None.

Poster

097. Imaging Advances: Cell Biology

Location: Halls A-C

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Topic: G.03. Staining, Tracing, and Imaging Techniques

Support: General User Grant to J.M.F.

DOE grant to U Chicago, DE FG02 92ER14244

DOE to NSLS DE AC02-98Ch10886

Title: Microprobe synchrotron X-ray fluorescence shows spatial distribution of copper, iron, and zinc in rat hippocampus

Authors: *K. BOGGS[1], A. LANZIROTTI[2], J. FLINN[1];
[1]George Mason Univ., Fairfax, VA; [2]Univ. of Chicago, Upton, IL

Abstract: Copper (Cu), iron (Fe), and zinc (Zn) are found in small quantities in the brain, where they play a vital role in human health. Typically these trace metals are bound to proteins forming metalloproteins in neurons and glia, although some are found loosely bound or free in synaptic

vesicles. In the hippocampus, vesicular zinc has been shown to modulate synaptic transmission and plasticity by affecting the activity of both GABA and NMDA receptors thereby influencing inhibitory and excitatory transmission in the brain. While zinc is important for neuromodulation, excess zinc can be deleterious to health and has been found to reduce copper absorption. Like zinc, copper too plays a role in synaptic transmission and it's intracellular/extracellular levels are regulated by the ATP7A copper pump, which is found in large quantities in hippocampal pyramidal cells and interneurons. The role of iron in the hippocampus is less clear, though hippocampal pyramidal cells express a specific extracellular iron binding receptor, and studies have shown that knockout rodent models that fail to express this receptor have deficits in learning and memory. Because these metals are so vital to the brain's physiological state, insufficient intake or excess intake of Cu, Fe, and Zn can adversely affect human health. In fact, dyshomeostasis of these metals in the brain have been implicated in many neurodegenerative diseases such as Alzheimer's, Parkinson's, and Huntington's, by increasing production of reactive oxygen species and increasing the rate of cell death. It is important to gain greater insight into how these metals are distributed in the brain to gain greater understanding of both their physiological role, as well as their role in the diseased brain. With the use of beamline X-26A National Synchrotron Light Source at Brookhaven National Laboratory, we were able to visualize the spatial distribution of copper, iron, and zinc in rat hippocampus at concentrations below 20 ppm. In contrast to other methods, X-26A allows for the simultaneous visualization of metals at a spatial resolution of 5μm within a given region of the brain, and allows for visualization of both bound and free metal ions without tissue destruction. Thionin staining was performed in addition to synchrotron fluorescence to confirm the distinct separation of metal ion distribution in different cellular layers of the hippocampus. The results reveal that zinc is mostly distributed in dentate gyrus and CA3, with a similar pattern of distribution for iron, though iron appears to extend into CA1. Copper on the other hand appears to be restricted to areas of entorhinal cortex rather than dentate gyrus and CA regions.

Disclosures: **K. Boggs:** None. **A. Lanzirotti:** None. **J. Flinn:** None.

Poster

097. Imaging Advances: Cell Biology

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 97.24/TT59

Topic: G.03. Staining, Tracing, and Imaging Techniques

Support: NIH Grant NS081629

Title: Identifying the intracellular zinc stores

Authors: Q. LU[1], H. HARAGOPAL[2], *Y. V. LI[3];
[1]Mol. and Cell. Biol. program, [2]Biol. Sci., [3]Biomed. Sci., Ohio Univ., Athens, OH

Abstract: Zinc is known to play an important role in regulating various physiological processes. In protein- bound form, it is involved in DNA and protein synthesis, hormone packaging, mitosis, apoptosis, and many other cellular functions. Unbound/free zinc is currently proposed to be a key modulator of intra- and intercellular neuronal signaling similar to calcium. Interestingly, the level of free zinc in neurons is tightly regulated. Indeed, like calcium, copper (II) and iron (III), disruptions in neuronal zinc homeostasis have been directly correlated with various neuropathological disorders like epilepsy, Alzheimer's disease, and Parkinson's disease. Outside of nervous system, cellular free zinc regulates insulin secretion, bone mineral density, and inflammation. Therefore, knowledge on intracellular zinc distribution is critical to understanding cellular zinc homeostasis. However, there is still a lack of comprehensive imaging data for it. Here, we used a membrane permeable zinc fluorescent indicator, Zinpyr1 along with organelle-specific fluorescent dyes to visualize the subcellular free zinc in 3 cell lines: Cath.a (neuronal), HIT-T15 (pancreatic beta cell-like), and HeLa (cervical cancer) cells. In the study, we treated the cells with 10uM of Zinpyr1 that was paired with 10uM of ER Tracker Red (stains endoplasmic reticulum/ER), 0.5uM MitoFluor Red 589 (stains mitochondria) or 10uM of BODIPY TR-ceramide (stains Golgi apparatus). We report from our colocalization data that free zinc is present within the ER, the Golgi apparatus and mitochondria but not the nucleus. Our studies in both neuronal and non-neuronal cell lines suggest that ER, mitochondria and Golgi apparatus are the general intracellular storage depots for free zinc.

Disclosures: **Q. Lu:** None. **Y.V. Li:** None. **H. Haragopal:** None.

Poster

097. Imaging Advances: Cell Biology

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 97.25/TT60

Topic: G.03. Staining, Tracing, and Imaging Techniques

Title: Synthesis and initial *in vivo* evaluation of [125I]iodoASEM, a radioligand for the α7 nicotinic acetylcholine receptor

Authors: R. MEASE[1], Y. GAO[1], T. TRAN[2], K. KELLAR[2], D. WONG[1], R. DANNALS[1,2], *M. POMPER[1], A. HORTI[1];
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Abstract: The α7 nicotinic cholinergic receptor (α7-nAChR) is a cationic, ligand-gated calcium channel with five identical ligand-binding subunits. The α7-nAChR system has been implicated in a wide variety of pathologies of the central nervous system, including Alzheimer's disease, schizophrenia and neuroinflammation. Non-invasive study of the α7-nAChR may uncover new aspects of the physiology of this receptor system, enable characterization of disease states with which it is associated and may hasten drug development. We recently reported a series of 3-(1,4-diazabicyclo[3.2.2]nonan-4-yl)dibenzo[b,d]-thiophene-5,5-dioxide derivatives, many of which possess sub-nanomolar affinity towards α7-nAChR. Within that series is [18F]ASEM, the first target-selective radiopharmaceutical for imaging α7-nAChR in primate brain with positron emission tomography (PET). Also within that series is 3-(1,4-diazabicyclo[3.2.2]nonan-4-yl)-6-iodobenzo[b,d]thiophene-5,5-dioxide (iodoSEM, Kd < 300 pM), the radioiodinated form of which we describe here. Precursor 3-1,4-diazabicyclo[3.2.2-nonan-4-yl)-6-(3,3-dibutyltriazeno)dibenzo[b,d]thiophene dioxide (A-55) was synthesized either from diazotination of 6-amino-3-(1,4-diazabicyclo[3.2.2]nonan-4-yL)-dibenzo[b,d]-thiophene-5,5-dioxide followed by reaction with dibutylamine, or by Pd coupling of 3-bromo-6-(3,3-dibutyltriazeno)dibenzo[b,d]thiophene-5,5-dioxide with 1,4-diazabicyclo[3.2.2]nonane. Radioiodination was performed from A-55 in acetonitrile followed by addition of a solution of [125I]NaI then trifluoroacetic acid, with heating at 80oC for 20 min. The reaction mixture was purified by reverse phase radio-HPLC to produce [125I]iodoASEM. Radiochemical yields approximated 27% with specific radioactivities on the order of 55.5 GBq/μmole. Intravenous administration of [125I]iodoASEM to CD-1 mice generated brain uptake values over 5% ID/g in cortex and superior colliculus, known α7-nAChR target regions, at 1 h post-injection. Receptor blockade using the known α7-nAChR ligand, SSR180711 (s.c., 2 mg/kg), decreased uptake in superior colliculus to < 1% (P = 0.007), indicating binding selectivity. No blockade was evident in cerebellum. [125I]iodoASEM demonstrates high, regionally specific binding to α7-nAChR in rodent brain and can be used for quantification of α7-nAChR *in vitro* or *in vivo* with single photon emission computed tomography (SPECT) in pre-clinical experimental models. Notably, isotopic substitution with 123I or 124I could enable clinical translation for SPECT and PET, respectively.

Disclosures: **R. Mease:** A. Employment/Salary (full or part-time):; Johns Hopkins University. **M. Pomper:** A. Employment/Salary (full or part-time):; Johns Hopkins University. **A. Horti:** A. Employment/Salary (full or part-time):; Johns Hopkins University. **Y. Gao:** None. **D. Wong:** A. Employment/Salary (full or part-time):; Johns Hopkins University. **R. Dannals:** A. Employment/Salary (full or part-time):; Johns Hopkins University. **K. Kellar:** A. Employment/Salary (full or part-time):; Georgetown University. **T. Tran:** A. Employment/Salary (full or part-time):; Georgetown University.

Poster

097. Imaging Advances: Cell Biology

Location: Halls A-C

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Topic: G.03. Staining, Tracing, and Imaging Techniques

Support: NIH Grant DA23957-01

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NIH Grant MH045372

Veterans Health Administration Merit Review and Career Scientist Programs

Title: Characterization of [3H]LS-3-134, a novel arylamide phenylpiperazine D3 dopamine receptor selective radioligand

Authors: *R. R. LUEDTKE, Ph.D.[1], C. RANGEL-BARAJAS[1], M. MALIK[1], M. TAYLOR[1], K. A. NEVE[2], R. H. MACH[3];
[1]Dept Pharmacol & Neurosci, Univ. North Texas Hlth. Sci. Cter, FORT WORTH, TX; [2]DVA, Oregon Hlth. & Sci. Univ., Portland, OR; [3]Radiology, Univ. of Pennsylvania Sch. of Med., Philadelphia, PA

Abstract: LS-3-134 is a substituted N-phenylpiperazine derivative that has been reported to exhibit a) high affinity binding (Ki value 0.2 nM) at human D3 dopamine receptors, b) >100-fold D3 vs. D2 dopamine receptor subtype binding selectivity and c) low affinity binding (Ki values >5,000 nM) at sigma 1 and sigma 2 receptors. Based upon a forskolin-dependent activation of adenylyl cyclase inhibition assay, LS-3-134 is a weak partial agonist at both D2 and D3 dopamine receptor subtypes (29% and 35% of full agonist activity, respectively). [^{3}H]-labeled LS-3-134 was prepared and evaluated to further characterize its use as a D3 dopamine receptor selective radioligand. Kinetic and equilibrium radioligand binding studies were performed. This radioligand rapidly reaches equilibrium (10-15 min at 37°C) and binds with high affinity to both human (Kd = 0.06 ± 0.01 nM) and rat (Kd = 0.2 ± 0.02 nM) D3 receptors expressed in HEK-293 cells. Direct and competitive radioligand binding studies using rat caudate and nucleus accumbens tissue indicate that [^{3}H]LS-3-134 selectively binds a homogeneous population of binding sites with a dopamine D3 receptor pharmacological profile. Based upon these studies we

propose that [^{3}H]LS-3-134 represents a novel D3 dopamine receptor selective radioligand that can be used for studying the expression and regulation of the D3 dopamine receptor subtype.

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Poster

097. Imaging Advances: Cell Biology

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 97.27/TT62

Topic: G.03. Staining, Tracing, and Imaging Techniques

Support: KAKENHI 25750400

Title: Segmented analysis of astrocytic restructuring induced by chronic hypoxia in the adult mouse cortex

Authors: H. MAEDA[1], M. NITTA[1], T. SUGASHI[1], H. KAWAGUCHI[2], H. TAKUWA[2], H. ITO[2], I. KANNO[2], *K. MASAMOTO[1,2];
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Abstract: The astrocytes play a major role in the maintenance of neural and vascular homeostasis in the brain. In response to hypoxia, the astrocytic processes change their shapes and increase contacts with the neighborhood vessels. These morphological changes are considered as an important mechanism in adaptation to brain hypoxia. In the present study, to characterize the compartmentalized differences in the structural plasticity of the astrocytes, we quantify the spatiotemporal dynamic changes in the astrocytic soma and process morphologies during adaptation to hypoxia in *in vivo* mouse cortex. Animal use and experimental protocols were approved by the Institutional Animal Care and Use Committee. The 3D structures of the astrocytes were repeatedly imaged with multi-photon microscopy (TCS SP5MP, Leica Microsystems) at 900-nm excitation, while the animals were exposed to limited oxygen environment (8-9% oxygen conditions) for 3 weeks. On each imaging date, the astrocytes were labeled with sulforhodamine 101 (5-10 mM in saline, 8 μL/g body weight i.p.), and the cortical image (1,024 pixel × 1,024 pixel, 0.3 μm/pixel) was taken through cranial window with a step size of 2.5 μm or 4 μm over 0 to 400 μm depths from the cortical surface. The obtained images were analyzed with a custom-written software in MATLAB (MATLAB2012b). First, a square region of interest (ROI; approximately 50 μm × 50 μm) was manually placed around the target

astrocytes at a center slice of the depth stack astrocytic images, and a total of 10 sequential slices were three-dimensionally extracted within ROI for each single astrocyte image. Then, the extracted images were three-dimensionally reconstructed. Finally, the number and area of the astrocytic processes, and somatic area were measured for each astrocyte. A total of 202 astrocytes were randomly extracted over depths of 0-300 μm in the mouse somatomotor cortex (N = 3 animals). We observed the maximum areas of the both astrocytic soma and processes at a depth of 150 μm in the cortex; 84 μm^2 and 136 μm^2, respectively, under pre-hypoxia treatment control conditions, while the number of the processes monotonically decreased with increasing depths in the measured cortex. After adaptation to 3-week hypoxia, the astrocytic soma was enlarged 1.5 to 2.0 fold relative to the pre-hypoxia control. In addition, a number of the processes also increased 1.5 to 2.0 fold. These data showed that the increase of the astrocytic cell volume was caused by the both increase of somatic volume and a number of the processes. In conclusion, the present analytical method allows for quantification of the compartmentalized differences of the astrocytic structure plasticity.

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Poster

097. Imaging Advances: Cell Biology

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 97.28/TT63

Topic: G.03. Staining, Tracing, and Imaging Techniques

Support: NIH Grant GM48677

Title: Combining molecular genetics with constellation pharmacology to identify neuronal cell types

Authors: ***R. W. TEICHERT**[1], Y. ZHENG[2], D. D. GINTY[3], B. M. OLIVERA[1];
[1]Dept. of Biol., Univ. of Utah, Salt Lake City, UT; [2]Dept. of Neurobio., Harvard Med. Sch., Cambridge, MA; [3]Dept. of Neurobio., Harvard Med. Sch. and Howard Hughes Med. Inst., Cambridge, MA

Abstract: Recently we have published several papers that outline a pharmacological approach to identify and classify neuronal cell types in the mammalian nervous system. We call this approach “constellation pharmacology” because we use pharmacology to elucidate the cell-

specific combinations or “constellations” of receptors and ion channels that define each neuronal cell type. Notably, each neuronal cell type is distinguished by its unique expression pattern of plasma-membrane receptors and ion-channel subtypes that are functionally integrated to produce cell-type-specific physiological properties. We have developed and validated pharmacological assays that can interrogate a broad spectrum of G-protein coupled receptors (GPCRs), ligand-gated ion channels and voltage-gated ion channels in ~200 individual cells simultaneously, using changes in cytosolic calcium concentration as a read-out. In essence, constellation pharmacology incorporates functional calcium imaging with a large toolkit of highly selective pharmacological agents, including target-selective peptides, toxins, drugs, other small molecules and natural products. The cellular responses to each pharmacological agent can be used to parse heterogeneous neuronal/glial cell populations into subpopulations or subclasses. Such phenotypic responses also elucidate the specific receptor and ion-channel subtypes that are expressed in each cell type. The available toolkit of selective pharmacological agents is vast and continuously growing, but there are still conspicuous gaps in the toolkit that do not allow us to identify the entire constellation of receptors and ion channels in each neuronal cell type. However, we can overcome this problem by combining constellation pharmacology with other methods that also identify neuronal cell types and that can be used to further elucidate their cell-specific constellations. We are currently combining constellation pharmacology with mouse molecular genetics to create a more integrated and comprehensive definition of cell types in the nervous system. We illustrate the coupling of constellation pharmacology with several examples of genetically labeled (fluorescent) somatosensory neuronal subclasses. Among many results, we highlight a functional difference in KV1.2 potassium channels between proprioceptors and low-threshold mechanoreceptor neurons. We also demonstrate key differences in the functional expression of GPCRs between various somatosensory neuronal subclasses, using a combination of molecular genetics and constellation pharmacology.

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Poster

097. Imaging Advances: Cell Biology

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 97.29/TT64

Topic: G.03. Staining, Tracing, and Imaging Techniques

Title: Generation of a transgenic rat for Cre-dependent expression of the infrared fluorescent protein (iRFP) in neurons

Authors: *H. A. BALDWIN[1], C. T. RICHIE[1], L. V. FORTUNO[1], D. B. HOWARD[1], Y.-J. ZHANG[1], M. A. VERDECIA[1], L. R. WHITAKER[1], J. J. HINKLE[1], J. C. SMITH[2], J. M. PICKEL[3], B. T. HOPE[1], B. K. HARVEY[1];
[1]NIDA IRP, Baltimore, MD; [2]NINDS IRP, Bethesda, MD; [3]NIMH IRP, Bethesda, MD

Abstract: Genetically encoded fluorescent reporters are a common and invaluable tool for neuroscience. Many of the well-known and frequently used reporters such as green fluorescent protein (GFP), yellow fluorescent protein (YFP), mCherry, etc. have certain limitations for imaging neuronal populations. For example, they have low signal to background ratios, photobleach easily, and experience high tissue absorption when attempting deep tissue imaging (>500 μm). Infrared fluorescent protein (iRFP) is a genetically encoded fluorescent probe engineered from a bacterial phytochrome photoreceptor that has excitation/emission spectra (690 nm/ 713 nm) inside the near-infrared window, thus allowing for deeper tissue penetration and lower tissue auto-fluorescence because of its minimal absorption by hemoglobin, melanin, and water. iRFP's unique structure gives it high photostability as compared to the other known fluorescent reporter proteins. These properties of iRFP make it potentially useful for studies of neuronal function in the intact or slice-prepared brain. We have previously reported several adeno-associated viral (AAV) vectors containing the iRFP gene, including a membrane-restricted expression (mem-iRFP), and two translationally fused with the optogenetic proteins channelrhodopsin (ChR2) and halorhodopsin (eNpHR3.0) which demonstrated the successful transduction and expression of iRFP in neurons both *in vitro* and *in vivo*. We have now created several transgenic rat lines, on both the Sprague-Dawley and Long Evans backgrounds, that express iRFP in a Cre-dependent manner. We have crossed our line of dopaminergic transporter (DAT) promoter driven Cre expressing rats with our Cre-dependent iRFP (DIO-iRFP) rats. These double transgenic rats may facilitate neuroscience research, particularly in models of dopaminergic neuron dysfunction that occurs in drug addiction and Parkinson's disease. Overall, we have demonstrated that iRFP is a useful reporter protein for studying and manipulating rat neurons.

Disclosures: **H.A. Baldwin:** None. **C.T. Richie:** None. **L.V. Fortuno:** None. **D.B. Howard:** None. **Y. Zhang:** None. **M.A. Verdecia:** None. **L.R. Whitaker:** None. **J.J. Hinkle:** None. **J.C. Smith:** None. **J.M. Pickel:** None. **B.T. Hope:** None. **B.K. Harvey:** None.

Poster

097. Imaging Advances: Cell Biology

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 97.30/TT65

Topic: G.03. Staining, Tracing, and Imaging Techniques

Support: JSPS 25350998

Title: *In vivo* imaging of CREB phosphorylation using a novel transgenic mouse line expressing bioluminescence probes

Authors: *T. ISHIMOTO, H. MANO, H. MORI;
Univ. of Toyama, Toyama 930-0194, Japan

Abstract: Cyclic adenosine monophosphate response element binding protein (CREB) is a transcription factor that is considered important for memory consolidation and recovery process of depression. Once serine 133 of CREB is phosphorylated, kinase inducible domain (KID) of CREB binds to KIX domain of CREB binding protein, and the downstream gene expression is induced. However, the spatiotemporal pattern of the phosphorylation of CREB *in vivo* has not been fully analyzed because of technical difficulty. We employed the split luciferase technique to monitor the phosphorylation of CREB in live cells and animals. In this technique, firefly luciferase was cleaved into N-terminal and C-terminal segments. The KID and KIX domains were fused with N-terminal and C-terminal segments of luciferase respectively. By the interaction between these two fusion proteins mediated by serine 133 phosphorylation of KID, split segments complement each other to be a functional luciferase that can emit light. The light emission was increased in response to forskolin treatment that up-regulated cAMP in HEK293T cells expressing probe proteins. The increase was cancelled by replacement of the serine 133 to alanine in the probe protein suggests the process is phosphorylation-dependent. To generate transgenic mouse line that expresses probe proteins, the bacterial artificial chromosome clone containing beta actin promoter and protein-coding region was used to insert the probe protein genes flanking IRES sequence by homologous recombination at the start codon was used. We found increased light emission from cerebral cortex of live transgenic mouse after the acute treatment of imipramine, a tricyclic antidepressant. Increase in CREB phosphorylation in cerebral cortex by the same treatment was confirmed using western blotting. These results demonstrate this transgenic mouse strain can be used for the imaging of CREB phosphorylation in live mouse brain. Furthermore, we also show the changes in pattern of CREB phosphorylation after reserpine treatment that is known to induce depression-like symptoms in mouse.

Disclosures: **T. Ishimoto:** None. **H. Mano:** None. **H. Mori:** None.

Poster

098. Imaging Advances: Neural Ultrastructure

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 98.01/TT66

Topic: G.03. Staining, Tracing, and Imaging Techniques

Support: CellNetworks Excellence Cluster EXC81

Title: Resin-free electron microscopy of nervous tissue: Preserved cytokeleton and immunogold labeling in a 3D ultrastructural landscape

Authors: V. VENKATARAMANI, H. HORSTMANN, *T. KUNER;
Functional Neuroanatomy, Heidelberg Univ., Heidelberg, Germany

Abstract: Conventional electron microscopy of cytoskeletal elements embedded in resin suffers from at least two fundamental drawbacks: fine filamentous structures are poorly visible and immunogold labeling is incompatible with detailed ultrastrucure. Here we introduce a novel approach applicable for scanning electron microscopy revealing intact cellular cytoskeleton and immunogold labeling in a highly detailed ultrastructural landscape of cells in their natural context. We sought to overcome charging effects in the scanning electron microscope which distort the image and hence lower the resolution. With a resin-free preparation we were able to enhance the electrical conductivity significantly thus achieving higher resolution scanning electron microscopy images of tissue blocks (~150 um thick) and Tokuyasu cryosections (300 nm thick). The resin-free method combines the modified rOTO-lead aspartate block stain with subsequent critical point drying. The surface of tissue blocks or sections was imaged with a SEM equipped with a field emission gun. To characterize the ultrastructure of nervous tissue on a molecular level we established pre-embedding immunogold labelling for tissue blocks and sections. We applied this approach to study the ultrastructure of the presynaptic calyx of Held and its postsynaptic principal neuron. The images reveal a complex filamentous cytomatrix with embedded synaptic vesicles, clathrin-coated vesicles, mitochondria and endosomal structures. The plasma membranes can be readily identified and active zone matrix as well as postsynaptic densities are clearly visible. Synaptic vesicles appear interconnected by filamentous structures and some are tethered to the active zone by short filaments. Postsynaptically, mitochondria, Golgi apparatus and endoplasmatic reticulum are suspended in a dense cytoskeletal network. Synapsin antibodies and immunogold labeling show the specific synaptic vesicle-associated distribution of 10 nm gold particles thoughout the vesicle clusters of the terminal with fully intact ultrastructure. Thus, our preparation method allows, for the first time, the molecular characterization of tissue on the ultrastructural level with preserved structural details.

Disclosures: **V. Venkataramani:** None. **H. Horstmann:** None. **T. Kuner:** None.

Poster

098. Imaging Advances: Neural Ultrastructure

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 98.02/TT67

Topic: G.03. Staining, Tracing, and Imaging Techniques

Title: 3D isotropic reconstruction of biological samples through cycles of physical and virtual sectioning in electron microscopy

Authors: *B. H. LICH[1,2], F. BOUGHORBEL[1], P. POTOCEK[1], R. VAN DEN BOOGAARD[1], L. HEKKING[1,3], E. KORKMAZ[1], P. CERNOHORSKY[2], M. HOVORKA[2], M. LANGHORST[3]; [1]AAE III, FEI Electron Optics BV, Eindhoven, Netherlands; [2]FEI Brno, Brno, Czech Republic; [3]FEI Munich, Munich, Germany

Abstract: In recent years there has been a considerable advancement in SEM-based methods for 3D reconstruction of large tissue volumes. Serial Block-Face SEM (SBF-SEM) involves combination of imaging and in-situ sectioning of plastic embedded tissue blocks within the SEM vacuum chamber1, allowing for automated imaging and subsequent reconstruction of volumes of tissue. The use of low electron energies for imaging limits sample charging which can be further mitigated with imaging in low vacuum mode and by further increasing the conductivity of the sample through sufficient amount of heavy metal staining and in-situ metal coating of the block face. Here we introduce a novel solution for high spatial resolution and throughput SEM volume imaging overcoming the resolution limits set by mechanical slicing by combining it with virtual sectioning. Virtual slicing is realized by Multi-Energy Deconvolution SEM (MED-SEM), a non-destructive technique that allows high resolution reconstruction of the top layers of the sample.2 After cutting a thin layer of the blockface using a diamond knife, freshly exposed tissue is imaged several times using various accelerating voltages. These images are subsequently used for deconvolving the information into several virtual subsurface layers. This cycle of physical and virtual sectioning offers isotropic datasets with excellent z-resolution and can be fully integrated and automated

Disclosures: **B.H. Lich:** A. Employment/Salary (full or part-time):; FEI Electron Optics. **F. Boughorbel:** A. Employment/Salary (full or part-time):; FEI Electron Optics. **P. Potocek:** A. Employment/Salary (full or part-time):; FEI Electron Optics. **R. van den Boogaard:** A. Employment/Salary (full or part-time):; FEI Electron Optics. **L. Hekking:** A. Employment/Salary (full or part-time):; FEI Electron Optics. **M. Langhorst:** A. Employment/Salary (full or part-time):; FEI Electron Optics. **E. Korkmaz:** A. Employment/Salary (full or part-time):; FEI Electron Optics. **P. Cernohorsky:** A.

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Employment/Salary (full or part-time):; FEI Electron Optics.

Poster

098. Imaging Advances: Neural Ultrastructure

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 98.03/TT68

Topic: G.03. Staining, Tracing, and Imaging Techniques

Title: Compatibility of long-range fluorescent tracers with locally dense 3D electron microscopy reconstruction

Authors: *A. EMENARI, C. HICKS, K. L. BRIGGMAN;
Natl. Inst. of Neurolog. Disorders and Stroke, NIH, Bethesda, MD

Abstract: Dense reconstruction of neural circuits is currently limited in scope by the size of realistic 3D electron microscopy (EM) volumes. However, neural circuits in most brain regions span volumes significantly larger than current EM volumes. This limits the information content within an EM volume for the case in which an axon exits the volume and is therefore not able to be traced to its source or target. We aim to combine long-range fluorescent axon tracing throughout the brain with local dense 3D EM reconstruction. One option is to inject widely available adeno-associated virus (AAV) constructs encoding fluorescent proteins into brain regions that we think project through the EM volume. This approach is however biased by the selection of these regions and limited to the number of discriminable fluorescent protein colors. We sought a less biased approach by injecting lipophilic tracers into the center of the EM target volume, under the assumption that lipophilic tracers will indiscriminately label membranes within the injection volume and diffuse along axons passing through the volume. For testing purposes, we have initially targeted the mouse dorsal lateral geniculate nucleus (dLGN) of the thalamus. The goal is to label both afferent and efferent axons passing through the volume that we will ultimately reconstruct by EM. In this study, we tested different injection methods that would both widely label the injection volume but also preserve ultrastructure. We tested both iontophoretic and pressure injections and a variety of solvents for the lipophilic dyes. Following injection and after a pre-determined diffusion duration we perfused animals and sectioned the brain into 300 um vibratome sections. The section containing the injection volume was stained for EM and assayed for ultrastructural preservation. We demonstrated that lipophilic dyes are able to diffuse to brain regions known to connect to the dLGN within 1-2 weeks *in vivo*. This

approach provides a method to incorporate long-range connectivity information with local dense 3D EM reconstruction.

Disclosures: **A. Emenari:** None. **C. Hicks:** None. **K.L. Briggman:** None.

Poster

098. Imaging Advances: Neural Ultrastructure

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Program#/Poster#: 98.04/TT69

Topic: G.03. Staining, Tracing, and Imaging Techniques

Title: Automated segmentation of nervous tissue from densely stained serial blockface electron microscopy data

Authors: *M. BERNING, M. HELMSTAEDTER;
Neocortical Circuits Group, Max Planck Inst. of Neurobio., Munich, Germany

Abstract: So far, dense semi-automated circuit reconstruction from serial blockface EM (SBEM) data relied on a tissue staining which enhanced plasma membranes, but made the direct detection of synapses impossible. Here we present automated classifiers based on semi-automated screening of convolutional neuronal network architectures that enable the reconstruction of fully stained 3-dimensional EM neuropil data. Using these classifiers, we pre-segmented a volume of 55 x 66 x 140 µm3 SBEM data from mouse retina and volume-reconstructed bipolar cell axons, ganglion cell and amacrine cell dendrites from their respective skeletons and detected all chemical synapses between these neurons. We then investigated how our classifiers transfer to novel SBEM datasets and developed a CNN screening algorithm that works successfully on novel large-scale SBEM datasets from mouse neocortex. Our results resolve the tradeoff between synapse detection and semi-automated reconstruction performance in high-resolution connectomics and provide the tools for efficient circuit reconstruction in fully-stained SBEM datasets.

Disclosures: **M. Berning:** A. Employment/Salary (full or part-time):; Max Planck Institute of Neurobiology. **M. Helmstaedter:** A. Employment/Salary (full or part-time):; Max Planck Institute of Neurobiology.

Poster

098. Imaging Advances: Neural Ultrastructure

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 98.05/TT70

Topic: G.03. Staining, Tracing, and Imaging Techniques

Title: Project Brainflight: Scaling connectomic reconstruction via lay-audience targeted image analysis

Authors: *E. DOW[1,2], Y. BUCKLEY[2], M. BERNING[2], T. BOCKLISCH[3], D. BRÄUNLEIN[3], T. HEROLD[3], N. RZEPKA[3], T. WERKMEISTER[3], M. HELMSTAEDTER[2];
[1]The Rockefeller Univ., New York, NY; [2]Max Planck Inst. of Neurobio., Martinsried, Germany; [3]Scalable Minds Inc., Potsdam, Germany

Abstract: A key bottleneck for large-scale electron-microscopy (EM)-based connectomics is data analysis throughput. In the past, dense circuit reconstructions have been made possible by the efficient combination of automated and human annotation. But the scale of possible reconstructions was limited by the expense and organization of human employees: so far up to 20,000 work hours of paid annotation has been achieved. One possible approach for scaling connectomic reconstruction is the recruitment of voluntary public annotators via online interfaces. Here we present Project Brainflight (http://brainflight.org), a set of online browser and mobile games created for different lay target audiences to annotate serial blockface EM datasets. Key innovations were the development of a simple human computation task, which reduced training times from tens of hours to minutes; focused annotation of regions that could not be reliably segmented by algorithms, selected in an active-learning classifier setting; image manipulation and software optimization to allow the distribution of high-resolution data to standard mobile devices; true game elements that support continuous attention and enhanced motivation in lay audiences; and creation of thematic game elements for selected target audiences. Together, these developments allow us to crowd-source connectomic data analysis in the mouse cerebral cortex and other large EM data sets.

Disclosures: **E. Dow:** A. Employment/Salary (full or part-time):; Max Planck Institute of Neurobiology. **Y. Buckley:** A. Employment/Salary (full or part-time):; Max Planck Institute of Neurobiology. **M. Berning:** A. Employment/Salary (full or part-time):; Max Planck Institute of Neurobiology. **T. Bocklisch:** 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.; Max Planck Institute of Neurobiology. **D. Bräunlein:** 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.; Max Planck Institute of Neurobiology. **T. Herold:** 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.; Max Planck Institute of Neurobiology. **N. Rzepka:** 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.; Max Planck Institute of Neurobiology. **T. Werkmeister:** 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.; Max Planck Institute of Neurobiology. **M. Helmstaedter:** A. Employment/Salary (full or part-time):; Max Planck Institute of Neurobiology.

Poster

098. Imaging Advances: Neural Ultrastructure

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 98.06/TT71

Topic: G.03. Staining, Tracing, and Imaging Techniques

Title: Large volume en bloc EM staining for high resolution connectomics

Authors: *Y. HUA, M. HELMSTAEDTER;
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Abstract: Large-scale connectomics requires dense staining of neuronal tissue blocks for automated volume EM imaging. Available protocols, however, only allow either small volume staining or selective staining of myelinated neurites. Here we report a large-volume dense en-bloc EM staining protocol that overcomes this limitation, making it possible to obtain tissue blocks sized more than a millimeter in their smallest dimension with homogeneous and high-contrast dense neuropil staining. Our protocol can be applied to multiple species and brain structures, as exemplified for samples from mouse neocortex, thalamus, and turtle cortex. The

protocol also reduces the previously substantial staining failure rate in en-bloc protocols, facilitating correlated large-volume functional and structural imaging in the neocortex.

Disclosures: **Y. Hua:** None. **M. Helmstaedter:** None.

Poster

098. Imaging Advances: Neural Ultrastructure

Location: Halls A-C

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Program#/Poster#: 98.07/TT72

Topic: G.03. Staining, Tracing, and Imaging Techniques

Title: Automated synapse detection in large-scale serial block-face electron microcopy data

Authors: *B. STAFFLER[1], P. VAN DER SMAGT[2], M. HELMSTAEDTER[1];
[1]MPI of Neurobio., Martinsried, Germany; [2]TUM, Munich, Germany

Abstract: Serial block-face electron microscopy (SBEM) provides image volumes of the size and resolution sufficient for reconstructing all axons and dendrites densely in modules of a cortical column in mouse neocortex. For connectomic analysis, chemical synapses have to be identified in addition to neurite reconstruction. Blocks of, e.g., 500 µm x 500 µm x 500 µm volume in mouse barrel cortex contain several tens of millions of synapses. Human annotation would consume hundreds of thousands of work hours. Therefore, automated synapse detectors are required. While automated synapse detectors have been developed for data obtained by high-resolution FIB-SEM or high in-plane resolution ssTEM imaging, automated synapse detection in fully stained SBEM data is missing. Here we developed such classifiers. We first pre-segment the imaged volume into neurite segments of ~5µm average length (Berning & MH, unpublished). We then classify contact interfaces between neighboring neurites as synaptic or non-synaptic. We first manually labeled 30000 neurite-to-neurite contact interfaces. We then trained a boosted ensemble classifier for identifying synapses and their directionality. The so far best classifier yielded precision and recall rates of 59% and 85%, respectively. This performance already provides a reduction in manual annotation effort of factor 34 with a neuron-to-neuron connection recovery rate of about 97%. We are currently improving the classifiers to allow synapse detection at tolerable annotation cost, fully automated synapse detection at an error rate tolerable for connectomic analysis, and an extension to the detection of synapse types.

Disclosures: **B. Staffler:** None. **P. van der Smagt:** None. **M. Helmstaedter:** None.

Poster

098. Imaging Advances: Neural Ultrastructure

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Program#/Poster#: 98.08/TT73

Topic: G.03. Staining, Tracing, and Imaging Techniques

Support: Max Planck Society

Title: Towards whole-mouse-brain serial block-face electron microscopy

Authors: *S. MIKULA, W. DENK;
Max-Planck Inst. For Med. Res., Heidelberg, Germany

Abstract: Volume electron microscopy techniques have proven successful for imaging volumes of a few hundred microns in size. However, almost all neural circuits are considerably larger than this, curtailing useful functional inference. To image the adult whole mouse brain, which measures 450 mm^3, at a resolution such that the entire circuit can be reconstructed, new methods are required. Current efforts to scale serial block-face electron microscopy (SBEM) to the whole mouse brain face three fundamental technical challenges: 1) preparing the whole mouse brain such that every neurite is traceable and every synapse is identifiable, 2) imaging with high-throughput scanning electron microscopy, and 3) reliably sectioning the sample at 20 - 30 nm thickness using an automated in-chamber microtome. We present results that the first challenge, sample preparation, has been addressed through our development of the BRAX (Brain-wide Reduced osmium staining with Amplification and eXtracellular space preservation) protocol, which produces samples that are suitable for mapping of the whole mouse brain. The second challenge, high-throughput imaging, seems to have been addressed with the recent introduction of the multibeam SEM, which we have used to acquire images from BRAX brains at >1 GHz pixel acquisition rate. The final challenge is reliable in-chamber thin cutting. While progress has been made on this front, reliably cutting whole-brain samples at 20 - 30 nm thickness is an as yet unsolved problem.

Disclosures: **S. Mikula:** None. **W. Denk:** None.

Poster

098. Imaging Advances: Neural Ultrastructure

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Topic: G.03. Staining, Tracing, and Imaging Techniques

Support: NIH grant MH067105

Title: Toward connectomic analysis of the zebra finch song system

Authors: *J. KORNFELD[1], F. SVARA[1], M. PICARDO[2], M. STETNER[3], G. KOSCHE[2], S. BENEZRA[2], M. S. FEE[3], M. LONG[2], W. DENK[1];
[1]BMO, Max Planck Inst. For Med. Res., Heidelberg, Germany; [2]Neurosci., New York Univ., New York City, NY; [3]McGovern Inst. and Dept. of Brain and Cognitive Sci., MIT, Cambridge, MA

Abstract: Connectomic analysis of neuronal circuits promises to provide insights into songbird questions such as HVC's mechanism of ultra-sparse code generation or the mechanism of song learning in Area X. Serial block-face electron microscopy (SBEM) can be used to acquire 3D EM datasets, at synaptic resolution, that span hundreds of micrometers in all dimensions and therefore contain entire microcircuits. We acquired several teravoxel-sized SBEM datasets of Area X and HVC (different parts of the zebra finch song control system) and are in the process of testing anatomical predictions made by various functional models. Circuit reconstruction is performed using a combination of human annotations and machine learning.

Disclosures: **J. Kornfeld:** None. **M.S. Fee:** None. **F. Svara:** None. **W. Denk:** Other; License income from SBEM / Gatan Inc.. **M. Stetner:** None. **M. Long:** None. **S. Benezra:** None. **G. Kosche:** None. **M. Picardo:** None.

Poster

098. Imaging Advances: Neural Ultrastructure

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Topic: G.03. Staining, Tracing, and Imaging Techniques

Support: Swiss National Foundation Sinergia

Title: A correlative light and electron microscopy approach for reconstructing syringeal motor neuron circuits in a songbird

Authors: *T. TEMPLIER[1,2], R. H. R. HAHNLOSER[1,2];
[1]Inst. of Neuroinformatics, Univ. of Zurich and ETH Zurich, Zurich, Switzerland; [2]Neurosci. Ctr. Zurich, University of Zurich and ETH Zurich, Switzerland

Abstract: What are the identities of postsynaptic partners of a given neuron? Correlative array tomography (CAT) is a candidate technique to address this question; it consists of imaging hundreds of consecutive ultrathin sections of brain tissue using both light (LM) and electron (EM) microscopy, combining the strengths of the two imaging modalities, namely fast acquisition and multicolor imaging for LM and nanometer resolution for EM. We show that five conventional neuroanatomical tracers (fluorescent dextrans and biotinylated dextran amine) previously injected *in vivo* into a nervous system can be simultaneously visualized with LM after a single step of postembedding immunohistochemistry against fluorophores and biotin. The LM imaging is performed in an unattended manner after mapping of the coordinates of hundreds of consecutive sections present on a single conductive silicon wafer. Sections are further poststained and the wafer is placed into the imaging chamber of a scanning electron microscope, where unattended EM imaging is done at 5 MHz, providing images with excellent ultrastructure for reliable neuron tracing and identification of synaptic contacts. Finally, LM and EM images are automatically registered, yielding a 3-dimensional correlative LM-EM stack. In a songbird we aim to characterize how cortical and brainstem neurons project to different motoneuron pools that control the muscles of the vocal organ. Contextual information about projection neuron type is provided by LM visualization of neuroanatomical tracers injected *in vivo* into the different muscles of the vocal organ, into the brainstem, and into the motor cortex. By contrast, neuron tracing and unambiguous identification of synaptic contacts is achieved using EM.

Disclosures: **T. Templier:** None. **R.H.R. Hahnloser:** None.

Poster

098. Imaging Advances: Neural Ultrastructure

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Title: Combined whole-brain optical and electron microscopic imaging in the larval zebrafish

Authors: *D. G. C. HILDEBRAND[1,2], G. S. PLUMMER[1], R. PORTUGUES[1], I. H. BIANCO[1], T. M. QUAN[2], W.-K. JEONG[2], J. W. LICHTMAN[1], F. ENGERT[1];
[1]Harvard Univ., Cambridge, MA; [2]Ulsan Natl. Inst. of Sci. and Technol., Ulsan, Korea, Republic of

Abstract: A central goal of neuroscience is to determine how neuronal connectivity enables the information processing that underlies perception and action. Modern light- and electron-based imaging technologies enable detailed examination of the functional and structural features of neurons. When combined and scaled, these methods permit interrogation of structure-function relationships in neuronal circuits. For most model organisms, however, it remains difficult to perform such studies at the whole-brain level. The larval zebrafish is a vertebrate model organism that offers convenient optical access to its entire nervous system. Additionally, its small size makes it an excellent system for whole-brain examination with serial-section electron microscopy. We describe new methods that take advantage of the larval zebrafish model to enable structure-function studies in a complete vertebrate brain, present data that is being used to generate a proof-of-concept high-resolution larval zebrafish brain atlas, and show correspondence of neuron identity across imaging modalities.

Disclosures: D.G.C. Hildebrand: None. **G.S. Plummer:** None. **R. Portugues:** None. **I.H. Bianco:** None. **T.M. Quan:** None. **W. Jeong:** None. **J.W. Lichtman:** None. **F. Engert:** None.

Poster

098. Imaging Advances: Neural Ultrastructure

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CNPq Bolsa Jovine Talento Class A

Title: Microtubules imaged in three dimensions by electron microscopy without averaging

Authors: *A. FERA[1,3], T. S. REESE[3], M. VALE DE SOUSA[1], B. M. RIBEIRO[2], D. L. SACKETT[4];
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Abstract: We report here the application to microtubules of a straightforward method to perform high-resolution EM tomography, including measurements. Under conditions where absorption contrast makes the largest contribution to an electron image, the analysis of the data can be done in real space without squaring individual contributions as is generally done. Eliminating the dependence on the imaginary part of the scattering wave function allows a much more direct combination of the images. Therefore, with a negative stain able to sustain a high electron dose, tomograms can be collected that allow a resolution directly at the pixel size smaller than 1 nm. It proved necessary to collect images above 160 keV in order for the negative stain not to rearrange after irradiation, as shown by Fera et al., 2012. Given that our observation likely depends on the negative stain making a closed shell of metallo-organic salt, the improvement could be realized with other staining methods. Here, we apply this method to a well-known cytoskeleton element, previously characterized by averaging methods. Microtubules reconstituted *in vitro* from rat-brain tubulin were deposited on carbon-only coated 200 mesh copper grids, negative stained with methyl-amine tungstate and imaged with a 200 keV Tecnai TEM equipped with a high-sensitivity 4096 pixel CCD camera. The round individual tubulin polymers appear much less uniform and more fragile than with averaging methods, and show a variable number of protofilaments and hence diameter, as also shown by AFM. As expected, microtubules typically appeared circular and hollow but with a height-to-width ratio of ~0.6 in accordance with recent finding on single microtubules. We suggest that these data open the way for studying more directly how enzymes cytoskeletonal components bind to microtubules.

Disclosures: **A. Fera:** A. Employment/Salary (full or part-time):; Universidade Federal de Brasilia, Brazil. **T.S. Reese:** A. Employment/Salary (full or part-time):; National Institutes of Health. **M. Vale de Sousa:** A. Employment/Salary (full or part-time):; Universidade Federal de

Brasilia, Brazil. **B.M. Ribeiro:** A. Employment/Salary (full or part-time):; Universidade Federal de Brasilia, Brazil. **D.L. Sackett:** A. Employment/Salary (full or part-time):; Eunice Kennedy Shriver National Institute of Child Health and Human Development.

Poster

098. Imaging Advances: Neural Ultrastructure

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Eranet-Neuron

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Departamento Salud, Gobierno de Navarra

Title: Light and electron microscopic detection of GPCR heteromeric complexes in the macaque basal ganglia using the *in situ* proximity ligation assay

Authors: *J. L. LANCIEGO[1,3], I. G. DOPESO-REYES[2,3], A. J. RICO[2,3], S. SIERRA-SAN NICOLAS[2,3], E. RODA[2,3], M. LANZ[2], D. PIGNATARO[2,3], D. SUCUNZA[2], D. FARRE[5,4], R. FRANCO[5];
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Abstract: Here the *in situ* proximity ligation assay (PLA) was used to characterize a number of G protein-coupled receptor heteromeric complexes across different basal ganglia nuclei in macaques, comprising control animals as well as animals rendered parkinsonian (with and without levodopa-induced dyskinesia). The PLA technique enabled us to demonstrate the presence of a number of GPCR receptor heteromers in both the input and the output basal ganglia nuclei. At the level of the caudate-putamen (input nuclei), different types of GPCR

heteromers were found, those comprising dopaminergic D1-D3 and cannabinoid CB1-GPR55 receptor heteromers. When considering the internal division of the globus pallidus (GPi, output nucleus), we focused on cannabinoid CB1-CB2 heteromers as well as on receptor heteromers made of adenosine 2A (A2A) and cannabinoid receptor types 1 and 2 (CB1 and CB2). The putative changes in the number of all these different types of GPCR heteromers in the different diseased states was qualitatively assessed with the confocal microscope. Most importantly, ultrastructural confirmation of the pre- and/or post-synaptic localization of cannabinoid receptor heteromers was provided. To the very best of our knowledge, this represents the first time in which the PLA technique was taken to the electron microscope. Throughout these studies, we took advantage of different technical recipes for the PLA assay, including: (i) fluorescent detection by labeling secondary antibodies with PLA probes, (ii) fluorescent detection following direct labeling of primary antibodies and (iii) ultrastructural detection of PLA-labeled primary antibodies using peroxidase-labeled oligonucleotides, the latter incubated with colloidal gold-tagged goat anti-peroxidase antibody and finally visualized with a silver enhancement solution.

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Poster

098. Imaging Advances: Neural Ultrastructure

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Topic: G.03. Staining, Tracing, and Imaging Techniques

Support: SfN Neuroscience Scholars Program

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P30EY014801

Title: A new laser mediated traumatic optic neuropathy model

Authors: *G. C. MUNGUBA[1], R. K. LEE[2];
[2]Bascom Palmer Eye Inst., [1]Univ. of Miami Miller Sch. of Med., Miami, FL

Abstract: Purpose: Current models of traumatic ocular neuropathy rely on blunt force trauma delivered by mechanical mechanisms. These models generate gross generalized damage that can

often very severe and lack good correlation to injuries observed with humans. We have developed a novel retinal injury animal model using a Nd:YAG laser generating a photodisruptive force above the retina to focus injury of a determined magnitude reliably into the rodent eye which is reproducible, can be followed longitudinally *in vivo*, is limited to the eye and does not result in mortality. This novel method more closely mimics the types of retinal injury and structural damage observed with human traumatic optic neuropathy. Methods: A Nd: YAG l laser was used to generate a photodisruptive blast injury with 0.4 mJ of energy above the level of the retina and behind the lens in the peripapillary region in Thy1-ChR2/EYFP murine eyes (n=45). Eyes were imaged using spectral domain optical coherence tomography (SD-OCT) and a confocal scanning laser ophthalmoscope (CSLO) before laser treatment and up to 20 weeks after treatment along with IOP measurements. Mice were sacrificed at various time points and perfused transcardially with paraformaldehyde. Histology of whole mount retina and retinal sections was determined with hematoxylin/eosin (HE) and immunostaining with RGCs markers, such as brn3b and thy-1. Results: After one week, the retina ganglion cells (RGCS) were observed to be significantly diminished in number in a wedge shape origination from the circumpapillary region of laser treatment as measured by CSLO (HRT). By 20 weeks, a similar pattern of ganglion cell layer loss was measured using SD-OCT. IOP was not statistically different at all measured time points. Histologically, HE staining demonstrated a significant loss of RGCs and nerve fiber layer, consistent with the imaging results from SD-OCT and CSLO only in laser associated regions. Similarly, immunohistological staining for RGC markers confirmed RGC loss in the regions of laser treatment compared to adjacent non-lasered regions of the retina. Conclusions: We developed a new model using a photodisruptive force generated by a Nd:YAG laser to reliably deliver blunt trauma to the retina and cause loss of RGCs and nerve fibers similar to that observed in traumatic optic neuropathy that is amenable to study of neuroprotective strategies *in vivo*.

Disclosures: **G.C. Munguba:** None. **R.K. Lee:** None.

Poster

099. Network Models and Computational Studies

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

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Topic: G.06. Computation, Modeling, and Simulation

Support: Spanish Government Grant RYC-2011-08755

Title: Exact firing rate equations for all-to-all connected networks of quadratic integrate-and-fire neurons

Authors: *A. C. ROXIN[1], D. PAZÓ[2], E. MONTBRIÓ[3];
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Abstract: Spiking network models are an important theoretical tool for understanding the collective dynamics of large numbers of recurrently coupled neurons. Most studies of network dynamics have been numerical, while in a few cases it has been possible to calculate stationary network states as well as their stability, e.g. [1,2]. Unfortunately a general analysis of non-stationary dynamical states in spiking network models has not been possible. Such an analysis would be valuable given that non-stationarity in brain activity is likely the norm and not the exception. Here we show that the dynamics of networks of all-to-all connected quadratic integrate-and-fire neurons is described exactly by a low-dimensional set of equations, in the limit of large system size. Specifically, if heterogeneity in the system is purely quenched (i.e. no external noise injection), then the dynamics for each network in the model evolves along a two-dimensional manifold which is globally attracting. The two dimensions are the mean firing rate and mean subthreshold potential, the dynamics of which obey coupled, nonlinear differential equations. These equations capture the response of the network to arbitrary external inputs, as well as intrinsically generated dynamical states. [1] Amit and Brunel, Network 1997. [2] Brunel and Hansel, Neural Computation 2006.

Disclosures: **A.C. Roxin:** None. **D. Pazó:** None. **E. Montbrió:** None.

Poster

099. Network Models and Computational Studies

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

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Title: Emergence of spatially localized slow activity in structured leaky-integrate- and-fire networks

Authors: *Y. N. BILLEH[1], M. T. SCHAUB[2], C. A. ANASTASSIOU[3], M. BARAHONA[2], C. KOCH[3];
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Abstract: Unraveling the interplay between neuronal connectivity and the spatio-temporal dynamics exhibited by neuronal networks is a key direction to advance our current understanding of neuronal information processing. Here we investigate how certain topological features of leaky-integrate-and-fire (LIF) networks underpin the propensity of the network to generate *spatially localized slow* (SLS) activity, defined as the simultaneous increase in the firing frequency and in the coherence of action potentials over particular sub-groups of neurons [1]. Using full-scale dynamical simulations, we show that the ability of LIF networks to support SLS dynamics is pre-determined by spectral properties of the asymmetric synaptic weight matrix. In particular, when it exhibits an eigenvalue gap and the leading eigenvalues are almost real, the observed SLS activity is approximately proportional to the gap. Furthermore, the separation of the leading eigenvalues is linked to a localization of the associated Schur vectors on groups of neurons, leading to spatially coherent dynamical activity on those groups. To understand the origin of the eigenvalue gap, we consider stylized analytical rate models and use the insights gained to develop new network topologies with alternative connectivity paradigms that display SLS activity. Specifically, such dynamics can be achieved by modifying merely the connectivity patterns *between* excitatory and inhibitory neurons leading to SLS dynamics involving both excitatory and inhibitory neurons. We also show that SLS activity can be exhibited on multiple hierarchical timescales. Our work provides a step towards understanding the influence of network structure (increasingly uncovered through advancements in neuroanatomy and connectomics) on spatio-temporal neural activity and the dynamical roles of such neuronal circuits. **References** [1] A. Litwin-Kumar and B. Doiron (2012), “Slow dynamics and high variability in balanced cortical networks with clustered connections”, Nat Neurosci, 15, 1498

Disclosures: **Y.N. Billeh:** None. **M.T. Schaub:** None. **C.A. Anastassiou:** None. **M. Barahona:** None. **C. Koch:** None.

Poster

099. Network Models and Computational Studies

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Topic: G.06. Computation, Modeling, and Simulation

Support: ONR MURI Grant N00014100072

ONR Grant N000141310672

Title: Feedback connections stabilize propagation of synchronous spiking in cortical neural networks

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Abstract: Precisely timed action potentials have been related to stimuli and behavior in monkeys, indicating that the neural coding may be based on precise spike timing of cortical neurons, synfire chains. An assumption that sensory information may be encoded by precise spike timing has been challenged by the critical question of whether synfire chains can successfully propagate through hierarchies of cortical areas. It is possible that noise would destroy millisecond precision during transmission of synfire chains through many layers of cortical networks. Previous studies demonstrated that synfire chains can propagate through the layers of a feedforward network. In the feedforward models, a spike precision sharpens as it propagates through the network. A separatrix divides the state space into two areas: stable and unstable. In the stable area, all trajectories converge into an attractor state representing successful propagation of synfire chains, and neural activity starting anywhere inside this area reaches a stable state with millisecond precision. Synfire chains starting outside the stable area decay after a few steps of transmission. These observations suggest that only strong enough stimuli, which evoke high number of spikes would successfully propagate through the cortical layers without degradation of temporal precision, while neural activities that are too weak will die out. Here we show that inclusion of feedback connections into a feedforward model enhanced propagation of synfire chains through the network layers, while preserving temporal precision. The enhancement of synfire chains propagation was due to feedback inputs increasing the number of spikes in the synfire chains. This moved the initial state of the stimulus into the basin of attractor representing successful synfire chains propagation. In addition, the feedback inputs changed the position of the separatrix, moving it downward and, therefore, increasing the basin of the attractor of the propagation regime.

Disclosures: **S. Moldakarimov:** None. **M. Bazhenov:** None. **T.J. Sejnowski:** None.

Poster

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Program#/Poster#: 99.04/TT83

Topic: G.06. Computation, Modeling, and Simulation

Support: NIH-1R01NS070865-01A1

NSF-DMS-1313225

Title: Breaking asynchrony in balanced networks with spatially dependent recurrent connections

Authors: *R. ROSENBAUM[1,2], J. E. RUBIN[1], B. DOIRON[1];
[1]Mathematics, Univ. of Pittsburgh, Pittsburgh, PA; [2]Applied and Compuational Mathematics and Statistics, Univ. of Notre Dame, Notre Dame, IN

Abstract: The spiking dynamics of networks of cortical neurons exhibit strong spatiotemporal variability. Balanced network models -- characterized by a dynamically stable balance between strong excitatory and inhibitory synaptic currents -- offer an appealing theoretical framework for studying this neural variability. Balanced networks produce intrinsically noisy spiking dynamics with statistical features similar to those observed in cortical recordings, including an asynchronous state in which pairwise correlations between neuronal spike trains are extremely small on average [1]. While this asynchronous state is consistent with some experimental recordings [2], it is inconsistent with many other recordings that show moderate correlations between the spike trains of cortical cells [3]. This raises the question of whether recurrent networks can exhibit moderate correlations while maintaining a stable balance between excitation and inhibition. We show that balanced networks can, in fact, exhibit moderate pairwise correlations if the spatial heterogeneity of recurrent connections and correlated inputs is taken into account. We first derive concise conditions on the spatial profile of recurrent connections that is required for the asynchronous state to be realized in balanced networks. We then show that breaking these conditions can give rise to moderate spike train correlations, similar to those observed in many cortical recordings [3], while maintaining balance between excitation and inhibition. Our results indicate that the same balanced network can exhibit moderate or extremely weak correlations, depending on the statistical structure of the external input to the network. These findings could potentially resolve the ongoing debate over the magnitude of correlations in cortical networks [2,3]. 1. Renart, A., de la Rocha, J. et al. The Asynchronous State in Cortical Circuits. Science 327(5965): 587-590 (2010). 2. Ecker, A. et al.

Decorrelated Neuronal Firing in Cortical Microcircuits. Science 327(5965): 584-587 (2010). 3. Cohen, M.R. and Kohn, A. Measuring and interpreting neuronal correlations. Nat Neurosci 14(7): 811-819 (2011).

Disclosures: **R. Rosenbaum:** None. **J.E. Rubin:** None. **B. Doiron:** None.

Poster

099. Network Models and Computational Studies

Location: Halls A-C

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Topic: G.06. Computation, Modeling, and Simulation

Support: KAKENHI25430009

Title: Gene-Matched Network: A micro-circuit model constructed by combinatorial matching of neuronal diverse attributes

Authors: ***T. KITSUKAWA**, T. YAGI;
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Abstract: Micro-circuits in the brain are foundation of neuronal information processing. Recent physiological and anatomical researches have clarified that the micro-circuits are not random and not uniform in the connection among neurons. Rather, they are rich in bidirectional connections and clusters, and show small world property. These facts indicate that the brain micro-circuits consist of overlapping groups of neurons which are densely connected in each group. In order to model such micro-circuits, we constructed an artificial micro-circuit network model named "Gene-Matched Network (GMN)" by the matching of neuronal attributes. First, multiple attributes were randomly assigned to each neuron (e.g. attributes numbered as 5, 19, 32 and 46 out of numbers 1~50). These numbers are called ‘genes’ since this matching rule was inspired by the fact that neurons expressing the same recognition genes form connections. Second, therefore, connections were formed among neurons that expressed the same ‘gene’ (e.g. connecting all neurons with the number, '5'). The GMN contained high density of clusters and the small world property, similar to the brain micro-circuits. Further, the layered GMN could retain information even when the information was passed through 10 layers, while random networks with the same numbers of connections lost the information. This fact suggests that the GMN can be a basis of the information transfer in parallel information processing. In addition, we demonstrate that the information in the network can be represented as a vector using the gene numbers which were

used for making connection. This fact indicates that the matching rule is a novel method to represent information contained in networks. Further, we show how the GMNs could transform the information by concatenating GMN layers with various thresholds.

Disclosures: **T. Kitsukawa:** None. **T. Yagi:** None.

Poster

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Topic: G.06. Computation, Modeling, and Simulation

Support: DFG Grant IRTG 1740

FAPESP Grant 2011/50151-0

Title: A computational study on irregular, self-sustained activity states in cortical network models

Authors: ***A. C. ROQUE**[1], P. TOMOV[2], R. F. O. PENA[1], M. ZAKS[2];
[1]Physics, Univ. de Sao Paulo, Ribeirao Preto, Brazil; [2]Mathematics, Humboldt Univ. of Berlin, Berlin, Germany

Abstract: A question that arises when studying the self-sustained activity (SSA) of the cortex in the brain's resting state is what are the mechanisms responsible for SSA in the absence of external input. Most previous theoretical studies have tackled this question by considering random networks of integrate-and-fire neurons. They showed that SSA can exist under a balanced state in which excitatory and inhibitory inputs to a neuron mutually cancel each other. Here, we study this problem using a cortical network model with more realistic architecture and more realistic neuron models. Our model has hierarchical and modular architecture and is made of excitatory and inhibitory neurons that belong to 5 electrophysiological cortical cell classes: regular spiking (RS), intrinsically bursting (IB), chattering (CH), fast spiking (FS) and neurons that produce low threshold spikes (LTS). The first 3 neuron types are excitatory and the other 2 are inhibitory. Neurons were modeled by the Izhikevich model and synapses were modeled in a conductance-based way so that when a presynaptic neuron fires a fixed synaptic conductance increment (gex/gin) is added to the corresponding synaptic conductance and, after that, the synaptic conductance decays exponentially. We considered different versions of our model with

different number of modules and neuronal compositions. The population of excitatory neurons (80% of total) was composed of a mixture of up to two cell types, RS cells (always present at a variable proportion) and either CH or IB cells (at the remaining proportion), and the population of inhibitory neurons (20% of total) was composed of only one cell type, either FS or LTS. The models received external current injection of variable amplitude applied to a variable fraction of the neurons for a variable short time interval (initial conditions) and were left to evolve freely after the end of this current. For each network realization and different initial conditions we determined the region of the gex-gin parameter space for which the system exhibited SSA with firing properties similar to the ones observed experimentally. For all studied networks this region was highly fragmented but concentrated in an area of the parameter space in which the ratio gin/gex is between about 4 and 12. This suggests that the balance mechanism is also operating in our models. However, we observed additional mechanisms that favor SSA. The SSA lifetime increased with the number of modules and when the network was made of RS and CH excitatory neurons and LTS inhibitory neurons. These new mechanisms point to a synergy between network topology and neuronal composition of the network in the generation of SSA cortical states.

Disclosures: **A.C. Roque:** None. **P. Tomov:** None. **M. Zaks:** None. **R.F.O. Pena:** None.

Poster

099. Network Models and Computational Studies

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Program#/Poster#: 99.07/TT86

Topic: G.06. Computation, Modeling, and Simulation

Support: NSF-DMS-1313225

Title: How STDP shapes the microcircuit structure of neuronal networks

Authors: *G. K. OCKER[1], A. LITWIN-KUMAR[2], B. DOIRON[2];
[1]Neurosci., [2]Mathematics, Univ. of Pittsburgh, Pittsburgh, PA

Abstract: In neocortex, specific microcircuits of connected pairs and triplets of neurons are over-represented compared to unstructured, random networks[1, 2]. These connectivity patterns are related to stimulus preference and activity levels[3]. Furthermore, theoretical studies show that they play an important role in shaping spiking activity[4, 5]. Understanding how microcircuit structure arises is an important open question with implications for how neuronal networks

develop, learn and process information. We have developed a mathematical framework for how spike timing-dependent plasticity (STDP) shapes the microcircuit structure of neuronal networks. We have combined a linear response theory for spiking statistics in networks of integrate-and-fire neurons[6] and an adiabatic theory for STDP[7]. Our theory self-consistently relates the evolution of spiking activity to the plasticity of the network structure. We then use this theory to derive a low-dimensional, closed dynamical system for the strengths of divergent, convergent and chain microcircuit motifs in the network. The form of this system naturally divides STDP rules into three classes, based on the relative shapes of the potentiation and depression windows and how the total amount of STDP relates to the synaptic weights. We show how the class of the STDP rule strongly determines the formation of microcircuit structure in the network. In particular, for additive STDP with balanced potentiation and depression, the underlying structural anatomy of the network is a crucial factor in determining the coordinated evolution of convergent, divergent and chain motifs across the network. References 1. Song S, Sjöström PJ, Reigl M, Nelson S, Chklovskii D: **Highly nonrandom features of synaptic connectivity in local cortical circuits.** *PLoS Biol.* 2005, **3.3**:e68 2. Perin R, Berger TK, Markram H: **A synaptic organizing principle for cortical neuronal groups.** *PNAS* 2011, **108.13**: 5419-5424 3. Ko H, Hofer S et al: **Functional specificity of local synaptic connections in neocortical networks.** *Nature* 2011, **000**:1-5 4. Hu Y, Trousdale J, Josić K, Shea-Brown E: **Motif statistics and spike correlations in neuronal networks.** *J. Stat. Mech.* 2013, **P03012** 5. Zhao L, Beverlin B II, Kakalios J, Nykamp DK: **Synchronization from second order connectivity statistics.** *Front. Comp. Neuro.* 2011, **5**:28 6. Trousdale J, Hu Y, Shea-Brown E, Josić K: **Impact of network structure and cellular response on spike time correlations.** *PLoS Comp. Biol.* 2012, **8**:e1002408 7. Kempter R, Gerstner W, van Hemmen JL: **Hebbian learning and spiking neurons.** *Phys. Rev. E* 1999, **59.4**: 4498-4514

Disclosures: **G.K. Ocker:** None. **A. Litwin-Kumar:** None. **B. Doiron:** None.

Poster

099. Network Models and Computational Studies

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Topic: G.06. Computation, Modeling, and Simulation

Support: NSF Grant IIS-1116530

Title: Non-random network structure of recurrent networks with STDP and potentiation decay

Authors: *A. J. MILLER[1], D. Z. JIN[2];
[1]Bridgewater Col., Bridgewater, VA; [2]Physics, Penn State Univ., State College, PA

Abstract: Spike-timing dependent plasticity (STDP) applied to synapses in recurrent networks of spontaneously active neurons leads to unstable synaptic growth. The instability is caused by feedback-driven growth along randomly strengthened synapses. Therefore, an additional homeostatic mechanism must be added to the dynamics of the synapses in order for recurrent networks to remain in an asynchronously spiking state. One possible homeostatic mechanism that can counteract the feedback-driven synaptic growth is a gradual, activity-independent potentiation decay. This combination of synaptic rules produces networks characterized by unimodal, statistically stationary distributions of synaptic strengths. While the distribution of strengths remains stationary, an individual synaptic connection fluctuates through a range of values. It has been previously shown that this combination of learning rules contributes to emergent synfire chain synaptic topology upon repeated external stimulation of recurrent networks initially in the stationary state. However, little else is known about network structure of these networks. Here we explore the network structure of statistically stationary recurrent networks, applying network measures such as strength and degree distributions, and clustering coefficients. We compare these measurements to the corresponding measures of random networks with identical distributions of synaptic strengths. Sample stationary networks for the analyses are produced by simulations of pulse-coupled leaky integrate-and-fire (LIF) neurons subjected to noisy external inputs and feedback inhibition. An additive-LTP, multiplicative-LTD STDP rule is applied to synapses along with a slow potentiation decay. For a range of potentiation decay rates and LTP strengths, various network measures are applied to samples of N=50 statistically stationary synaptic connectivities. Network measures averaged over each sample set suggest that the networks are not randomly structured, but rather contain a modular structure that depends on the potentiation decay rate.

Disclosures: **A.J. Miller:** None. **D.Z. Jin:** None.

Poster

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Topic: G.06. Computation, Modeling, and Simulation

Support: NIH grant R01 NS049288-06

Title: Modeling spike timing dependent plasticity in large cortical networks of biologically realistic neurons with GENESIS 2.4

Authors: *D. BEEMAN[1], H. CORNELIS[2];
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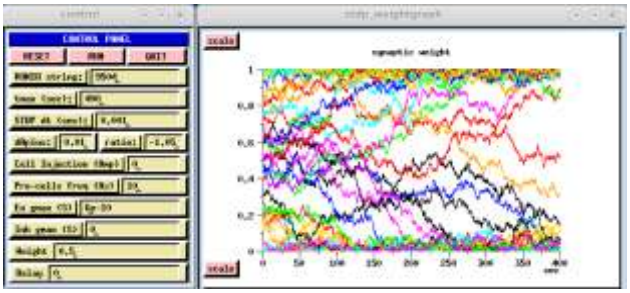
Abstract: The Song, Miller, and Abbott (SMA) model of spike timing dependent plasticity (STDP) is a popular simple model of synaptic plasticity. It is typically implemented on networks of point integrate and fire neurons that behave differently than structurally realistic multicompartmental models. Until now neither of the two most popular simulators for modeling multicompartmental neurons (GENESIS and NEURON) provided a command or class of objects to model this type of plasticity. The object oriented paradigm for scripting simulations in GENESIS 2 allows modelers easily to modify/share/reuse pieces of simulation scripts or to extend the simulator itself. To implement this paradigm efficiently, GENESIS makes use of an 'hsolve' object that can increase the speed of network simulations by a factor of 10 to 20. However, this efficiency limits the ability to easily add new synaptic models. Inflexibilities of the architectures of 1990s era simulators were the reason that GENESIS development shifted to GENESIS 3 (G-3) after the release of GENESIS 2.3. G-3 is still in a development stage, and it was necessary to give GENESIS 2 a mechanism for hsolvable models of synaptic plasticity. This was achieved with changes to the core simulator code with an 'stdp_update' object that implements modifiable SMA mechanisms to act on GENESIS 'synchan' objects. It allows for axonal conduction delays and can easily be extended by users to add other types of plasticity. Although GENESIS 2.3 was intended as a final release of the GENESIS 2 series, it has continued to be widely used for large network models of multicompartmental neurons and for projects in modeling courses such as LASCON. Thus, there will be a 2.4 release in November 2014. It contains recent improvements, user contributions, tutorials, and new capabilities for modeling large networks, including hsolvable synaptic plasticity. GENESIS may be downloaded from http://genesis-sim.org/GENESIS. Figure 1 shows the development of 32 synaptic weights in a GENESIS modified SMA model.



Disclosures: **D. Beeman:** None. **H. Cornelis:** None.

Poster

099. Network Models and Computational Studies

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Topic: G.06. Computation, Modeling, and Simulation

Support: Army Research Laboratory contract no. W911NF-10-2-0022

Alfred P. Sloan Foundation

Title: Using stimulation to reveal structure-function relationships in dynamic brain networks

Authors: *S. E. FELDT MULDOON[1,2], J. M. VETTEL[2], D. S. BASSETT[1];
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Abstract: Understanding the brain as a complex network of interacting components can provide insight into cognitive function. From this perspective, one can study two types of networks: the anatomical network composed of physical connections between neurons or brain regions, and the functional networks constructed from coherent neurophysiological activity. The relationship between these two types of networks is far from understood, and many questions therefore remain. Do underlying anatomical networks drive functional networks and if so how? What constraints do anatomical connections play in potential patterns of functional activations? In a novel approach to addressing this relationship, we examine the effects of stimulation on functional network structure in settings with a known underlying connectivity using a joint approach combining meso-scale computational modeling with comparisons to experimental stimulation data. We first derive a spatially embedded network model of brain dynamics using biologically motivated Wilson-Cowan oscillators, connected using a realistic inter-regional connectivity map obtained from diffusion spectrum imaging (DSI) of white matter tracts. In silico, we then systematically stimulate different nodes (brain regions) of our network to determine how structural node properties (such as the degree, betweenness, clustering, etc.) influence the ability of the node to modify functional network structure. These results are compared to experimental ECoG data obtained from epilepsy patients undergoing cortical stimulation mapping prior to resective surgery. We examine the relationships among structural connections, functional connections, and physical proximity to understand how these different networks interact as a function of stimulus amplitude and location.

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Poster

099. Network Models and Computational Studies

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Topic: G.06. Computation, Modeling, and Simulation

Support: DFG Collaborative Research Center SFB910

Title: Spike rate dynamics of coupled adaptive model neurons

Authors: *J. LADENBAUER[1,2], M. AUGUSTIN[1,2], K. OBERMAYER[1,2];
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Abstract: How the properties of single neurons and their coupling give rise to different types of functionally relevant collective dynamics can be effectively studied using population activity models derived from recurrently coupled spiking model neurons and mathematical analysis techniques (see, for example, [1-3]). Here we first derive a low-dimensional model for instantaneous population spike rates from a network of adaptive spiking model neurons and then use this reduction method to examine how changes in neuronal excitability can (de)stabilize different network states. Specifically, we consider adaptive integrate-and-fire neurons [4], that can well reproduce the activity of cortical neurons, and in-vivo like fluctuating inputs. This neuron model includes a description of slowly decaying potassium currents (so-called adaptation currents), which have been shown to strongly affect neuronal spiking activity [5,6]. We extend different reduction techniques based on the Fokker-Planck equation [2,7] to take into account adaptation currents and we evaluate the reduced population activity models in terms of spike rate reproduction accuracy for a range of biologically plausible input statistics, computational demand and implementation complexity. This approach allows for the application of powerful methods to analyze the stability of network states, where, for example, stability bounds can be calculated for general coupling topologies [8,3]. Additionally, a direct link between macroscopic quantities (network activity) and microscopic properties (neuron biophysics) is retained. Using this framework we demonstrate how changes in neuronal excitability via adaptation currents lead to the (de)stabilization of asynchronous states as well as fast and slow oscillatory activity generated by different mechanisms. In this way we identify network regimes where switching between different dynamical states can be mediated by (top-down) neuromodulatory signals that target adaptation currents [9]. [1] X.-J. Wang, Physiol. Rev. 90 (2010) [2] E. Schaffer, S. Ostojic,

L. Abbott, PLOS Comput. Biol. 9 (2013) [3] S. Ostojic, Nat. Neurosci. 17 (2014) [4] R. Brette and W. Gerstner, J. Neurophysiol. 94 (2005) [5] J. Ladenbauer, M. Augustin, K. Obermayer, J. Neurophysiol. 111 (2014) [6] M. Augustin, J. Ladenbauer, K. Obermayer, Front. Comput. Neurosci. 7 (2013) [7] S. Ostojic and N. Brunel, PLOS Comput. Biol. 7 (2011) [8] J. Ladenbauer, J. Lehnert, H. Rankoohi et al., Phys. Rev. E 88 (2013) [9] D.A. McCormick, Progr. Neurobiol. 39 (1992)

Disclosures: **J. Ladenbauer:** None. **M. Augustin:** None. **K. Obermayer:** None.

Poster

099. Network Models and Computational Studies

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Topic: G.06. Computation, Modeling, and Simulation

Support: BACS FP6-IST-027140

BIND MECT-CT-20095-024831

ANR-10-LABX-0087 IEC

ANR-10-IDEX-0001-02 PSL*

Title: Learning linear dynamical systems in spiking networks

Authors: *R. BOURDOUKAN, S. DENÈVE;
Ecole Normale Supérieure, Paris, France

Abstract: We live in a perpetually changing world where timescales of perceptual decisions or motor actions vary from a few milliseconds to several minutes. These tasks require constant temporal predictions of the future sensory inputs and motor trajectories. Many have been formalized as dynamical systems. It is however unclear how spiking networks can learn to implement such dynamics. Using a purely top down approach, we derive local current-based Hebbian plasticity rules allowing spiking neural networks to learn to implement a wide range of linear dynamical systems. The trained network exhibits many observations seen in the cortex such as irregular spiking and balance between excitation and inhibition. Learning in recurrent spiking networks is a notoriously difficult problem. Local changes in connectivity may have an unpredictable effect on the global dynamics. In addition, reproducing the Poisson-like statistics

of neural responses require the use of networks with balanced excitation and inhibition. These networks are chaotic and highly sensitive to initial conditions or perturbations. Here we overcome these limitations by combining a spike-based predictive coding approach with supervised learning. We use a recurrent network of LIF neurons that displays two types of connectivity: fast and slow. Fast connections are trained to balance excitation and inhibition. This produces Poisson-like spike train statistics, but also enforces a maximally efficient spike-based code. On top of that, a "sensory feedback" loop provides a very strong error signal to the untrained network, serving as a teaching current for slow connections. Under the effect of this strong feedback loop, the output of the neurons becomes effectively independent of the recurrent connectivity. This allows us to derive a simple Hebbian plasticity rule for slow connections from a gradient descent over a loss function. Over the course of learning, the prediction error, and thus the teaching signal gradually attenuate and disappear, as the network becomes autonomous. We found that these networks could learn extremely efficient (i.e. accurate and with a very small number of spikes) implementation of a wide variety of linear dynamical systems such as oscillators or integrators. The resulting network can be considered as "minimal" (in terms of connectivity and activity) in order to implement a given function. This contrasts with approaches based on liquid computing, where very large and dynamically complex networks have to be used.

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Poster

099. Network Models and Computational Studies

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Topic: G.06. Computation, Modeling, and Simulation

Title: Feedforward supervised learning for deep neural networks with sparse dynamics

Authors: *T. SHINOZAKI, Y. NARUSE;
BIT laboratory, NICT, Kobe City, Hyogo, Japan

Abstract: A multilayered deep neural network is one of the most powerful methods for human-like recognition tasks, image (Le et al., 2012), and speech recognition (Dahl et al., 2012). Some previous studies demonstrated great performances with supervised learning in signal classification tasks (LeCun et al., 1989; Krizhevsky et al., 2012). Supervised learning for multilayered neural network generally uses gradient-based learning rules, in particular, back-

propagation learning (Rumelhart et al., 1986). However, the amount of supervisory information in the last layer is insufficient to supervise the whole deep neural network because the information is selected and reduced from layer to layer. This tendency is more serious in signal classification tasks which have limited discrete output. Thus, layer-wised learning is generally used for both mutually connected (Bengio et al., 2007) and feedforward networks, resulting in difficulties in incremental learning and online updating. This study proposes a novel supervised learning method for multilayered neural networks that uses feedforward supervisory signal. The learning method requires sparse dynamics, and uses additional advance input as a supervisory signal to learn the target input. Before the target input, advance input, which produces the required classification label, is propagated through the whole network, and then the target input is processed with the after-effect of the advance input. We validated the efficiency of the proposed method by the visual recognition task of MNIST handwritten image dataset (LeCun & Cortes) with a five-layer feedforward network. The proposed method improved the error rate from 15.6±0.7 % just after the pre-training, to 4.2±0.2 % after 20 iterations of the training set (n=10). One of the interesting point the proposed learning method is that it could seamlessly incorporate both reinforcement learning and competitive learning. Reinforcement learning emerges if there is no advance input, and the usual competitive learning emerges if there is no correct/incorrect signal. Therefore, the learning mode could be selected by the sequence of input and correct/incorrect signals.

Disclosures: **T. Shinozaki:** None. **Y. Naruse:** None.

Poster

099. Network Models and Computational Studies

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 99.14/UU1

Topic: G.06. Computation, Modeling, and Simulation

Support: KAKENHI (22115013)

JSPS fellowship

Title: Learning higher-order structure of correlated input by excitatory and inhibitory spike-timing-dependent plasticity

Authors: N. HIRATANI[1,2], *T. FUKAI[3,1,4];
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Abstract: Spike-timing-dependent plasticity (STDP) is ubiquitously observed at synapses of the mammalian brain, and is considered to be critical for both developmental and adulthood neural plasticity. Previous studies suggest that STDP enables neurons to capture the principal component of input stimulus patterns and to learn the major feature of stimuli. However, it is still unclear how neurons capture higher-order components, especially when these features are encoded by synchronization of neural activity, not by firing rate. For example, the brain may extract faint sounds from a mixture of non-independent auditory sources, but the underlying mechanism remains unknown. Furthermore, it is unknown how the structure of spike correlation and various types of noise influence the learning process. In addition, recent experimental studies suggest that inhibitory synapses also show spike-timing-dependent synaptic plasticity, although its functional role is still uncertain. Here, we constructed a computational circuit model with linear Poisson neuron model to analytically investigate how and when STDP can detect higher-order structures. We show that lateral inhibition and inhibitory STDP are crucial for improving the performance of learning process.

Disclosures: **N. Hiratani:** None. **T. Fukai:** None.

Poster

099. Network Models and Computational Studies

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Topic: G.06. Computation, Modeling, and Simulation

Support: NSF Grant No DGE-1321851

Title: Autaptic connections shift network excitability and bursting

Authors: *L. K. WILES, D. S. BASSETT, D. F. MEANEY;
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Abstract: Although neurons receive thousands of inputs from other neurons in the brain, in some cases, a neuron synapses onto itself to form an autapse. Autapses are found in multiple brain areas, including pyramidal neurons in the cortex, but appear more commonly on inhibitory

neurons such as fast-spiking interneurons. Despite the evidence that they appear throughout the brain circuitry, the function of autapses on network dynamics is largely unexplored. We use a simple spiking model of neuronal activity in which neurons are connected in a distance dependent fashion and systematically adjust the fraction of autaptic connections to evaluate if these connections shift the network activity pattern. Moreover, we test if placing autaptic connections on highly connected neurons, which are thought to be influential on network dynamics, further shift network behavior. Based on studies in dissociated cortical neuronal networks, simulations consisted of networks of 800 excitatory and 200 inhibitory neurons, varying synaptic strengths, and averages of 75 excitatory and 18.75 inhibitory inputs onto a neuron. Spiking activity was analyzed for numbers of events per neuron and for coordinated firing across large numbers of neurons within a brief time period (network-wide bursting) defined in comparison to interspike-interval-shuffled surrogate data. We added a varying number of autapses either uniformly at random to neurons or preferentially to highly connected neurons, with appropriate statistical controls. In simulations without autapses, fast-spiking interneurons fired more frequently than excitatory neurons. Spontaneous bursting was significantly less common in networks where the excitatory synaptic strength was weaker than the inhibitory synaptic strength. In comparison, adding autaptic connections to excitatory neurons led to an increased number of spiking events in the network and to network-wide bursts in simulations where excitatory synapses were stronger than inhibitory synapses. Bursting behavior in the network occurred more readily with autaptic connections when autapses were selectively added to highly connected excitatory neurons rather than added to a random subset of neurons. Collectively, our results demonstrate that autapses on excitatory neurons increase network excitability and bursting. If selectively targeted to highly connected excitatory neurons in the network, the transition to bursting behavior will occur with fewer autaptic connections. Autapses provide an important network control mechanism that could play a key role in the excitability of healthy and diseased cortical networks.

Disclosures: **L.K. Wiles:** None. **D.S. Bassett:** None. **D.F. Meaney:** None.

Poster

099. Network Models and Computational Studies

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Program#/Poster#: 99.16/UU3

Topic: G.06. Computation, Modeling, and Simulation

Support: IRP, NEI, NIH, DHHS

Title: A new reduction method for setting synaptic weights of conductance-based neurons with slow and/or fast receptors to simulate behavior in a spiking neural network

Authors: P. M. DAYE[1], *L. M. OPTICAN[2];
[1]Movement disorders and basal ganglia laboratory, Inst. du Cerveau et de la Moelle épinière, Paris, France; [2]Natl. Eye Inst., BETHESDA, MD

Abstract: To simulate the function carried out by a neural area, using a large population of conductance-based neurons, one needs to compute a set of synaptic weights that will reproduce the behavior of that area. However, computing these weights is difficult, because of the non-linear dynamics of the mathematical model representing each neuron, and because the number of weights increases with the number of neurons. Therefore, approaches based on a reduced representation of the full conductance-based neuron model have been proposed (e.g., Ermentrout, 1994; Seung et al, 2001). These methods estimate an averaged representation of neuronal dynamics using the fact that slow receptors (e.g., NMDA, GABAB) dominate the overall dynamical behavior of the network. However, a model with neurons composed of either slow and/or fast receptors is required if we are to understand how drugs work in patients with brain diseases. Unfortunately, prior methods perform poorly with fast receptors (e.g., GLY, GABAA, AMPA). We developed an extension of the previous averaging techniques to compute the weights in networks composed of neurons with slow and/or fast synapses. Our new method reduces the conductance-based model of a neuron to a simpler dynamical model. The reduction technique is based on the total amount of neurotransmitter produced by the neuron over a period of time. The averaged model then estimates the amount of neurotransmitter produced by a neuron as a function of its synaptic inputs. The network weights are then computed by evaluating the change of synaptic conductance in a post-synaptic neuron generated by an afferent neuron. We demonstrate our new model reduction technique by computing the weights of a bilateral neural integrator. Then, we simulate the model of the integrator using either the full or the reduced representation of the neurons. To assess the quality of the reduced model, we first compare its dynamics to the dynamics of the full model (e.g., response to a step or a ramp input of neurotransmitter). Then we compare the integrator network built with either simplified or full representations of neurons (e.g., time constant of the persistent firing, sensitivity of persistent activity to eccentricity). Finally, using the integrator as benchmark, we compare our new technique to previous ones. We show that both approximate equally well the dynamics for slow receptors but our new technique better approximates the dynamics of fast receptors.

Disclosures: **P.M. Daye:** None. **L.M. Optican:** None.

Poster

099. Network Models and Computational Studies

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Topic: G.06. Computation, Modeling, and Simulation

Support: EPSRC Grant I005102

Wellcome Trust Grant 095668

Title: Learning in recurrent networks with the Neural Marketplace algorithm

Authors: S. N. LEWIS[1], P. YGER[2], *K. D. HARRIS[1];
[1]Univ. Col. London, London, United Kingdom; [2]Inst. de la Vision, Paris, France

Abstract: The brain consists of billions of neurons, which self-organize into the world's most powerful information-processing machine. Outside of biology, the only system known in which comparable numbers of autonomous units organize into productive networks, is the global economy. In the economy, money can be interpreted as a signal passing in the opposite direction to goods, that informs firms that their products are useful to consumers. By competing for limited quantities of this retrograde signal, firms therefore increase the benefit they play to the economy as a whole. In the brain, the classical form of communication between neurons is anterograde, carried by action potentials. But signals also pass backward along axons, carried by slow chemical messengers such as neurotrophins. During development, competition for such retroaxonal factors determines whether cells live or die. But retroaxonal signals can play additional roles in developmental plasticity, including controlling the strength of input synapses to the presynaptic cell [Du & Poo, Nature 2004; Du et al, PNAS 2009, Sharma et al, Neuron 2010]. We have hypothesized that in adult learning, retroaxonal signals carried by messagers such as neurotrophins may play an analogous role by stabilizing recent changes to neuronal input synapses [Harris, TINS 2008]. Competition for these factors would therefore reward neurons that have found information useful to downstream targets, and cause their representations to become stable. We describe a mathematical model for such a form of self-organization, in which a small number of "consumer" neurons receive explicit fast error signals, while a larger number of "producer" neurons compete to supply them with information, guided by retroaxonal signals from the consumers and from each other. We show how slow retroaxonal signals can allow producers to estimate their value to the global network, and how these estimates allow the network to perform a form of parallel search over multiple producer cells. We validate our approximations and demonstrate the proposed learning rule using simulations of firing-rate and spiking neural networks performing a real-world speech recognition task.

Disclosures: **S.N. Lewis:** None. **P. Yger:** None. **K.D. Harris:** None.

Poster

099. Network Models and Computational Studies

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Program#/Poster#: 99.18/UU5

Topic: G.06. Computation, Modeling, and Simulation

Support: NSF-DMS-1313225

Title: Dynamics of recurrent networks with multiple inhibitory subpopulations

Authors: *A. LITWIN-KUMAR[1,2], R. ROSENBAUM[2], B. DOIRON[2];
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Abstract: Computational studies have typically treated inhibitory interneurons as a homogeneous population, but recent advances in the characterization of interneuron subclasses reveal heterogeneous functional and connectivity properties. In the mouse visual system, three major interneuron subtypes, parvalbumin-expressing (PV), somatostatin-expressing (SOM), and vasointestinal peptide-expressing (VIP) neurons comprise 80-90% of inhibitory neurons. Connectivity between these subtypes follows stereotyped rules: for example, SOM neurons are inhibited only by VIP neurons and VIP neurons only inhibit SOM neurons (Pfeffer et al. 2013). Further, these neurons perform distinct computational roles: VIP neurons disinhibit the pyramidal neuron population during locomotion via suppression of SOM neurons (Fu et al. 2014) while activation of PV or SOM neurons divides or subtracts pyramidal neuron responses to oriented bars, respectively (Wilson et al. 2012, Atallah et al. 2012). We study the dynamics of networks with inhibitory neuron connectivity that matches experimental recordings. Our self-consistent theory describes conditions under which the dynamics of such networks are stable and how perturbations of distinct neuronal subclasses recruit activity changes through recurrence. We apply these conclusions to study disinhibition, surround suppression, and subtraction or division of orientation tuning curves in a model network. Our calculations and simulations establish conditions under which activity consistent with experiment are possible. They also lead to predictions concerning connectivity and network dynamics that can be tested via optogenetic manipulations. Finally, they illustrate that the recurrent dynamics of these networks must be taken into account to fully understand many effects reported in the literature. Pfeffer, C.K. et al. Inhibition of inhibition in visual cortex: The logic of connections between molecularly distinct interneurons. Nature Neuroscience 16(8): 1068-76 (2013). Fu, Y. et al. A cortical circuit for gain control by behavioral state. Cell 156(6), 1139-1152 (2014). Wilson, N.R., et al. Division and

subtraction by distinct cortical inhibitory networks *in vivo*. Nature 488(7411), 343-348 (2012).
Atallah, B.V. et al. Parvalbumin-expressing interneurons linearly transform cortical responses to visual stimuli. Neuron 73(1), 159-170 (2012).

Disclosures: **A. Litwin-Kumar:** None. **R. Rosenbaum:** None. **B. Doiron:** None.

Poster

099. Network Models and Computational Studies

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Title: Irregular and uncorrelated activity can arise as a natural consequence of synaptic inhibition

Authors: *J. E. RUBIN[1], C. O. DIEKMAN[2], D. TERMAN[3];
[1]Mathematics, Univ. of Pittsburgh, Pittsburgh, PA; [2]Mathematical Sci., New Jersey Inst. of Technol., Newark, NJ; [3]Mathematics, The Ohio State Univ., Columbus, OH

Abstract: Normal brain states are often characterized by irregular spiking activity, with little correlation among neurons within particular brain regions. In particular, there are many examples of inhibitory networks involved in such irregular activity. For example, experiments have revealed that, within the globus pallidus of the basal ganglia, the spike times across pairs of neurons are rather completely uncorrelated under normal resting conditions. Indeed, a hallmark of the Parkinsonian state is the replacement of this uncorrelated activity by more correlated firing. Another example is cortical networks in the dorsolateral prefrontal cortex, which have been identified as playing a key role in working memory tasks. Experiments have demonstrated that during persistent activity, neurons in this area exhibit highly irregular firing patterns with a

Poisson-like spike time distribution. Although there have been numerous theoretical and experimental studies of each of these brain regions, the mechanisms underlying their irregular and/or uncorrelated activity remain poorly understood.// Here, we introduce a novel mechanism for irregular dynamics in Hodgkin-Huxley-like conductance-based models for neuronal activity. Unlike many previous studies, it does not rely on a balance of excitation and inhibition. Nor does it require large-scale networks. In fact, the irregular spiking can be generated in purely inhibitory networks consisting of just two cells, from which it scales up to larger purely inhibitory or excitatory-inhibitory networks. Mathematical analysis of the model demonstrates that the irregular dynamics arises naturally through interactions between standard ionic currents and synaptic inhibition. The analysis leads to rather precise conditions on parameters for when irregular dynamics emerge.// We also consider how changing parameters in the model may switch the network activity between phase-locked and uncorrelated spiking. Such a switch in network activity is often associated with the switch between a normal brain state and a pathological one. For example, these results may offer useful insights into how Parkinsonian conditions lead to abnormally high correlations in activity within the globus pallidus, which in turn could influence activity downstream from the globus pallidus and throughout the basal ganglia-thalamo-cortical network.

Disclosures: **J.E. Rubin:** None. **C.O. Diekman:** None. **D. Terman:** None.

Poster

099. Network Models and Computational Studies

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Topic: G.06. Computation, Modeling, and Simulation

Support: NSF Grant CCF-1162449

Title: An energy efficient neuron model with excitatory and inhibitory inputs

Authors: ***J. XING**[1,2], T. BERGER[1], T. J. SEJNOWSKI[2];
[1]Dept. of Electrical and Computer Engin., Univ. of Virginia, Charlottesville, VA; [2]Salk Inst. for Biol. Studies, La Jolla, CA

Abstract: Neural information processing within neural microcircuitry is determined by the balance between excitation and inhibition (E/I balance). Toward understanding it better from an information-energy efficiency standpoint, we earlier calculated the maximum Shannon mutual

information transfer per unit of energy expenditure of an idealized integrate-and-fire (IIF) neuron all of whose synapses are excitatory but have differing weights. Since it is well known from neuroscientific experiments that the postsynaptic potential (PSP) accumulation in real neurons is not monotonically increasing but behaves like a diffusion process that possesses both local decrease and local increase, in this paper we extend the IIF model to a biophysically more realistic one in which synaptic weights not only are assumed unequal but also can be inhibitory. Such inhibitory signals are also widely considered essential for keeping signal processing in the brain from becoming unstable. Based on information theory and stationary processes, we have derived the formula for the long-term average mutual information rate. We also establish that the probability density function (pdf) of interspike interval (ISI) duration induced by the bits per joule (bpj) maximizing pdf of the combined excitatory and inhibitory postsynaptic potentials (EPSPs/IPSPs) remains a delayed gamma distribution as in the IIF model with only excitatory synapses. The bpj optimizing input density, in the case of inhibitory as well as excitatory unequal weights, still satisfies an inhomogeneous Cauchy-Euler equation with variable coefficients.

Disclosures: **J. Xing:** None. **T. Berger:** 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.; US National Science Foundation under Grant No. CCF-1162449. **T.J. Sejnowski:** None.

Poster

099. Network Models and Computational Studies

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Topic: G.06. Computation, Modeling, and Simulation

Support: SNI-61244

Title: System size resonance in a Fitzhugh-Nagumo artificial neural network

Authors: *J. A. TAPIA[1,2], E. MANJARREZ[1];
[1]Inst. de Fisiología, Benemérita Univ. Autónoma de Puebla, Puebla, Mexico; [2]Escuela de Biología - BUAP, Puebla, Mexico

Abstract: The stochastic resonance refers to an enhancement of the signal perception by a nonlinear system with the addition of an optimal noise. This phenomena has been observed in

artificial neurons by means of computational simulations (Goldbach et al, 2008) and within electronic devices (Calvo et al, 2006). Furthermore, there is a stochastic resonance phenomenon in the spinal monosynaptic reflex observed during the simultaneous arrival of a subtrheshold signal and a noisy input to sensory pathways (Manjarrez et al 2005). The aim of the following work was to develop an artificial neuronal network with an architecture similar to that of the monosynaptic spinal circuit which exhibited a stochastic resonance signal enhancement. We developed an electronic circuit based on the Fitzhugh - Nagumo equations. This artificial network consisted of a layer of interneurons (AIN) and a layer of motoneurons (AMN). The final output is the sum of all the artificial motoneurons. We applied a subthreshold signal simultaneously with a white Gaussian noise input to all the artificial interneurons, this noise was applied at ten different levels. The signal to noise ratio (SNR) was calculated in the bandwidth of the subtreshold signal. The number of interneuron-mononeuron pairs was increased from 1 to 10.We observed an increase in the SNR when applying an optimal intermediate noise (S=[36 - 60.9]mV). By increasing the number of AIN/AMN pairs the optimal noise was decreased. Furthermore, the amplitude of the calculated SNR was increased with the increase of the number of neurons. This electronic circuit provides insight of a possible system size resonance phenomenon in the biological systems. Also, this device could lead to a new type of signal processing unit, in which environmental noise cannot be reduced by traditional methods; or in which we could take advantage of the noise itself. Furthermore, we could fine tune the “sensorial circuit” to increase the SNR by having the amount of needed neurons according to the environmental noise.

Disclosures: **J.A. Tapia:** None. **E. Manjarrez:** None.

Poster

099. Network Models and Computational Studies

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Topic: G.06. Computation, Modeling, and Simulation

Support: REA-MCAFP7 Marie Curie Action, European Comission

ANR-11-0001-02 PSL

ANR-10-LABX-0087

Title: Dynamics of large networks of excitatory and inhibitory units with sparse, partially symmetric couplings

Authors: *D. MARTÍ[1,2], N. BRUNEL[3], S. OSTOJIC[1,4];
[1]Group For Neural Theory, DEC, Paris, France; [2]INSERM, Paris, France; [3]Dept. of Statistics and Neurobio., Univ. of Chicago, Chicago, IL; [4]CNRS, Paris, France

Abstract: Over the past ten years, several studies based on multielectrode recordings have revealed that cortical pyramidal neurons connect with each other according to patterns that display a non-trivial statistical structure. One prominent example of such structure is the fact that bidirectional connections between neurons appear more often than one would expect if connections were random. It is still unclear how this prevalence of bidirectional connections affects the dynamics of cortical circuits, and what possible functional role it plays. To elucidate this question we study the dynamics of large-scale networks of excitatory and inhibitory units with unclustered, random, sparse, and partially symmetric connections. We investigate networks of both rate units and spiking neurons and show that, in both cases, the nature of the network dynamics is strongly determined by the spectrum of eigenvalues of the connectivity matrix. For weak couplings, the real part of the eigenvalues is small and the network is in an equilibrium state with constant firing rates. For increasing connection strengths, the real part of the eigenvalues gets larger and the network eventually undergoes an instability that leads to a rate-chaotic regime, characterized by heterogeneous and fluctuating firing rates with a characteristic timescale that depends only mildly on the coupling strength. Using numerical simulations and mathematical analysis, we show that introducing partial symmetry in the couplings slows down the rate fluctuations in the chaotic regime, a result that can be qualitatively explained by the appearance of connection that contribute to activity reverberation. More quantitatively, this slowing down is brought about by the flattening of the eigenspectrum along the real axis and the subsequent concentration of low-frequency modes. For the non-chaotic regime, we compute how symmetry modulates the timescale of the noise filtered by the network operating at the transition point. In that case symmetry increases the characteristic asymptotic decay time of the autocorrelation function. Furthermore, for sufficiently symmetric connections the system operating in the chaotic regime exhibits aging effects, by which the timescale of the rate fluctuations slowly grows as time evolves, and which is a characteristic of systems out of equilibrium exhibiting a very large relaxation time.

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Poster

099. Network Models and Computational Studies

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Program#/Poster#: 99.23/UU10

Topic: G.06. Computation, Modeling, and Simulation

Title: Temporal expectation in chaotic balanced networks

Authors: *A. P. PONZI, J. R. WICKENS;
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Abstract: Neuronal activity becomes anticipatory in predictable sensory environments. In streaming perceptual discrimination tasks performance improves with predictability of both what stimuli will occur and when [1]. Temporally regular sensory streams entrain ongoing brain oscillations but how this happens and why it enhances discrimination is unknown. Here we show that temporal expectation emerges naturally from the dynamics of recurrent balanced neural networks. This is because the weakly chaotic oscillations they generate can be partially or fully stabilized by repetitive stimulus sequences in sensory driving streams. Periodicities in driving phase lock periodic orbits embedded in the underlying chaotic set, resulting in phase synchronized [2] stimulus stream specific network trajectories which though strongly varying have a stable phase evolution. This occurs even when stimulus streams realistically consist of brief 50 ms cue stimuli trial presentations separated by 800 ms long inter-trial-interval (ITI) periods of cue-independent static background input. Studies show that recurrent networks display temporally extended stimulus specific dynamical activity patterns as reactive responses to input stimuli and that these evolving trajectories provide a substrate for working memory and represent elapsed time [3]. We here extend this to demonstrate that in phase synchronized networks the temporally evolving response profiles of sequentially activated cells are specific to the stream context the stimuli are embedded in and thus anticipatory of the type and timing of upcoming stimuli. We show that such single cell activity patterns provide greater discriminatory information of the preceding cue during the subsequent ITI than purely reactive responses. This is because the more chaos is stabilized by the driving stream the more reproducible the ITI period cue response across multiple trials. We show that while phase synchronization and therefore ITI period stimulus discriminability increase continuously with both the temporal regularity of the stream and the predictability of the stimulus type these two kinds of anticipation interact in a non-linear way. The more temporally irregular the stream the less type predictability enhances discriminability. Our results are reliable with significant noise in the network dynamics, occur at natural streaming frequencies although networks possess only biological timescales and are consistent with multiple studies of streaming perceptual discrimination tasks.
[1] Nobre AC et al. (2007) Curr. Op. Neurobio. 17:465. [2] Pikovsky A et al. (1997) Chaos 7:680 [3] Buonomano DV, Merzenich MM (1995) Science 267:1028

Disclosures: **A.P. Ponzi:** None. **J.R. Wickens:** None.

Poster

099. Network Models and Computational Studies

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Topic: G.06. Computation, Modeling, and Simulation

Title: A modified kinetic Inverse Ising method for the inference of synaptic spatial structure and characteristic times

Authors: *P. DEL GIUDICE[1], C. CAPONE[2], C. FILOSA[3], G. GIGANTE[4], F. RICCI TERSENGHI[3];
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Abstract: A long standing problem in neuroscience, both in modeling and in data analysis, is the one of inferring synaptic couplings from correlations of the sampled neural activities. The recent availability of techniques allowing simultaneous recording from several tens electrodes, in-vitro as well as in-vivo, gave new momentum to research in this direction. In particular, much effort has been devoted to develop and refine inference methods inspired by the statistical mechanics of spin systems (so called 'inverse Ising' methods). In the original proposal ([1]), simultaneously recorded data are binned in time, binarized and interpreted as successive configurations of a spin system with pairwise interactions at equilibrium. Inference proceeds then as the solution of a constrained optimization problem: determine the spin couplings providing the maximum entropy (Gibbs) distribution compatible with the observed mean activities and pair spatial correlations, used as constraints. 'Brute-force' solutions can be obtained by iterative procedures akin to learning algorithms in Boltzmann machines; the need to reduce the computational load for large networks motivated the use of various forms of mean-field estimates of the correlations from the measured mean activities. The interest in relaxing the assumption of equilibrium later led to the development of inference methods based on kinetic Ising models; for a review of the state of the art see [2]. In this work, using simulations of networks of integrate-and-fire neurons, we incorporate in kinetic inverse Ising inference methods the important notion that spikes are transmitted between neurons with delays, which are estimated from the profile of the cross-correlation function prior to the inference procedure, and inform the choice of the time bin used in the inference algorithm. A method is also developed to take into account a finite time of integration of the synaptic input. Finally, we analytically and numerically study the relationship between the inferred and the real synaptic efficacies, and how the choice of the time bin affects

it. Such relationships turns out to be quadratic both for excitatory and inhibitory synapses, but it depends critically on the time bin for the excitatory synapses only, whilst being essentially independent of the time bin for the inhibitory ones. Preliminary results on the application of the inference method to data from neural cultures will be reported. [1] Schneidman E, Berry MJ, Segev R and Bialek W, Nature 440, 1007-1012, 2006 [2] Hertz J, Roudi Y, Tyrcha J, in "Principle of Neural Coding" S. Panzeri and R. Q. Quiroga eds, CRC Press 2013

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Poster

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Topic: G.06. Computation, Modeling, and Simulation

Support: NASA NNX10AD59G

NIH Grant NS35915

Title: Context-driven generation of virtual dendritic morphologies enables complete population-level construction and analysis

Authors: *C. SCHNEIDER[1], H. CUNTZ[2,3], I. SOLTESZ[1];
[1]Anat. and Neurobio., Univ. of California, Irvine, Irvine, CA; [2]Ernst Strüngmann Inst. (ESI) for Neurosci. in Cooperation with Max Planck Society, Frankfurt/Main, Germany; [3]Inst. of Clin. Neuroanatomy, Goethe Univ. Frankfurt, Frankfurt/Main, Germany

Abstract: Dendritic morphology has been shown to have a dramatic impact on neuronal function, and there is an inherent variability in this morphology that is often overlooked when studying computation in neural networks. While detailed models for morphology and electrophysiology exist for many types of single neurons, the role of detailed single cell morphology in the population has not been studied in detail anatomically or computationally. Current techniques for the construction of morphological models largely ignore the structural location and context that the morphologies originate from, focusing on independent branching statistics. The current study instead uses the structural context of the neural tissue in which dendritic trees grow to drive the morphological generation process. Utilizing parallel computing,

the complete population of the most numerous cell type in the hippocampus, the dentate gyrus granule cell, was generated within a realistic structural context, with a granule cell layer that contains all somata and a molecular layer that contains the dendritic trees. By growing dendrites within elliptical cones, which are characteristic of the dendritic field of dentate granule cells, in the realistic structural context of the volume of these neural layers, dendritic trees could be generated that were statistically and visually indistinguishable from experimental reconstructions. Branching statistics can now be linked to larger scale neuroanatomical features and the input organization of granule cells can be studied at the level of the population. This also provides a framework by which to populate network models with detailed single-cell morphological models and three-dimensional, intersection-based connectivity. This procedure can be repeated for other cell types within the dentate gyrus and for other brain regions, and represents a major advancement in the development of realistic large-scale neural network models. This work was funded by NASA NNX10AD59G and NIH grant NS35915 to I.S.

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Poster

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Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 99.26/UU13

Topic: G.06. Computation, Modeling, and Simulation

Support: JSPS Research Abroad, MEXT

NSF (grant numbers 0904912 and 1058291)

Title: Hubs, clusters and communities of hundreds cortical neurons

Authors: *M. SHIMONO, J. M. BEGGS;
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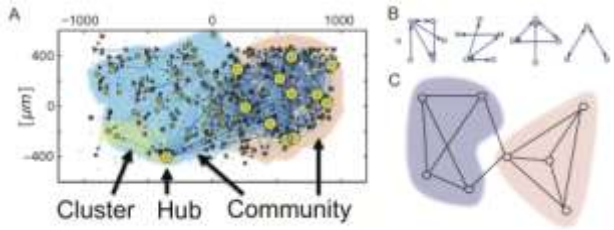
Abstract: Neuronal networks are essentially non-random. At same times, although neurons show multiple-scale behaviors on both of temporal and spatial scales, how non-randomness in several different topological sizes relate each other has not been demonstrated experimentally before. This study aimed to clarify important relations between non-randomness in groups of 3-6 neurons and non-randomness in groups of 50-100 neurons through five steps. First, we recorded spontaneous activity of up to 500 neurons from rodent somatosensory cortex using 512ch. multi-

electrode system over one hour. Second, we prepared an analysis scheme to reconstruct effective networks using transfer entropy. Third, after evaluating similarities of topologies of effective networks in 3-6 neuron scales (clusters including motifs [Figure1-B]) with topologies of synaptic connections measured using cutting edge patch-clamp experiments recording from up to 12 neurons simultaneously. Fourth, we constructed community or modular structures representing non-randomness of larger group of neurons [Figure1-C]. Fifth, we evaluated how much these two kinds of non-randomness are fragile when distributing connections connecting with several percentages of high-degree neurons (hubs) to many other low-degree neurons. From these procedures, we found three things. First, effective networks consisting of hundreds of cortical neurons have distinctive non-random structures of connectivity at two different scales. Second, the degree-distribution showed power-low distributions. This supported the existence of "hubs" in the cortex. Third, structure at these scales showed different dependencies on connections from hub neurons with the smaller groups being relatively more fragile than the larger groups because large-scale nonrandom structure can exist even after small-scale nonrandom structure is completely disrupted.



Disclosures: **M. Shimono:** None. **J.M. Beggs:** None.

Poster

099. Network Models and Computational Studies

Location: Halls A-C

Time: Saturday, November 15, 2014, 1:00 PM - 5:00 PM

Program#/Poster#: 99.27/UU14

Topic: G.06. Computation, Modeling, and Simulation

Support: JSPS Research Fellowship (DC2)

KAKENHI 22115013

Title: Network structure generates priors for internal probabilistic model

Authors: *N. HIRATANI[1,2], T. FUKAI[1,2];
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Abstract: Many experimental results suggest that connections in neural circuits often have characteristic structure that is arguably genetically determined. For example, in the olfactory bulb of *Drosophila*, some connections are highly stereotyped while others are random. In somatosensory cortex of rats, there is an intrinsically clustered connection structure. However, functional roles of these structures remain elusive. In particular, each synapse can show a wide range of plasticity depending on spike timing and firing rate, and theoretical results suggest that various structures can be learned by randomly connected networks. In addition, recent experiments revealed that EPSPs typically follow long-tailed distributions in cortex. In particular, strong synapses have significant effects on the post synaptic neuron. This means that the network structure defined by strong synapses can override the structure defined by connectivity, and possibly play a major role in information processing. Thus, we studied the functional role of network structure under the presence of synaptic plasticity and synaptic weight variability. By assuming that neuronal circuits provide internal models of probabilistic phenomena, we constructed network models with synaptic plasticity. Our results suggest that network structures provide priors for internal probabilistic models, and enables learning even from noisy or incomplete stimuli. As a result, the learned network can perform inference nearly optimally. We further discuss the functional roles of synaptic rewiring, especially when the external environment shows variability.

Disclosures: **N. Hiratani:** None. **T. Fukai:** None.

Poster

099. Network Models and Computational Studies

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Topic: G.06. Computation, Modeling, and Simulation

Support: S.C. Holds a Career Award at the Scientific Interface from the Burroughs-Wellcome Fund

Title: Modeling neural-metabolic homeostatic coupling in burst suppression

Authors: S. LIU[1], *S. CHING[1,2];
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Abstract: Recent interest has grown around burst suppression (BS), a pattern of the electroencephalogram characterized by quasi-periodic alternation of high-voltage activity (burst) and isoelectric silence (suppression). Burst suppression is associated with significant reduction of neural activity, such as in deep general anesthesia and certain types of coma. A recent computational model (Ching et. al., 2012) has suggested a role for cerebral metabolism in the slow - up to tens of seconds - time-scale process that governs the termination and re-initiation of bursts. Specifically, in the model, reductions in cerebral metabolic rate, typical of the etiologies of BS, lead to the formation of a slow metabolic depletion-recovery cycle. That is, activity-dependent substrate depletion during bursts, followed by substrate recovery during suppressions. In this sense, the mechanism involves a slow, metabolic process that gates the neural activity. Missing from the model, however, is a role for homeostatic coupling between neural and metabolic processes, i.e., a supply-demand feedback effect wherein the neural activity itself gates metabolism. Here, we develop a general, low-dimensional neural mass model for burst suppression that contains homeostatic coupling between the neural and metabolic dynamical processes. Specifically, we add cerebral metabolic dynamics to a neural mass model of a cortical population. The metabolic dynamics involve a variable corresponding to substrate that gates the neural activity, the intensity of which then determines the metabolic consumption and recovery rates via a homeostatic coupling function. This creates a physiologically consistent, reciprocal interaction between the neural and metabolic processes that, in baseline conditions, supports continuous activity. Through detailed computational simulation and analysis, we subsequently show that burst-like activity can manifest in the neural dynamics through downregulation of either metabolism or neuronal activity, independently. The latter result, in particular, fills an important gap in explaining how burst suppression might arise through the actions of anesthetic drugs on neural dynamics, absent a direct effect on metabolism. For example, it characterizes why increasing inhibition leads to bistable, i.e., burst-like activity, rather than a gradual 'tapering off'. Our modeling results are fully consistent with empirical characterizations of burst suppression across etiologies. Moreover, the model highlights the importance of including the biophysical dynamics of homeostatically coupled processes, such as metabolism, in computational neural models.

Disclosures: **S. Liu:** None. **S. Ching:** None.